Mushroom cultivation 3rd edition
Appropriate technology for mushroom growers

Peter Oei
This manual provides essential information on how to grow mushrooms, and gives details on the most cultivated mushrooms worldwide: Button mushroom, Oyster mushroom and Shiitake. Furthermore, the cultivation practices for ten other mushrooms are explained, as well as marketing aspects, feasibility studies, mechanisation, climate control, farm management and post-harvest handling. This third edition has been updated to include modern high-tech developments such as indoor composting, energy efficiency and certified organic cultivation. In addition to addressing the more robust procedures for developing countries, it now contains six new chapters on White button mushroom cultivation.
Mushroom Cultivation
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appropriate technology
for mushroom growers

by

Peter Oei

Backhuys Publishers, Leiden
The Netherlands
The Technical Centre for Agricultural and Rural Cooperation (CTA) was established in 1983 under the Lomé Convention between the ACP (African, Caribbean and Pacific) Group of States and the European Union Member States. Since 2000, it has operated within the framework of the ACP-EC Cotonou Agreement.

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PRINTED IN BELGIUM
Caught by the mushroom virus in 1985 and later working for the former Mushroom Tower, I was asked to write a practical manual on mushroom growing for developing countries in 1991. When I compiled the first edition, I hadn’t expected to rewrite it for a second edition in 1996 and now a third edition in 2003. Over 6000 copies have been distributed all over the world, both in French and English.

Intended originally for developing countries, the book has evolved to its current state. The second book was already distributed more in Western countries than in the third world because of its detailed instructions on growing ‘exotic’ mushrooms. This third edition now gives a detailed view on growing the most important mushrooms of the world. It also covers recent developments in the highly specialised sector of White button mushrooms. These new chapters have been provided by experts in their specific fields: Marc Maas (Cpoint), Luc Klunder (Gicom), and Ruud Thielen (Thirolot). Peter van Erp and Anton Sonnenberg, Applied Plant Research, kindly permitted me to use parts of the book Mushroom Cultivation, edited by Van Griensven in 1988, which deals exclusively with White button mushrooms.

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Tiel, June 2003

Peter Oei
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1 Benefits of mushrooms

The beneficial aspects of mushrooms can be divided in their use:
- as a food with specific nutritional value and a comparatively high price,
- as a means ‘to come closer to the gods’,
- as medicine or tonic to increase health,
- as natural dyes for fabrics,
- to bioremediate polluted soil or neutralise acidic runoff.

The first aspect is economically the most important, the last aspect (bioremediation) is still in the experimenting stage.

1.1 History and the origin of religion

Many *Psilocybe* mushrooms have a strong effect on the mind and cause hallucinations. (Courtesy PFE). Right: Meso American mushroom stones, relicts which show the importance of mushrooms in Latin America’s religions before the colonisation by the Spanish.

Mankind has harvested wild edible mushrooms for millennia. The mushrooms were either eaten directly or preserved for later use by drying. Mushrooms have been treated as a special kind of food since earliest times. Chinese and Japanese chronicles indicate that the Shiitake mushroom was collected in the wild and was given to the emperors as a tribute. The Romans ate mushrooms on special occasions. In some cultures however, all mushrooms were considered toadstools, poisonous gifts from the Devil. Nowadays, the migration of many millions of people with different food habits has spread the popularity of mushrooms. Palatability of mushrooms is considered further in the chapter Marketing.
Mankind also consumed mushrooms, which had hallucinogenic effects. According to Roger Wasson, a famous American banker who devoted much of his life to the study of this peculiar kind of mushroom, ‘entheogenic’ (carrying God within them) mushrooms formed the fundament of many religions. He managed to prove that theory for Mexican and North American Indians, and made it quite probable for Hinduism. In many parts of the world old rituals, where a shaman reaches a state of ecstasy after consuming mushrooms or specific plants, have long been replaced by rituals of other religions. In modern Western society, with its sharp boundary between culture and nature, the use of entheogenic mushrooms is often illegal; the chemicals which cause the hallucinogenic reactions are even considered hard drugs. A small group of people rediscovered the use of these mushrooms to raise their level of awareness and managed to develop a simple cultivation technique on the basis of captured spores.

1.2 Wild edible mushrooms

Much less controversial is the consumption of mushrooms as a food. Common edible mushrooms are still gathered in the wild in many cultures. The Romans were very fond of mushrooms; often it was the lord of the house himself who prepared mushrooms. Story goes that picking the Emperor’s Amanite (Amanita caesarea) was punished by cutting off the hands of the offender. In some cultures a tremendous fear of poisonous mushrooms exists. In England for example, people are very reluctant to pick wild mushrooms. The reason why some ethnic groups despise and other groups love mushrooms passionately has not yet been found.

To many people, mushrooms are strange phenomena. The actual organism lives most of the year unnoticed under the ground or inside wood; only by fruiting it reveals its presence. The peculiar morphology of many mushrooms has given rise to many fantastic stories, in which devils, witches and elves abound. In the 16th century, some European scientists discovered the function of the spores, but their knowledge did not spread much for centuries. In the early
seventeenth century, Count Marsigli described how a white, mouldlike web could be found when mushrooms were carefully dug up. Many wild species are being collected for consumption, far more than are cultivated at present. For a large number of species, the cultivation parameters are not yet known, especially for fungi which live in close association with other organisms. For these mushrooms, mankind has to rely on nature. More information on picking wild mushrooms can be found in the chapters ‘Biology’, ‘The collection and cultivation of Mycorrhizal mushrooms’ and the appendix ‘Termitomyces’, a genus of popular edible mushrooms in Asia and Africa.

1.3 Edible mushrooms and their cultivation

In early times cultivation failed because the biology of fungi was not understood. The Romans tried to cultivate Agrocybe aegerita on poplar wood without knowing how the mushrooms propagated. These attempts show, however, that the ecological situation for these mushrooms was known: thick trunks of hardwood were covered with soil in the hope of a future harvest.

The first professional growers of mushrooms were the Chinese; as early as 1313 a document was published which described the cultivation method for Shiitake on wood logs. The Chinese even erected a temple for the priest Wu San Kwung, who discovered that more harvest would follow after the (fully grown) wood logs were given a beating.

We now know that this physical shock triggers the fruit body formation and releases captured CO₂ inside the logs. The cultivation of the Rice straw mushroom is also centuries old; heaps of fermenting rice straw were stacked for this purpose; these early growers relied on spontaneous inoculation by spores from the air. Even older is the cultivation of the Wood ear mushroom (Auricularia spp.), of which sources indicate it was cultivated from the year 600 A.D. onwards.

The white button mushroom, Agaricus bisporus, was domesticated in France. The first descriptions date from 1707 and the technology was based on the fact that composted manure which produced mushrooms, could be used to inoculate new stacks of composting horse manure. At the end of the 19th century a multipore technique for spawn (mushroom ‘seed’) was developed in France, followed by tissue culture techniques by the American Duggar.

Around that time, Japanese scientists developed methods to inoculate wood logs for Shiitake production.

Cultivation of the White button mushroom rapidly spread after the second World War when reliable spawn became commonly available in a number of countries.

Mushroom growing has many advantages: no arable land is needed, agricultural waste is converted into fertilisers and soil conditioners, it is income-generating and the mushrooms provide an extra source of protein and valuable vitamins and minerals. Especially mainland China and Taiwan have developed many methods to grow mushrooms.
with limited input. Also, there is a growing interest in Africa and Latin America in the cultivation of mushrooms. Often the existing producers are very secretive about their production methods. This book is intended to distribute knowledge from various places. Some of the methods discussed in this book are published for the first time in the English language. Extension workers and mushroom growers can use the case studies from other parts of the world to broaden their view. However, they should always adapt techniques to local circumstances. For example, a technique to grow mushrooms on coffee pulp waste developed in Mexico can also be applied in coffee-producing regions in Africa, but the organisation behind the mushroom farm may be different, as well as the type of substrate containers.

1.4 Nutritional aspects

The nutritional value of a product should be considered in relation to the complete menu. Nothing is healthy in itself, it is the combination of different foodstuffs that can be sufficient or deficient in trace minerals, vitamins or protein.

Many mushrooms are considered to be healthy food because they contain large amounts of qualitatively good protein, vitamins (B1, B2, C) and minerals and have a low fat content. Furthermore, several species have a definitive effect on blood pressure, tumours and viruses. Most of the ingredients which determine the nutritional value are located within the cell walls. The cell wall itself is made up of chitin (and a number of other components), a difficult to degrade compound, which is also commonly encountered in the skin of insects. It is therefore important to break up the cell walls during the preparation of the food and to chew thoroughly. This will increase digestibility.

**Protein:** Protein is usually determined by determining nitrogen content and multiplying this figure by 6.25. This involves the assumption that nitrogen is only present in the form of protein. Some of the nitrogen, however, is contained in the cell walls (chitin). A factor of 70% × 6.25 = 4.38 is considered to better approximate the real protein content. Mushrooms are often referred to as valuable protein sources, but actually their protein content is rather low, normally 3 to 4% of their fresh weight. Water content is usually around 90%.

If protein content of the dry weight is considered, one will find that mushrooms contain between 19 and 35% protein. Mushrooms contain less protein than soy beans (39% of dry weight), but much more than rice, oranges or apples. The value of proteins is determined by the kinds of amino acids that form the protein. Mushrooms contain all the (for humans) essential amino acids, as well as most commonly occurring non-essential amino acids and amides. If one or more essential amino acid is in inadequate supply, the utilisation of all the others will be reduced with the same ratio. Animal food is generally better balanced than vegetable foods in proteins. Cereal grains for example, contain little lysine. Lysine, however, is the most abundant essential amino acid in mushrooms. Thus mushroom protein can be a valuable addition to the diet.

**Fat:** The table on the next page shows that the fat content on a dry weight basis is in the range of 1 to 8%, with an average of 4%. Unsaturated fatty acids make up at least 72% of the total fat content, mainly due to linoleic acid. Saturated acids abound in animal fats and are regarded as hazardous to health. The high content of linoleic acids is one of the reasons why mushrooms are considered healthy food.
Vitamins and minerals: Mushrooms are a good source of vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin, biotine, and ascorbic acid (vitamin C). Mushrooms in general contain significant amounts of phosphorus, sodium, potassium and a lesser amount of calcium.

Vitamin content in mushrooms (adapted from Eli V. Crisan and Anne Sands, in Chang & Hayes, 1978)

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<th>Riboflavin</th>
<th>Ascorbic acid</th>
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<td>55.7</td>
<td>5.0</td>
<td>81.9</td>
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<tr>
<td>Auricularia polytricha (fresh)</td>
<td>0.2</td>
<td>1.6</td>
<td>0.9</td>
<td>no data</td>
</tr>
<tr>
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<td>4.7</td>
<td>0.6</td>
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</tr>
<tr>
<td>Flammulina velutipes</td>
<td>6.1</td>
<td>106.5</td>
<td>5.2</td>
<td>46.3</td>
</tr>
<tr>
<td>Lentinula edodes (fresh)</td>
<td>7.8</td>
<td>54.9</td>
<td>4.9</td>
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<tr>
<td>Lentinula edodes (dried)</td>
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<td>11.9</td>
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<tr>
<td>Pleurotus spp.</td>
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<td>108.7</td>
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</tr>
<tr>
<td>Volvariella volvacea</td>
<td>0.35-1.2</td>
<td>4.88-91.9</td>
<td>1.63-3.30</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Carbohydrates and fibres: Fibres are also part of a healthy diet. In modern society some foodstuffs are refined (for example white bread) and thus contain less fibres. Fresh mushrooms contain relatively large amounts of fibre and carbohydrates.

The composition of mushrooms. The following data show the percentage of dry weight, except the initial moisture content (expressed as water content/total weight x 100%) and energy value in kcal. (Adapted from Eli V. Crisan and Anne Sands, The Biology and Cultivation of Mushrooms, in Chang & Hayes, 1978, and Bano et al., Mushroom Journal for the Tropics, 1(3)6, 1981)

<table>
<thead>
<tr>
<th></th>
<th>Initial moisture %</th>
<th>Crude protein N x 4.48</th>
<th>Fat</th>
<th>Total carbohydrates</th>
<th>N-free carbohydrates</th>
<th>Fibre</th>
<th>Ash</th>
<th>Energy (Kcal/100 g dry material)</th>
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</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>88-90</td>
<td>1</td>
<td>1.7-3.1</td>
<td>51.3-62.5</td>
<td>44.0-53.5</td>
<td>8.0-10.4</td>
<td>7.7-12</td>
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<tr>
<td>Auricularia polytricha</td>
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<td>24-34</td>
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<td>79.9-93.2</td>
<td>68.0-82.9</td>
<td>2.5-21.60</td>
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<td>17.6</td>
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<td>59.2</td>
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<tr>
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<td>58.0</td>
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<td>57.6-81.8</td>
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<td>90.1</td>
<td>10.5-30.4</td>
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<td>90.5</td>
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<td>2.4</td>
<td>45.3</td>
<td>9.3</td>
<td>8.8</td>
<td>276</td>
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<tr>
<td>Volvariella volvacea</td>
<td>89.1</td>
<td>25.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.5 Medicinal use

Lower fungi have yielded important medicines, like penicillin and other antibiotics from *Penicillium* (a common contaminant in mushroom cultivation). The use of mushrooms is much less widespread in the Western world. Sometimes the mycelium is used, but fruit bodies are considered better in Asia. In China alone, more than 700 medicinal products with mushrooms as the main ingredient are commercially available. According to a statistic from the ministry of Peoples Health, at least 106 medicinals contain *Ganoderma*, 43 *Cordyceps*, and 7 Shiitake. It was estimated that the market of medicinal and health products from mushrooms had a turnover of 5 to 6 billion US$ in 1995. These products are either sold as whole mushrooms (often dried), or in the form of powder, capsules or a bitter tea.

The health effects of mushrooms are mainly restricted to the prevention or outgrowth of diseases. They do not have much of a healing effect. That may, however, just be a matter of time as more and more research is done in this field.

1.5.1 Heart and coronary diseases

Heart and coronary diseases are often caused by high cholesterol levels. Mushrooms fit perfectly in a diet with many fibres and low fat. Experiments with Shiitake indicate, that especially the thicker ‘donko’ mushrooms have a cholesterol-lowering effect. Even a combination of Shiitake with fat pork decreased the cholesterol level. Oyster mushrooms also contain substances which lowered the cholesterol level in serum and liver of rats.

1.5.2 Cancer

Many mushrooms contain substances, which suppress the growth rate of tumours. Often these substances belong to the group of polysaccharides, like Lentinan from Shiitake (*Lentinula edodes*), Schizophyllan from *Schizophyllum commune* and Grifolan (from *Grifola frondosa*). An extract of the Monkey head mushroom (*Hericium erinaceus*) is used to enhance the effects of chemo- or radiotherapy and the well-being of the patients.

1.5.3 Diabetes

Research on animals indicates that mushrooms like *Cordyceps sinensis*, *Lentinula edodes*, *Grifola frondosa* and *Pleurotus ostreatus* all have a positive effect on diabetes. Older literature mentioned the use of the Shaggy mane, an Inkcap, for this purpose.
1.5.4 Protection against free radicals and infection
Free radicals can damage body cells and induce cancers. Free radicals are the result of specific transformation processes. Many bioactive compounds protect the body against free radicals. These substances are often called antioxidants and present in many mushrooms. A positive effect on infections has been shown in *Tremella fuciformis*, *Auricularia auricula-judae*, and *Ganoderma japonicum*.

1.6 Soil bioremediation
Most cultivated mushrooms, which are treated in this book, are so-called white rot basidiomycetes. These share the ability to degrade lignin, a major constituent of plant material. Wood consists of lignin fibres which enclose the more easily degradable cellulose. A third major constituent of wood is hemicellulose. Lignins are difficult to degrade, as are a number of xenobiotic pollutants. The structure of lignin is similar to that of PAH’s (poly-aromatic hydrocarbons), consisting of benzene rings linked with carbon and hydrogen atoms in linear, angular or clustered arrangements. A beneficial aspect of mushrooms is that they are able to break down organic pollutants, like PAH, PCB and dioxins. Bacteria have already been used for the remediation of gasoline-containing soils. They manage to break down a number of PAH’s but cannot degrade PAH’s with more than four benzene rings. This is due to the very low water solubility of the more complex PAH’s. The pollutants strongly adhere to organic substances and are difficult to reach for the bacteria.

The mycelium of white rot (lignin-degrading) basidiomycetes works quite differently. It produces extra-cellular enzymes, which can reach non-soluble pollutants, even if these adhere to humic substances. The organism most used in scientific studies is *Phanerochaete chrysosporium*, which has been shown to degrade anthracene and benzo(a)pyrene. It is not easy however to meet the specific requirements for the optimal growth of this fungus in the soil, like a high temperature (37 °C), and a high oxygen concentration. Another organism, *Coriolus versicolor*, has also been studied intensively. It has been shown to produce extracellular peroxidases, which are believed to degrade PAH’s.

The use of mushroom compost has also been mentioned for neutralising acidic runoff water from coal mines in Pennsylvania, USA. Research indicates that passive treatment of acid runoff in so-called ‘successive alkalinity producing systems’ gave good results in Pennsylvania. In these systems, limestone and a layer of 50–70 cm of spent mushroom compost are flooded with acid runoff water. The system will gradually increase the pH of the water and heavy metals will precipitate without clogging the pores of the limestone.
2 General biological information on mushrooms

To understand mushroom growing and the properties of mushrooms, some biological knowledge of the crop is necessary, as the production techniques will then make more sense and crop failures may be understood. This chapter treats the following subjects:

- taxonomy of fungi,
- fungus ecology,
- life cycle of fungi,
- mushroom physiology.

2.1 Taxonomy of fungi

All mushrooms belong to the kingdom of Fungi, a group very distinct from plants, animals and bacteria. Like plants, fungi have a distinct cellular structure but they lack the most important feature of plants: the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, as does mankind and in fact all animals. All fungi, with the exception of yeasts, form so-called hyphae, tiny threads that originate from the spores. These hyphae will branch out and form the mycelium. After fertilisation, they will enter a sexual phase and form spores. The larger spore-producing structures (bigger than about 1 mm) are called mushrooms. In nature this is the most striking part of the organism, but in fact it is just the fruit body. Many different forms have evolved to form structures that are capable of producing millions of spores. The major part of the living organism, however, is to be found under the ground or inside the wood.

Different ways to increase the surface and thus the potential spore release: (from left to right) tubes (Boletus), gills (Agaricus), spines (Hydnum).
Most of the cultivated mushrooms belong to the Basidiomycetes, which produce their spores on so-called basidia. Another important group are the Ascomycetes, which produce their spores in asci (‘bags’).

Fungal taxonomy is still a much disputed issue, mainly because there are so many different mushrooms of which relatively few have been described properly. Especially in developing countries there are thousands of species unknown to science, although sometimes well-known by the local population. It has been estimated that our planet harbours about 1.5 million different species of mushrooms. Only 64,000 species have been described so far. Many species from tropical rain forests and remote areas may have disappeared before science has had the opportunity to describe them. Apart from nature conservation, it is therefore particularly important to isolate native strains and deposit the cultures in a culture collection.

2.1.1 Scientific and colloquial names of mushrooms
In this book the scientific names of mushrooms are used, as they give rise to less confusion than colloquial names do. In the chapter Marketing some colloquial names are given.

Some people only consider the white cultivated mushroom to really be a ‘mushroom’ and call the rest ‘toadstools’. The name Oyster mushroom is applied to more than 20 different species of mushroom, each with its own cultivation characteristics like optimal temperature range, colour and growth rate. Within a region the name for the same mushroom can differ from province to province even within the same ethnic group. The giant *Termitomyces titanicus* is called Igihefu in Burundi, Chikolawa by the Bemba in Zambia, Tou Swen by the Lozi (meaning ‘WHITE elephant’) and Utale by the Chewa people. By using the scientific name and preferably the origin of the strain, one can make clear which species is meant.

In the chapter Marketing a table is given with both colloquial and scientific names, special marketing features of the different mushrooms like texture and taste, and the markets which can be aimed at.

2.1.2 Species concept in cultivated mushrooms
The scientific name of a mushroom (e.g. *Agaricus bisporus*) consists of two parts: the genus name, starting with a capital (*Agaricus*) and the specific epitheton (*bisporus*) in lower case. *Agaricus bisporus* thus belongs to the genus *Agaricus*. All *Agaricus*-species share the feature of purple or chocolate brown spores, show the presence of a veil in a young stage, that forms a ring on the stalk in most cases, and have gills free from the stem (see figure on top of next page).

Typical features of the genus *Agaricus*
Although the mycelium is the real organism and the mushrooms are merely its fruit bodies, the mycelium cannot serve to tell us which species it belongs to. The form of the fruit body is the traditional basis for classification in fungi. The colour of the spores, the shape and attachment of the gills, the presence of a volva, ring, etc., are all features to distinguish mushrooms from each other. Macroscopic features alone are insufficient for mushroom taxonomists. Spore shape
and size, the presence of clamp connections, and other microscopic features are required to differentiate between macroscopically similar mushrooms. Different species have to differ in physical and chemical characteristics. If, according to the biological concept of species, the offspring of two individuals is fertile, then they belong to the same species. For a large number of saprophytic fungi, this distinction works quite well. Some strains, however, are morphologically very similar, but not compatible. Other groups possess highly variable morphological characteristics, suggesting that more than one species may be involved, but different species cannot be simply identified.

The biological species concept, based on genetic isolation between species, is nowadays the most widely accepted concept. A population of a specific species constantly exchanges genetic material within itself. Thus, speciation can only proceed if part of a population becomes isolated from other populations of the same species, through ecological specialisation or geographical barriers, thus restricting gene flow to a small part of the population. If spores are only viable within a certain distance of their source, then such a population may specialise to benefit optimally from the specific environment. In this way a strain can gradually evolve into a new species. DNA sequencing nowadays offers the potential to recognise specific strains and species. It also enables breeders to develop strains much faster than in the past, as specific traits can be attributed to the presence of certain genes.

The most practical approach for mushroom growers to the subject of taxonomy is to rely on taxonomists. It is best to order strains from renowned institutions like culture collections. When referring to the strain, ideally the number under which it can be ordered from the culture collection should be mentioned. When obtaining spawn from a company, the company should be able to clarify questions about the identity of the strain involved.

### 2.2 Fungus ecology

Fungi depend on other organisms for their food. Three modes of living can be recognised:

- saprophytes: degrading already dead material,
- symbionts: living together with other organisms (especially trees) in a close, mutually beneficial relation,
- parasites: living at the expense of other organisms.

In reality species often do not confine themselves to one mode only. Several symbiotic mushrooms have saprophytic abilities, many parasitic mushrooms change to saprophytism after their host is killed.

The mode of living has nothing to do with edibility: in all three groups both edible or poisonous mushrooms can be found.
2.2.1 Saprophytes
Most of the fungi discussed in this book belong to the saprophytes. These need organic matter to decompose. In nature they will grow on fallen leaves, animal droppings, or stumps of dead wood. Some are specialised in breaking down the hairs of mammals, while others may decompose birds’ feathers. It is their role in nature to decompose the complex organic structures left behind by plants and animals. Subsequently plants or animals regain access to minerals and other nutrients present in the substrate. In regard to wood degraders, scientists often distinguish between ‘white rot’ and ‘brown rot’ fungi. The brown rot fungi are not well equipped to break down lignin, which gives the degrading wood a brown colour with a cubic structure. White rot fungi are capable of degrading both lignin and (hemi)cellulose and this process will turn the degrading wood white.

The mushroom species discussed in this book can roughly be divided into those living on a composted medium, or on wood (or substitutes like lignocellulose-rich agricultural wastes). After having decomposed their specific substrate, the spent compost can be applied to serve as a fertiliser or at least improve the structure of the soil.

Left: Brown rot is caused by mycelium which can not degrade the lignin in the wood; notice the cubic particles into which the wood falls apart. Middle: Flammulina velutipes, the Velvet stem mushroom, grows in nature on dead wood stumps. Right: Oyster mushrooms degrade dead wood, too.

2.2.2 Symbionts
The group of symbionts is very important for agricultural processes. The roots of most plants are covered with a sheath of mycelium of some kind of fungus. Many non-woody plants, amongst which all cereals, live in close association with several species of microfungi, not considered further in this book. Many symbiotic macrofungi are associated with a range of tree species, and are of great importance to forestry. The mycelium of symbiotic mushrooms will deliver water and salts in an efficient way to the plant and will receive easily accessible nutrients (carbohydrates) in return. The mycelium can also protect the plant in some ways, by producing antibiotics. The myc-
lial sheath functions as a shield against other (possibly harmful) fungi, and by lowering the carbohydrate content in the roots it makes the plant less susceptible to pathogens. Mycorrhizal mushrooms have great marketing potential in many parts of Africa: most species from the genera *Boletus* and *Cantharellus* are edible and can be picked and marketed. A typical aspect about harvesting mushrooms is that the forest is not rapidly overexploited, contrary to collecting ornamental plants or specific animals. Harvesting mushrooms does not affect the ecosystem, as has been shown in many scientific studies. Mushrooms do not become extinct because man picks their fruit bodies. The main reason for mushrooms becoming extinct is that they lose suitable habitat, which may have several causes.

Ectomycorrhizal species from genera like *Cantharellus* and *Boletus* grow in close relationship with trees. Both tree and mushroom mycelium benefit from each other. This symbiosis offers huge potential for environmental education to the local population. To put it bluntly: no trees, no mushrooms. Preserving the forest is necessary to secure mushroom harvests. Mushrooms can actually help in saving the forest. However, unregulated harvesting will also cause unwanted side effects; consult the chapter on mycorrhiza for more information.

It has proved to be very difficult to cultivate mycorrhizal mushrooms, although much research has been done. Some of these species are much appreciated in cuisine and high prices are paid for them. Fruiting of mycorrhizal fungi requires a very delicate balance in nutrients and environment. It is only for the most expensive mushrooms that a semi-natural cultivation is followed. Young trees are inoculated with Truffle or Matsutake mycelium and planted at suitable sites. Consult the chapter ‘Growing mycorrhizal mushrooms’ for more information.
2.2.3 Parasites
Parasites live at the expense of other living organisms. They may attack insects, trees, and even other mushrooms. A famous parasite is the Chinese \textit{Cordyceps sinensis}, a precious ‘herb’ in traditional Chinese medicine. The Chinese thought it was an insect first, which later turned into a plant. In fact the ‘plant’ is the fruit body of \textit{Cordyceps}, growing from an infected insect.

Tree-parasites vary in their ability to attack healthy trees. Some can only live on trees which are weakened by some other cause, while others can attack healthy trees. The much appreciated edible Honey mushroom (\textit{Armillaria mellea} s.l.: a complex of at least five species, each differing in their aggressiveness) is a very common parasite on many kinds of trees. It is therefore unsuitable for cultivation: trees in the vicinity of the farm would be harmed. Some \textit{Ganoderma} species are also parasitic in nature, but can be cultivated on sawdust media like saprophytic mushrooms. Their spores might harm trees in the vicinity of the farm, however.

2.3 Life cycle of fungi
In nature fungi multiply by producing millions and millions of spores. When a spore settles in a suitable environment, it can germinate and branch to form a mycelium.
2.3.1 Sexual patterns
The mycelium growing out of a single spore (of a heterothallic mushroom) is haploid (it contains only half of a complete set of chromosomes) and generally not capable of sexual reproduction. When two sexually compatible mycelia meet, they may fuse to form a so-called secondary mycelium, which is diploid (but dikaryotic: the nuclei do not fuse). There are some mushroom species, however, which are capable of forming fruit bodies on mycelium which has grown from a single basidiospore. These are called homothallic. Two types of homothallism are recognised: primary and secondary. In primary homothallism only a single uninucleate spore is involved. In secondary homothallism the spores carry at least two nuclei.

Heterothallism is thus a different mechanism: the mycelium from a single spore (monokaryotic mycelium) has to fuse with the mycelium originating from a compatible spore. The mating system may require one (unifactorial) or two (bifactorial) compatibility factors. It is important for scientists who perform breeding experiments to recognise the specific sexual pattern of the chosen mushroom species.

Mushrooms and their sexual patterns

<table>
<thead>
<tr>
<th>Mushroom</th>
<th>Sexual Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bitorquis</td>
<td>bifactorial homothallic</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>unifactorial heterothallic</td>
</tr>
<tr>
<td>Auricularia auricula-judae</td>
<td>unifactorial heterothallic</td>
</tr>
<tr>
<td>Auricularia polytricha</td>
<td>(probably) bifactorial heterothallic</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>bifactorial heterothallic, sometimes, however, with monokaryotic fruit bodies</td>
</tr>
<tr>
<td>Lentinula edodes</td>
<td>bifactorial heterothallic</td>
</tr>
<tr>
<td>Pleurotus spp.</td>
<td>bifactorial heterothallic</td>
</tr>
<tr>
<td>Stropharia rugoso-annulata</td>
<td>heterothallic</td>
</tr>
</tbody>
</table>
Tremella fuciformis  bifactorial heterothallic, complicated, asexual reproduction involving both mono- and dikaryons has been reported
Volvariella volvacea  primary homothallic

2.3.2 Mycelial growth and reproductive stage
In time the mycelium will colonise the substrate and use the available nutrients. When some nutrients run out, or when the weather changes, the mycelium will reach a different phase: the reproductive sexual stage. In the practice of cultivating mushrooms no use is made of spores. Their small size makes it difficult to handle them and their genetic characteristics may differ from those of their parent. Moreover, it takes some time for mushroom spores to germinate; other fungi, like green moulds, germinate and spread much faster.

The desired mushroom should be able to colonise the substrate before other fungi or bacteria do so. To achieve this, pre-grown mycelium (free of any contaminants) of the mushroom is mixed with a sterile substrate. This material is referred to as spawn. Using the spawn will give the cultivated mushroom an advantage in growth compared to other fungi. The rest of the process is actually a duplication of natural conditions to achieve highest yields.

Like in nature the mycelium will colonise the substrate. This is commonly referred to as spawn run. For most species a temperature of about 25 °C is optimal for their spawn run. Furthermore, the environment can enhance the growth of the desired mycelium: a high CO₂ concentration is favourable for mycelial growth (but not for cropping).

After having colonised the substrate, the mycelium is capable of producing fruit bodies. The number and quality of the fruit bodies depend on the environment. Key factors in the induction of fruit bodies are:

- changing temperature,
- high humidity,
- deficiency of a nutrient,
- CO₂ concentration in the air,
- light,
- physical shock.
These factors differ from mushroom to mushroom. Most of the changes to stimulate fruiting have a negative effect on vegetative growth of the mycelium. They should therefore only be applied when the mycelium has fully grown through the substrate. It is actually the less favourable condition for vegetative growth that will stimulate the mycelium to fruit.

Some examples to illustrate the manipulation of key factors in order to induce fruiting in different mushrooms:

- some Oyster mushrooms (for example *Pleurotus ostreatus* strains) will fruit reliably when they experience a cold shock (a difference of 5 °C to 10 °C) after mycelial growth. The CO₂ concentration has to be lowered as well. Mycelial growth can take place in the dark, but for fruiting light must be present.
- the common White button mushroom, *Agaricus bisporus*, needs a nutrient-poor casing soil on top of the nutrient-rich compost. In addition, the temperature should be lowered, the concentration of CO₂ reduced and watering intensified.
- full-grown logs with Shiitake, *Lentinula edodes*, mycelium are soaked in water for one or two days and receive a physical shock to stimulate fruiting. The shock will remove captured CO₂.

Small primordia will be formed at the beginning of the reproductive phase. Under the right climatic conditions, these primordia will develop into fruit bodies. Nutrients are transported from the mycelium to the fruit bodies by a steady moisture flow. Water has to evaporate on the surface of the mushrooms in order to allow the flow to continue. This explains why spraying water on maturing mushrooms or a too high relative humidity of the air can spoil the crop. It depends on the market at which stage the mushrooms should be picked: when they are still quite young and biologically immature (as is the case in White button mushrooms), or when they reach maturity and disperse large quantities of spores.

### 2.4 Mushroom physiology

The mushroom mycelium is triggered to form small primordia: hyphal knots with astonishing vigour. The Pink Oyster, which is one of the fastest growing mushrooms, forms primordia within ten days after spawning; it is able to produce ready-to-pick fruit bodies within 15 days after spawning. As the primordia grow, the cells in the primordia will start to differentiate: they will form stem, cap, gills and basidia, each with different hyphae.

The nutrients for the mushrooms are transported through the mycelium water; evaporation is thus essential for good growth. This explains why mushrooms may start to rot in an environment which is kept at 100% relative humidity for a prolonged period. As a rule of thumb, three litres of water have to be evaporated for each kilo of mushrooms. The function of fruit bodies is clear: to produce and disperse as many spores as possible. Some mushrooms form ripe spores even when the fruit bodies are still very small (e.g. many strains of Winter Oyster mushrooms). Others, like the White button mushrooms, release no spores until the cap comes free from the stem.

Post-harvest physiology has mainly been studied in White button mushrooms (*Agaricus bisporus* and *A. bitorquus*). This paragraph describes the development of White
buttons after they have been picked. Other cultivated mushrooms may behave similarly, though there are some differences between the species.

2.4.1 Developmental stages
When young mushrooms are kept at temperatures at which their mycelium can grow actively, they will mature in a matter of days. Mushrooms picked at a younger state will stay fresh longer. Often less yield will be obtained if only small mushrooms are picked, but this is not a fixed rule. The grower has to consider whether he will receive a higher price for a better quality product.

**Carbohydrates.** Soluble carbohydrates account for 30-50% of the dry weight in just picked White button mushrooms. The three main carbohydrates are: mannitol (considered to be the major osmotica for cell expansion), trehalose and glycogen. If the mushrooms are kept at 18 °C for four days, mannitol levels will drop from 24.5% to only 4%; similarly glycogen levels fall from 11.8% to 4.5%. The changes in composition are not uniform. The mannitol levels decline rapidly in the cap, but peak after two days in the stipe. It seems the carbohydrates in the cap are mainly used for spore production and gill formation.

**Nitrogen metabolism.** The level of soluble protein in White button mushrooms declines to 30-70% in five day old mushrooms compared to fresh mushrooms. Protein is the main source of nitrogen after harvest. Levels of urea, chitin and cell-wall associated protein increase after harvest. It has been speculated that breakdown of DNA and RNA could provide another nitrogen source, but levels of nucleic acids have been shown to rise in samples from whole mushrooms. DNA/RNA-content in gill tissue has been shown to rise significantly (because of the ongoing production of gill tissue and spores), while the amount in stipe and pileus generally decreases.

2.4.2 Respiration
Prior to picking, the mushroom receives its nutrients and water from the mycelium in the substrate and/or casing soil. When this supply is cut off at the moment of picking, the metabolism adapts to the new situation. The primary function of mushrooms in nature is to produce and disperse spores. The metabolism of harvested mushrooms is adapted in such a way that gill formation and spore production are sustained. Picked mushrooms will thus still produce CO₂ and evaporate water. The respiration rate of mushrooms is rather high compared to that of vegetables, which is not surprising if one considers how fast they grow. The high respiration rate causes a rapid change in the concentrations of soluble nutrients in the mushrooms, like carbohydrates and proteins. Also, there is a considerable loss in weight of harvested mushrooms because of evaporation.
Average respiration rate of different crops (mg CO₂ production/kg/hour) at similar temperatures (after Burton, 1991).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Temperature of measurement</th>
<th>Respiration rate (mg CO₂/kg/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White button mushroom</td>
<td>18 °C</td>
<td>600</td>
</tr>
<tr>
<td>Sprouting broccoli</td>
<td>20 °C</td>
<td>425</td>
</tr>
<tr>
<td>Calabrese</td>
<td>20 °C</td>
<td>240</td>
</tr>
<tr>
<td>Sweetcorn</td>
<td>20 °C</td>
<td>210</td>
</tr>
<tr>
<td>Spinach</td>
<td>20 °C</td>
<td>150</td>
</tr>
<tr>
<td>Asparagus</td>
<td>20 °C</td>
<td>127</td>
</tr>
<tr>
<td>Leeks</td>
<td>20 °C</td>
<td>110</td>
</tr>
<tr>
<td>Parsnip</td>
<td>20 °C</td>
<td>492</td>
</tr>
</tbody>
</table>

The respiration rate of mushrooms is much faster than vegetables; they therefore have a shorter shelf life.
3 Substrate characteristics

The material on which the mycelium grows is called substrate. The properties of a substrate determine which fungi and microbes can grow in it. The environment also plays an important role: humidity of the air, ventilation, shade or sun, and temperature, together with the internal condition of the substrate, determine whether a mycelium can grow into the substrate. The better the substrate meets the demands of a specific mushroom and the less suitable it is for others, the more selective it is. Selectivity depends on:
- available nutrients,
- compactness,
- water content and water activity,
- pH (acidity),
- microbial activity.

To create a selective substrate, the following aspects are important:
- thorough mixing,
- fermentation,
- heat treatments.

The last paragraph in this chapter discusses the advantages and disadvantages of different substrate preparation techniques.

3.1 Physical and biochemical characteristics

3.1.1 Nutrients
Some fungi can use a broad range of substrates, while others are very selective. Some Oyster mushrooms, for example, will grow on almost any broad-leaved tree wood as well as on straws, corn cobs, and distillers grain waste, while Shiitake requires specific trees (or sawdust) to support its growth. The bulk substrate material for many mushrooms usually consists of agricultural waste with a high lignocellulose content. Other nutrients may include sugars and proteins. The more easily accessible nutrients are available, the higher the yield. The risk of contamination also increases, as selectivity drops. Especially green moulds (feared contaminants in mushroom cultivation) are capable of breaking down available sugars at a fast rate. If the infection pressure is high, it might be more profitable to add less nutrients, thus getting a lower yield, but less infections.

3.1.2 Compactness
If the substrate is either too tight or too loose the mycelium will have difficulties in colonising it. If it is too loose the mycelium needs more energy to reach the next bit of
sawdust or straw. If it is too tight the mycelium cannot breathe: it needs oxygen and releases carbon dioxide. A low $O_2$ concentration will slow down its growth rate. Good aeration is therefore necessary to avoid anaerobic conditions. Species differ in their aeration needs. *Dictyophora indusiata* (the Veiled lady) and *Stropharia*, for example, need a very loose substrate, while Shiitake can be grown on a dense sawdust substrate with small particles only.

### 3.1.3 Water content and water activity

A too high water content results in clogging of air flow. A too low water content also prevents growth, because of deprivation of water. Under anaerobic conditions, microbes will develop that give a foul smell. They may also produce toxic metabolites during their growth. Actually it is not the exact water content but the water activity that is important. Water activity is related to the amount of suction required to extract water from the substrate, and as such it is directly related to the availability of water to a mycelium living in the substrate. Each particle in the substrate has a film of water surrounding it. The water in this film matters, because it is available to organisms in the substrate, hence its name *free water*. Furthermore, the availability of water is influenced by its osmotic value. If many molecules are dissolved in the water (for instance salt or sugar), then only little water will be available.

Squeeze test: if a lot of water leaks out of the straw, then it is certainly too wet. Just a few drops of water should be released with some pressure. The same test can also be done with sawdust: take a fistful of sawdust and squeeze tightly.

This principle is used in conserving food in brine or in the preparation of marmalade. Water content gives the percentage of total water in a substrate. Only a fraction of this water can be used by the mycelium. Therefore the water activity would be a better measure. Water activity has to be regarded in relation to the water holding capacity. A
very fine sawdust can hold more water than relatively coarse sawdust. The simple squeeze test reveals how much pressure has to be applied to release a few water drops from a fistful of substrate. If the size of the particles used for the substrate is always the same, then a given water content will always result in more or less the same water activity. The water activity is in equilibrium with the relative humidity at the surface of the substrate.

3.1.4 pH (acidity) of a medium
The acidity of a medium is expressed in terms of its pH value. A neutral solution has a pH of 7; lower values indicate acidic, higher values alkaline conditions. During the growth of the mycelium the pH of its substrate can change considerably. Shiitake mycelium in wood logs, for example, will decrease the pH value from 5.5 to 3.8. Most cultivated mushrooms prefer a slightly acidic medium to grow in. The pH can be stabilised with buffers. The most commonly used buffers to achieve a suitable pH in the substrate are gypsum and chalk. Measuring the pH value of the substrate should be a routine operation. Take a representative sample of the substrate of 5 to 10 g and put it in distilled water. Crush the substrate materials and shake the water. Two methods can be used to determine the pH value of this solution: with paper indicators or with an electric pH meter. The paper indicators contain a number of substances that change colour at different pH values. By comparing the paper with a colour table, the pH value can easily be determined. Electric pH meters are more expensive. A small, convenient pen-like pH meter costs about US$ 50. Electric meters should always be calibrated with a buffer solution. Do not consider pH as an absolute measure. A low pH can be an indication of microbial activity, which is influenced by the substrate contents. If the real problem is microbial activity because of improper pasteurisation and conditioning, then increasing the pH value by adding a buffer (chalk) is not the solution; rather, a better heat treatment should be attempted.

3.1.5 Microbial activity
The microbial activity is determined by the nature of the substrate, the organisms which have access to the substrate and the employed heat treatment. Sterilised substrates are supposed to be sterile, but often bacterial endospores will have survived the treatment. As long as their numbers are limited, they will do little harm as long as the substrate is left undisturbed. If it is mixed, however, they may get the chance to proliferate abundantly and cause a bacterial bloom. The situation in pasteurised substrates is more complex. Much depends on the substrate ingredients and on the microflora present in them. The spore count of the same supplement can vary widely, thus affecting the reliability of pasteurisation. If a high spore count is encountered, then the pasteurisation may have to continue for a longer period. Pasteurised substrates should be free of spores of unwanted fungi; thermotolerant bacteria are usually present in great numbers. It is important to realise that this microflora can be helpful. Bacteria in substrates often produce substances that inhibit the growth of unwanted green moulds. This can easily be observed if some substrate is sterilised with formalin: the biological vacuum will be quickly filled by green moulds.
The most complex situation can be found in fermented and pasteurised substrates. The rapid degradation of easily degradable substances like polysaccharides, cellulose and hemi-cellulose leads to a quickly rising temperature. The substrate, piled up in dikes or heaps, will have a hot core inside. A thermophilous microflora replaces the original mesophilous microflora system. After pasteurisation most of the mesophilous microflora has been removed. Furthermore, one fungus is dominant throughout the substrate in this stage: *Scytalidium thermophilum*. It has been found that *Agaricus* (the White button mushroom) can grow quickly on the remnants of *S. thermophilum*. The latter is thus a good indicator of successful substrate preparation.

### 3.2 Fermentation

In nature some fleshy fungi which can be grown on composted substrate are usually found with their mycelium in organic debris, which has been worked over by worms, insects and other fungi. They don’t grow on fresh substrates. For these species (mainly *Agaricus*, but sometimes also *Volvariella*, Shiitake and *Pleurotus*) the substrate needs to be fermented in order to become suitable.

During fermentation the easily accessible nutrients are assimilated by microbial activity. The heat released during this process would kill the mushroom mycelium if this were to be spawned in the fresh substrate. Rather, the substrate is spawned only after the microbial activity has declined (usually after a subsequent pasteurisation to eliminate contaminants and insect larvae). The fermentation tends to make the substrate more suitable for the growth of the desired mushroom than for other fungi. The substrate has become *selective*. To achieve a good fermentation, the size of the substrate heaps is important. If the heaps are too small, the temperature will not reach the desired height. Usually a heap has to measure at least $1 \times 1 \times 1.20$ m$^3$. Solid wastes have a poor heat conductivity. The heat produced inside is thus not easily conducted to the outside environment. Heat and water loss from the heaps can further be prevented by covering them with plastic. The temperature in the core can rise to 60 °C and even 80 °C. The outside of the heap, however, will stay relatively cool. Therefore the heap has to be turned. The inner core has to be on the outside of the new heap and vice versa. Exact specifications on this procedure can be found in the chapters on the cultivation of *Agaricus*.

Fermentation is also applied to sawdust of coniferous trees to degrade resins, which hinder the growth of many wood-degrading fungi. The procedure is described in the case study on Shiitake cultivation on plastic bags in Taiwan.

### 3.3 Mixing

Usually a substrate consists of several ingredients. A uniform distribution of these is very important. If lumps of supplements occur, then these are much more selective to contaminants because the concentration of nutrients is very high at one spot. Mixing is also very important for the moisture distribution. Water should be available in the same concentration everywhere in the substrate. Sometimes a better distribution can be achieved if the substrate ingredients are mixed in a dry state (e.g. in sterilised substrates containing sawdust and supplements); water is added later.
Special machines have been developed to mix substrates thoroughly. Most used are ribbon mixers, cement mixers, and auger mixers. Ribbon mixers are much used in Taiwan, they consist of a fixed container with mixing blades, mounted on a central rotating shaft.

Cement mixers follow the opposite philosophy in mixing; here it is the container itself which is rotated; the blades inside are fixed. Auger mixers consist of a bin with two augers (screws) running in opposite directions.

Compost turning calls for a completely different design of mixing machines. The modern turning machines have a capacity of 200 tons per hour at a speed of 2 metres per minute. They have to be manufactured from material which can stand the aggressive chemicals released by the compost, like ammonia.

### 3.4 Heat treatments

Most substrates are given a heat treatment before spawning. It is an important measure to control pests and diseases. Several types of heat treatment are employed in mushroom cultivation:

- sterilisation under high pressure,
- semi-sterilisation under low pressure,
- heat treatment of dry material,
- pasteurisation by steam,
- pasteurisation by immersion in hot water.

Another phase which relates to substrate production is the so-called Conditioning.

**Sterilisation:** This will free the substrate from all living organisms. It requires sterile packing, a special autoclave, a high-pressure steam boiler and quite a lot of energy. The
3. SUBSTRATE CHARACTERISTICS

pressure is required to achieve a temperature of 121 °C inside the substrate. Some organisms (especially endospore-forming bacteria) can survive a treatment at 100 °C and these will grow very fast on the media used for cultures, like agar and grain. They would contaminate the media and render them worthless. For spawn and pure cultures sterilisation is absolutely necessary. New sterilisation techniques employ gamma-radiation; so far no commercial applications are known to the author.

**Semi-sterilisation:** This may require a less expensive steam boiler and steaming room, because no pressure is involved. Depending on the selectivity of the substrate, semi-sterilisation can be an effective control of contaminants. Many kinds of wood-degrading fungi can be cultivated after having applied either a complete sterilisation or a semi-sterilisation to the substrate.

In Taiwan for example, where farmers have the resources and the knowledge, substrates will be sterilised if only a few flushes can be harvested. The higher temperature will degrade more substrate materials, thus making it easier to break down for the mycelium. When more flushes over a longer period can be grown, semi-sterilisation is preferred.

**Heat treatment of dry material:** wood chips, straw or sawdust which has been heated above 200 °C can be used directly with water and (much) spawn. Consult the paragraph *Shiitake cultivation on pre-heated substrate* in chapter 22.

**Pasteurisation:** This is aimed at killing unwanted organisms but keeping the favourable ones alive. To reach this state a temperature of 60 °C to 70 °C has to be sustained for some time. Most pests and diseases will be eliminated at this temperature. When pasteurisation by steam is practised, it is often followed by conditioning.

**Immersion in hot water:** This is also a form of pasteurisation. It differs, however, from pasteurisation by steam in that the hot water will remove the easily soluble sugars and at the same time kill contaminants. Coffee pulp and different types of straw can be treated in this way for the cultivation of *Pleurotus*. This method is very easy: only hot water, containers and the means to keep the water hot are required. This method has great potential for developing countries, as it is simple and may employ solar energy.

**Conditioning:** This is aimed at creating a suitable environment for the desired mushroom by promoting the growth of thermophilic organisms after the pasteurisation phase. Some lower fungi and representatives from the group of Actinomycetes will benefit greatly from this treatment. They will transform the easily accessible sugars and ammonia (in case fresh straw has been used; no easily degradable sugars will be found in fermented compost) and discourage the growth of competitors on these nutrients. The period of conditioning depends on the concentration and type of nutrients to be transformed. The usual temperature is 48 °C. Conditioning is used in cultivating *Pleurotus* on fresh wheat straw and corncobs, *Lentinula* on fresh corncobs, and for *Agaricus* on a fermented and subsequently pasteurised and conditioned substrate.

### 3.5 Advantages and disadvantages of different substrate treatments

The different ways to create a suitable substrate can be divided according to the way the substrate is treated. In this manual four techniques are distinguished:

1. preparing wood logs,
2. fermenting and subsequently pasteurising substrates,
3. pasteurising substrates which have not been fermented, including the preheated substrates,
4. sterilising substrates.

The substrate preparation method has to be combined with
- the right species (check the tables in the chapter *Technical data* on temperature needs, possible cultivation techniques and substrate needs),
- suitable cultivation conditions: the right climate at the moment of fruiting.

<table>
<thead>
<tr>
<th>treatment</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>wood logs</td>
<td>low investment</td>
<td>wood can be used for other purposes</td>
</tr>
<tr>
<td></td>
<td>little use of resources</td>
<td>logs are difficult to handle</td>
</tr>
<tr>
<td>fermented and pasteurised substrate</td>
<td>use of agricultural wastes</td>
<td>process is difficult to mechanise</td>
</tr>
<tr>
<td></td>
<td>only method to grow <em>Agaricus</em></td>
<td>the fermentation process attracts many insects</td>
</tr>
<tr>
<td>fresh substrate</td>
<td>good method to handle large amounts of pasteurised by steam substrate</td>
<td>process demands expertise</td>
</tr>
<tr>
<td></td>
<td>use of agricultural wastes like straw, cornsobs, cotton seed hulls</td>
<td>steam boilers are necessary to provide pasteurisation</td>
</tr>
<tr>
<td>fresh substrate pasteurised by immersion in hot water</td>
<td>simple method, low investment, solar energy can easily be used</td>
<td>steam boiler and pasteurisation room necessary</td>
</tr>
<tr>
<td></td>
<td>little chance of contamination because easily soluble carbohydrates are removed by the immersion process</td>
<td>chances of contamination bigger than with immersed or sterilised substrate</td>
</tr>
<tr>
<td></td>
<td>feasible for several agricultural wastes, like coffee pulp waste, straw</td>
<td>containers for the hot water need to be installed</td>
</tr>
<tr>
<td>(semi)sterilised</td>
<td>easy to mechanise</td>
<td>investments in autoclave and steam boiler are relatively high</td>
</tr>
<tr>
<td></td>
<td>stable technique, little chances of contamination</td>
<td>high energy consumption during sterilisation process</td>
</tr>
<tr>
<td></td>
<td>less infection danger</td>
<td></td>
</tr>
<tr>
<td>pre-heated substrate</td>
<td>low investment</td>
<td>high costs of substrate ingredients because of high spawn percentage</td>
</tr>
<tr>
<td></td>
<td>simple technique</td>
<td></td>
</tr>
</tbody>
</table>
This chapter provides an overview of basic data like:
- temperature ranges of cultivated mushrooms,
- selecting bulk substrate materials,
- selecting supplements,
- which substrate preparation technique to apply.
With these data, mushroom species which suit the local conditions best can be selected.

4.1 Temperature ranges of cultivated mushrooms

Both investments in climate control and energy costs can be high if the mushrooms require a fruiting temperature which differs much from the prevailing temperatures. Lower costs are involved if the fruiting range is as close as possible to outside conditions. Growers may switch to high temperature strains in summer to maintain quality, but the table shows that there are actually few species suited for really tropical conditions. Only *Volvariella volvacea*, *Pleurotus tuberregium*, *Coprinus cinereus*, *Stropharia*...
rugoso-annulata and Pleurotus cystidiosus/abalonus are currently being cultivated at temperatures around or just below 30 °C. However, there are many more edible species in tropical regions that have not yet been tried for cultivation. It is therefore important that scientists in these countries put effort into collecting local species. Agaricus strains however, which are able to fruit at higher temperatures, often show a significantly lower yield than the normally used strains. When comparing wild local strains of Volvariella in Mexico with commercial strains from the Far East the commercial strains gave much higher yields. Still, it is important to increase the genetic pool and conserve local strains in culture collection centres. In some countries mushrooms are only seasonally cultivated; often limited climate control is applied then. This may seem profitable as lower investments are required, but marketing a seasonal (fresh) product is more difficult.

Table showing mushroom species, temperature ranges (all in °C) and which substrate preparation techniques can be applied.

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>T&lt;sub&gt;mg&lt;/sub&gt;</th>
<th>T&lt;sub&gt;optimal mg&lt;/sub&gt;</th>
<th>T&lt;sub&gt;fruited&lt;/sub&gt;</th>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>10-32</td>
<td>20-28</td>
<td>10-20</td>
<td>2</td>
</tr>
<tr>
<td>Agaricus bitoliquis</td>
<td>25-31</td>
<td>30</td>
<td>25-30</td>
<td>2</td>
</tr>
<tr>
<td>Agaricus blazei</td>
<td>n.d.</td>
<td>21-27</td>
<td>20-25</td>
<td>2, 4</td>
</tr>
<tr>
<td>Auricularia polytricha</td>
<td>20-33</td>
<td>25-28</td>
<td>23-28</td>
<td>1, 4</td>
</tr>
<tr>
<td>Coprinus comatus</td>
<td>10-35</td>
<td>20-28</td>
<td>12-21</td>
<td>2</td>
</tr>
<tr>
<td>Coprinus cinereus</td>
<td>n.d.</td>
<td>n.d.</td>
<td>estimated at 30-35</td>
<td>3</td>
</tr>
<tr>
<td>Dictyophora indusiata</td>
<td>17-30</td>
<td>22-24</td>
<td>17-29 (22 optimal)</td>
<td>3</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>3-33</td>
<td>22-26</td>
<td>3-15</td>
<td>4</td>
</tr>
<tr>
<td>Hericium erinaceus</td>
<td>&lt;35</td>
<td>25</td>
<td>15-25 or 20-28 *</td>
<td>4</td>
</tr>
<tr>
<td>Hirneola auricula-judae</td>
<td>n.d.</td>
<td>20-25</td>
<td>15-23</td>
<td>1, 4</td>
</tr>
<tr>
<td>Lentimula edodes</td>
<td>5-35</td>
<td>20-30</td>
<td>8-24 *</td>
<td>1, 4, 3</td>
</tr>
<tr>
<td>Lepista nuda</td>
<td>10-30</td>
<td>20-25</td>
<td>12-18</td>
<td>2</td>
</tr>
<tr>
<td>Pholiota nameko</td>
<td>10-30</td>
<td>22-30</td>
<td>12-18</td>
<td>3, 4</td>
</tr>
<tr>
<td>Pleurotus abalonus</td>
<td>15-35</td>
<td>20-30</td>
<td>25-30</td>
<td>3, 4</td>
</tr>
<tr>
<td>Pleurotus tuberregium</td>
<td>15-40</td>
<td>35</td>
<td>30</td>
<td>2, 3, 4</td>
</tr>
<tr>
<td>Pleurotus cystidiosus</td>
<td>10-35</td>
<td>25-28</td>
<td>25-30</td>
<td>3, 4</td>
</tr>
<tr>
<td>Pleurotus ostreatus</td>
<td>5-35</td>
<td>20-25</td>
<td>5-25</td>
<td>3, 4</td>
</tr>
<tr>
<td>Pleurotus pulmonarius</td>
<td>5-35</td>
<td>20-25</td>
<td>13-20</td>
<td>3, 4</td>
</tr>
<tr>
<td>Pleurotus cornucopiae #</td>
<td>15-35</td>
<td>24-30</td>
<td>20-30</td>
<td>3, 4</td>
</tr>
<tr>
<td>Pleurotus djamor ^</td>
<td>10-35</td>
<td>20-25</td>
<td>15-22</td>
<td>(3), 4</td>
</tr>
<tr>
<td>Pleurotus eryngii</td>
<td>10-35</td>
<td>21-27</td>
<td>10-32</td>
<td>3</td>
</tr>
<tr>
<td>Stropharia rugoso-annulata</td>
<td>10-35</td>
<td>20-25</td>
<td>20-27</td>
<td>1, 4</td>
</tr>
<tr>
<td>Tremella fuciformis</td>
<td>5-38</td>
<td>20-25</td>
<td>30-35</td>
<td>3</td>
</tr>
<tr>
<td>Volvariella volvacea</td>
<td>20-40</td>
<td>30-35</td>
<td>30-32</td>
<td>3</td>
</tr>
</tbody>
</table>

Key: T<sub>mg</sub>: the range at which the mycelium stays viable; at both high and low ends of this range the growth speed declines; T<sub>optimal mg</sub>: the optimal temperature range for spawn run; T<sub>fruited</sub>: temperature range required for fruiting; the most important temperature.

Techniques for substrate preparation: 1. wood logs; 2. fermented and pasteurised substrate; 3. pasteurised or pre-heated substrate; 4. sterilised substrate.

Notes: # including Pleurotus citrinicola; ^ including probable synonyms: P. ostreotoroseatus, P. salmono-stramineus, P. flavellatus; * significant difference between strains.
The data in this table have been gathered from different, sometimes conflicting sources. Strains within the same species may vary significantly in fruiting temperatures. It should be noted that the temperature for mycelial growth should be measured inside the substrate. Especially when using techniques 2 and 3 (fermented and pasteurised substrates) in larger containers, the temperature inside the substrate can be much higher than the surrounding air temperature!

4.1.1 Suitable bulk substrate materials
The following table lists the most commonly used substrates for mushroom cultivation. Other locally abundant lignocellulose wastes may prove to be suitable, too. Substrate materials that have proved to give a good yield are discussed first. For many types of waste material the best technique is not yet known. Often the addition of a limited amount of a supplement will increase yields. The substrate formulations can be adapted to the availability of local wastes, keeping the general aspects of substrate preparation (porosity, water holding capacity, acidity, suitable nutrients) in mind. More detailed formulations are given in the specific chapters on fermented, pasteurised and sterilised substrates.

<table>
<thead>
<tr>
<th>Substrate material</th>
<th>Mushroom species</th>
<th>Cultivation technique</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood logs</td>
<td>Auricularia spp.</td>
<td>1</td>
<td>Freshly cut, minimise bark damage.</td>
</tr>
<tr>
<td></td>
<td>Lentinula edodes</td>
<td>1</td>
<td>Consult the chapter Cultivation on wood logs for a list of suitable tree species.</td>
</tr>
<tr>
<td></td>
<td>Pleurotus spp.</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tremella fuciformis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Horse and chicken manure</td>
<td>Agaricus spp.</td>
<td>2</td>
<td>Consult a number of substrate formulations in the chapters on fermented substrates.</td>
</tr>
<tr>
<td></td>
<td>Coprinus comatus</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lepista nuda</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Rice straw</td>
<td>Volvariella volvacea</td>
<td>2</td>
<td>Stored under dry conditions.</td>
</tr>
<tr>
<td></td>
<td>Pleurotus spp.</td>
<td>3,4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Agaricus spp.</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Barley or wheat straw</td>
<td>Pleurotus spp.</td>
<td>3</td>
<td>Stored under dry conditions.</td>
</tr>
<tr>
<td></td>
<td>Agaricus spp.</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Syropharia rugosa-anulata</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Wood chips</td>
<td>Lentinula edodes</td>
<td>4 (3)</td>
<td>From broad-leaved trees. The chips should either be used directly after chopping or dried after chopping; an alternative treatment is pre-heating.</td>
</tr>
<tr>
<td></td>
<td>Pholiota nameko</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pleurotus spp.</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Syropharia rugosa-anulata</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dictyophora indusiata</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Sawdust</td>
<td>Auricularia spp.</td>
<td>4</td>
<td>Sawdust from broad-leaved trees can be used directly, sawdust from coniferous trees or tropical hardwoods should be fermented first.</td>
</tr>
<tr>
<td></td>
<td>Flammulina velutipes</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ganoderma spp.</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hericium erinaceus</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lentinula edodes</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pholiota nameko</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pleurotus spp.</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Substrate material</td>
<td>Mushroom species</td>
<td>Cultivation technique</td>
<td>Remarks</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------------</td>
<td>-----------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Coffee pulp</td>
<td><em>Pleurotus spp.</em></td>
<td>3</td>
<td>Fresh coffee pulp should be fermented first, dried coffee pulp can be used directly. Pasteurisation can only be applied if the corn cobs contain few pieces of corn.</td>
</tr>
<tr>
<td>Corn cobs</td>
<td><em>Lentinula edodes</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pholiota nameko</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pleurotus spp.</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cotton waste</td>
<td><em>Volvariella volvacea</em></td>
<td>3</td>
<td>Several types are suitable: short fibre, card sweepings, card drops, blow gutter, weaving hard oily waste. Only weaving sweeping has been reported to be unsuitable. Contains much cellulose.</td>
</tr>
<tr>
<td></td>
<td><em>Pleurotus spp.</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Distillers grain waste</td>
<td><em>Hericium erinaceus</em></td>
<td>4</td>
<td>The grain waste is dried and stored under dry conditions until it is used. Contains much cellulose.</td>
</tr>
<tr>
<td>Cotton seed hulls</td>
<td><em>Lentinula edodes</em></td>
<td>4</td>
<td>The cotton seed hulls should be dried and stored under dry conditions.</td>
</tr>
<tr>
<td></td>
<td><em>Hericium erinaceus</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pleurotus spp.</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Banana tree leaves</td>
<td><em>Volvariella volvacea</em></td>
<td>2</td>
<td>Only dead leaves hanging from the trees should be collected; too time-consuming for large scale production.</td>
</tr>
<tr>
<td>Crushed bagasse and other wastes from sugar industry</td>
<td><em>Pleurotus spp.</em></td>
<td>4</td>
<td>C/N ratio is high, supplements are necessary for good yields.</td>
</tr>
<tr>
<td></td>
<td><em>Auricularia spp.</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Water hyacinth/lily Eichhornia crassipes</td>
<td><em>Pleurotus spp.</em></td>
<td>3,4</td>
<td>Water hyacinth is a major pest in many lakes; plants should be dried for a week and subsequently stored under dry conditions.</td>
</tr>
<tr>
<td></td>
<td><em>Volvariella sp.</em></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cotton straw</td>
<td><em>Pleurotus spp.</em></td>
<td>3,4</td>
<td>Cotton straw has a high moisture content, which makes it more difficult to store; an anaerobic tank can store the moist straw.</td>
</tr>
<tr>
<td></td>
<td><em>Volvariella sp.</em></td>
<td>3</td>
<td>Contains much cellulose; check the paper regularly for heavy metals.</td>
</tr>
<tr>
<td>Used paper</td>
<td><em>Pleurotus spp.</em></td>
<td>3,4 *</td>
<td>The fibre which covers the coconut, including the adhering soil.</td>
</tr>
<tr>
<td></td>
<td><em>Volvariella sp.</em></td>
<td>3 *</td>
<td></td>
</tr>
</tbody>
</table>

Substrate preparation techniques codes: 1 wood logs; 2. fermented and pasteurised substrate 3. pasteurised or pre-heated substrate; 4. sterilised substrate  
* no commercial use known to the author

### 4.2 Supplements

Supplements are additives which increase yields by providing specific nutrients for the growth of the mycelium. When using wood logs as a substrate, no additional nutrients can be added to enrich the substrate. The three other techniques all offer the potential of adding supplements to the substrate. The supplements supply extra nitrogen or easily degradable carbohydrates (or both). If the bulk material has a high nitrogen content, less supplements are necessary. The average nitrogen content of proteins is 16%. If the protein content of the supplement is known, its nitrogen content can be calculated,
assuming that nitrogen is only present in the proteins. Actually the availability of nitrogen should be calculated, rather than the total nitrogen content, but there are no simple ways to do this. The mycelium can respond in three ways to a specific nitrogen supplement:
- it can readily utilise the supplement,
- it can utilise the supplement only after other micro-organisms have transformed it,
- the supplement cannot be utilised by the mycelium.
Specific supplements for each species and cultivation technique are given in the paragraphs with detailed information on cultivation practices. Practice has shown that the waste materials in these formulations can be utilised by the mycelium, or by micro-organisms during fermentation. Heavy supplementation increases the risks of contamination: other micro-organisms are likely to profit from the extra nutrients as well. Special products have been developed for the supplementation of *Agaricus* compost, e.g. soy bean meal which has been treated with formaldehyde to decrease the release rate of nutrients.

<table>
<thead>
<tr>
<th>Inorganic nitrogen sources</th>
<th>% Nitrogen</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ammonium sulphate</td>
<td>21%</td>
<td>this will lower the pH of the substrate; add CaCO&lt;sub&gt;3&lt;/sub&gt; to buffer the substrate at the desired pH</td>
</tr>
<tr>
<td>ammonium nitrate</td>
<td>26%</td>
<td>used in synthetic composts</td>
</tr>
<tr>
<td>urea</td>
<td>46%</td>
<td>used in synthetic composts</td>
</tr>
</tbody>
</table>

Inorganic supplements are mainly used for the cultivation of mushrooms on fermented substrates. The above mentioned supplements have all been used in *Agaricus* production. For other mushrooms it is not sure whether their mycelium can utilise these inorganic fertilisers. Urea can be used in sawdust for *Pleurotus* cultivation, if the mixture is fermented before sterilising. Normally, organic supplements are used in the sterilised plastic bag cultivation method. There is no fermentation phase in which micro-organisms convert the inorganic nitrogen to organic nitrogen compounds.

Other supplements include: soy bean meal, sugar industry waste (molasses), and spent tea leaves. Some of these materials also contain large amounts of carbohydrates. The exact composition of organic supplements (but also of bulk substrate material) fluctuates according to natural variations in the weather and due to handling after harvesting or processing. The initial spore count of organic supplements can vary enormously, even between batches of the same type. This may lead to interpretation problems when performing experiments with the same type of supplement.
4. TECHNICAL DATA OF MUSHROOM SPECIES: AN OVERVIEW

<table>
<thead>
<tr>
<th>Organic nitrogen sources</th>
<th>% Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood meal</td>
<td>13.5</td>
</tr>
<tr>
<td>fish meal</td>
<td>10.5</td>
</tr>
<tr>
<td>wheat bran</td>
<td>9.7</td>
</tr>
<tr>
<td>cotton seed meal</td>
<td>6.5</td>
</tr>
<tr>
<td>peanut meal</td>
<td>6.5</td>
</tr>
<tr>
<td>chicken manure</td>
<td>3-6</td>
</tr>
<tr>
<td>brewe grain</td>
<td>3-5</td>
</tr>
<tr>
<td>malt sprouts</td>
<td>4</td>
</tr>
<tr>
<td>rice bran</td>
<td>2</td>
</tr>
<tr>
<td>corn bran</td>
<td>2.5</td>
</tr>
</tbody>
</table>

4.2.1 Selecting the right technology: substrate production and climate control
The choice for a specific substrate preparation technique depends on the chosen mushroom, the availability of suitable substrate and available resources. The previous chapter Substrate contains a description of the different substrate preparation techniques and the pros and cons of each technique. Another aspect of technology is the way the climate is controlled once the substrate has been prepared. This is covered in several chapters, both high tech (chapter 13/14) and low investment options (chapters 11-12) are discussed.
5 Spawn, breeding and conservation of strains in general

5.1 Why use spawn?

The mushroom seed is generally referred to as spawn. In nature mushrooms use spores for generative multiplication. These spores are extremely small and therefore difficult to handle for mushroom growers. Spores need time to germinate as well, and competitor fungi might germinate and grow faster during that time. Therefore traditionally a pure culture of the desired mycelium is added to the substrate to give it an advantage. This procedure is called spawning, and the pure culture is called spawn.

It is necessary for each growing cycle to start with fresh spawn. If old mycelium (that yielded mushrooms) is used to inoculate a new crop, then all the contaminants that have accumulated will be inoculated together with the mycelium of the desired mushroom. This will spoil the crop. A second reason for using fresh spawn each time is that degeneration may occur when the mycelium grows old. The genetic properties of micro-organisms change at a rapid pace. Each time, therefore, new spawn should be made from the fresh mycelium of a healthy mushroom, or from a pure culture from a type culture collection. (Also refer to Appendix B: Conservation of strains and culture collections.) Hybrid strains of Agaricus are unstable and new spawn should only be made from viable mother cultures. Spawn production is a critical process. If the spawn is contaminated by bacteria or other fungi, no mushrooms can be harvested, no matter how well the substrate is prepared and all the other conditions are met.

In the western world, high quality spawn is easily available. It is therefore not advisable for a mushroom grower to produce his own spawn. Factors which are used to determine the spawn supplier are suitable strains, flexibility, service and of course price.

In many developing countries, the availability of a good quality spawn is the limiting factor for mushroom cultivation. Import is often hindered by bureaucracy at customs, high shipping costs and the difficulty to keep the spawn cooled from spawn plant to the grower. Therefore it can be necessary for the mushroom grower to produce his own spawn.

5.1.1 Spawn production in general

Spawn production requires a clean laboratory and some specialised knowledge. The most expensive part is usually the autoclave: metal containers in which substrate can be sterilised by heating to 121 °C under at least 1 bar overpressure. High pressure cookers can be used for small-scale enterprises. This kind of equipment is commonly
available in hospitals, research stations and universities. A second requirement is a clean room in which the sterilised substrate is spawned. It is best to import fresh mother cultures every three months, to avoid any degeneration of the strains used.

In countries lacking mushroom production, spawn may be supplied by a university at the start of a project. If the project is successful and more spawn is needed, a commercial spawn supplier can start to operate. Another possible provider of spawn is a local Rhizobium-manufacturer (the *Rhizobium*-bacteria live in the roots of soya, corn and other vegetables and provide extra nitrogen for the crop). The production of *Rhizobium*-bacteria is very similar to spawn production. Some *Rhizobium* projects have been implemented in Bolivia, Zimbabwe and other countries. The producers of the *Rhizobium*-bacteria might be interested in diversifying their activities by producing spawn. *Rhizobium* needs less aeration than the mycelium for edible fungi, so some adaptation of the technique is necessary.

### 5.1.2 Different steps in spawn production

The essential steps in spawn production are shown in the figure. With each step, the total mycelial mass is increased. A normal path is: mother culture on agar, inoculation cultures in petri-dishes, mother spawn on grain in bottles, final spawn on grain in plastic bags. It is possible to use the mother spawn also to inoculate wood plugs, wooden sticks or sawdust. Many alternatives exist, the objective always being to obtain vigorously growing mycelium of the desired mushroom without any contaminants. Purity should be maintained during the transfers by working under aseptic conditions with sterilised media. The vigour is affected by strain, substrate material, storage time and storage temperature. Spawn production is discussed in detail in a separate chapter: Practical aspects of spawn production.

### 5.1.3 Produce spawn or buy it?

Good quality of the spawn is the most important aspect to consider in answering this question. If a reliable source of spawn at reasonable prices can be found, then the potential mushroom grower should concentrate on other aspects of mushroom cultivation than spawn production. Sometimes the original quality of spawn may be good, but the circumstances during shipping may cause deterioration and thus failure of the crop.
Especially grain spawn is very sensitive to higher temperatures, so check shipping conditions carefully to ensure cooled transport.

If there is no local spawn producer and shipping is troublesome, then it is wise to start producing it yourself. Do not underestimate the problems to be solved before consistently high quality spawn can be produced. It takes at least one year if no prior experience with working under aseptic conditions is available. Producing spawn yourself may seem cheaper than buying ready-made spawn, but it is only a fraction of the total cost of mushroom growing. Switch only to self-prepared spawn if you are absolutely sure that its quality is better than available spawn. An experienced Shiitake grower from New Zealand went bankrupt after he had inoculated all his substrate with spawn from a degenerated strain (from which he had prepared spawn himself). If sterilised substrate producers, who distribute to several farmers, use a degenerated strain, all the farmers will suffer. Producing spawn yourself means that you will have to maintain the mother cultures in a proper condition or obtain them from reliable institutes.

5.2 Genetics and breeding

Breeding is rarely performed by mushroom growers, in contrast to other sectors in horticulture. This is due to the fact that a laboratory and special skills are needed to obtain new strains. Nowadays, DNA techniques form the basis of modern breeding and the use of these techniques is beyond the capacity of individual growers.

Cultures (either from tissue or spores) from wild mushrooms form the basis of the commercial strains. Often many different wild strains have to be collected to obtain one single commercial strain. The yield of wild mushrooms is usually rather low. Therefore selection is carried out to find strains that give sufficient yields. If strains are obtained from different institutes or spawn producers, then it is sometimes difficult to

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Life cycle of *Agaricus bisporus* (homothallic) compared to *Agaricus bitorquis* (heterothallic) shows that normal spores of *A. bisporus* contain more nuclei, which makes it more difficult to breed with them when compared to *A. bitorquis*. 
5. SPAWN, BREEDING AND CONSERVATION OF STRAINS IN GENERAL

determine whether they are actually different strains. Often a well-performing strain is
distributed by spawn manufacturers under different names.
A simple way to determine whether two strains of *Lentinula edodes* are actually one
and the same strain is to inoculate a petri dish with both strains. If the mycelia of the
two strains grow together, then it is the same strain (or very similar). If the strains are
different they will form a distinct zone between the two mycelia. Of the Shiitake strains
90% can be identified this way. The method becomes more accurate when a guaiacol
solution is poured on the plate. The antagonistic zone will show as a clear dark line. For
other mushrooms, like the many different strains of *Pleurotus*, other ways have to be
developed to differentiate between strains. Advanced techniques use iso-enzyme analysis
or DNA fragment analysis to determine the genetic variability of different strains. The
advantage of these techniques is that genetic markers can be linked with commercial
characteristics. But experiments like these can only be performed in well-equipped
laboratories. Sometimes the results of these kinds of tests are published by mushroom
journals.

5.2.1 Cross-breeding
By cross-breeding, favourable characteristics of different species can be combined.
For breeding, the sexual pattern of the mushroom has to be considered. The mush-
rooms discussed in this book differ in sexuality: some can form fertile mycelium from
only one spore, while others need two compatible spores. The heterothallic species are
easier to breed than the homothallic ones, since it is relatively easy to separate spores
from different strains and let them fuse. Special techniques have been developed to
breed the homothallic species (like *Agaricus bisporus*) also, since the economic value
of White button mushrooms is great. The sexuality of mushrooms is discussed in more
detail in the chapter General biological information on mushrooms.

5.2.2 Selection
Selection simply means growing the (mushrooms of different) strains and comparing
them. The strains may be commercially available as mycelium, isolated from geneti-
cally different individual mushrooms within the same species, or mycelium from spore
cultures. In selection, no efforts are made to combine the characteristics. The strains
can be compared in factors like growth rate, density, fruiting ability, fruiting tempera-
ture range, yield, etc. Fluffy mycelium in species that are normally strandy, as well as
slow or depressed growth rate usually indicate low yielding strains. Other strains should
be used to make spawn and actually grow the mushrooms. Fruiting trials are conducted
in small containers first, then suitable strains are selected and grown in larger quanti-
ties later. Yields are of course very important. Other features to select for are: size,
colour, form, keeping ability, sensitivity to CO₂, spore production, resistance to bruising,
concentrations of medicinal compounds, etc.

5.2.3 Cross-breeding and hybridisation
The objective of breeding is to combine favourable characteristics of different strains.
Such a combination is often referred to as a hybrid strain. Scientists use the term hybrid
for a cross-breeding of two different species, but spawn manufacturers are less exact in
their nomenclature. Although scientifically not correct, the term hybrid is often used by them for cross-breeding within the same species. In cross-breeding monokaryotic mycelium is used to obtain a dikaryotic mycelium. Some ‘hybrids’ (for example in Agaricus bisporus) are unstable and their mother cultures have to be kept under liquid nitrogen. Otherwise they might lose their characteristics within a few years. Breeding can be performed by simply inoculating a petri dish with two different strains and isolating the mycelium from the lines of juncture. The rate of success will be low, however. With modern biotechnology, scientists could actually take genetic material out of one strain and combine it with another strain. The society is reluctant in accepting genetically modified foods (GMO), and NGO’s (non governmental organisations) claim that the risks are unknown. For this reason spawn manufacturers are reluctant to apply genetic modification to mushroom strains.

5.2.4 Conservation of strains

If a strain is cultivated continuously it may eventually lose some of its genetic characteristics that made it desirable to grow. Cell degeneration in fungi can occur due to lack of nutrients or oxygen, infections (for instance by bacteria, viruses or other fungi), changes in the pH of the substrate, or the accumulation of unfavourable metabolites. Preserving the genetic stability and viability of the pure mycelium is the objective of strain preservation. Several techniques have been developed to preserve the cultures. A description of these can be found in the Appendix Strain conservation. Do not hesitate to contact one of the larger type culture collections to store possibly valuable mushroom strains; the addresses can also be found in the mentioned chapter. Make an accurate description of the fruit bodies and send some dried fruit bodies for their herbarium with the cultures. There are thousands of promising mushroom strains which are not yet known to science. Especially strains which perform well under tropical conditions are needed for use in developing countries.
6 Environmental care and certification schemes

Loss of biodiversity, erosion and loss of arable land, flooding, air pollution, climate changes and the ever growing population urge mankind to protect the environment. How do environmentally sound practices affect mushroom cultivators? A number of environmental measures (e.g. energy reduction) increase the economical efficiency of the operation. On a macro-economical level, environmental care is always cost-effective, as it decreases the costs which have to be made to restore the environment. On the level of an individual farmer however, some measures may actually have higher costs and reflect the fact that environmental effects are insufficiently priced.

Authorities may order specific measures for: storage of chemicals like pesticides and disinfectants, waste management of empty packaging of chemicals and other toxic wastes, license to use specific chemicals, the disposal of spent compost, soil sanitation, waste water disposal, the use of groundwater, maintenance and emissions of heating installations, storage of gasoline, maintenance of cooling machines with (H)CFK’s, odour control, and genetically modified organisms.

Some compost producers caused so much odour problems, that they were closed down by local authorities (e.g. Petaluma farm in the USA). Some topics are covered briefly in a general paragraph on environmental care, whereas the following subjects are treated in separate paragraphs:

- Plastic,
- Pesticides,
- Energy,
- Utilisation of spent substrate.

Two certification schemes are described: the MBT program (translated: Environmentally aware cultivation) from The Netherlands and organic standards from British Columbia in the USA.

6.1 Environmental care systems

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural wastes</td>
<td>→ Soil conditioner / fertiliser</td>
</tr>
<tr>
<td>Spawn</td>
<td>→ Mushrooms</td>
</tr>
<tr>
<td>Plastic</td>
<td>→ Waste water</td>
</tr>
<tr>
<td>Water</td>
<td>→ Odour</td>
</tr>
<tr>
<td>Energy</td>
<td>→ CO₂, NO₂</td>
</tr>
<tr>
<td>Chemicals</td>
<td>→ Mobility</td>
</tr>
<tr>
<td>Equipment</td>
<td>→ Residues</td>
</tr>
</tbody>
</table>

The mushroom cultivation process: inputs and outputs
Many larger companies nowadays implement environmental care systems to decrease pollution and use of non-renewable resources. A complete environmental care system consists of the following elements:

- a company statement to take environmental issues seriously and to work constantly at decreasing environmental stress,
- an environmental programme with priorities,
- integration of environmental care in all company activities,
- a monitoring and registration programme,
- internal checks,
- internal and external reports,
- regular audit of the complete system.

In simpler words: environmental care is dedicated to decreasing environmental stress by the company’s activities in a systematic way. In order to obtain figures about the amount of environmental stress, registration of input and output is necessary. Then priorities can be determined. An environmental care system is often linked to a quality control system. Both systems offer potential for substantial savings.

**Agricultural wastes**: the possible use of agricultural wastes is a positive aspect of mushroom growing. Lignin-cellulose rich wastes abound in developing countries. These include different types of straws, corn cobs, sawdust, cotton seed hulls, alcohol distillation wastes, etc. A few examples: Coffee pulp creates environmental stress because it is often dumped in rivers and lakes. If it is used to grow mushrooms first, it can afterwards serve as a good soil conditioner. Water hyacinth is a major pest in many lakes. By removing it from the lakes to serve as a substrate for mushroom growing, both wildlife in the lake and the farmers benefit. In this way, farmers can usually obtain the substrate for *Volvariella* cultivation for free.

**Equipment and machines** obviously last longer when they are maintained well, thus reducing both production cost and avoiding replacement by new ones. This third edition contains a new appendix Maintenance chart, which helps growers to inspect regularly.

Chemicals for cooling CFC’s are notorious ozone-killers; yet they are still much in use. Cooling is necessary to keep mushrooms for the fresh market in a good condition. Inspect the cooling unit regularly and make sure no leaks occur.

### 6.1.1 Plastic

**Selection of the type of plastic.** Currently the bulk of the plastics used in mushroom growing is made out of either polyethylene (PE: for pasteurised substrates) or polypropylene (PP: for sterilised substrates). The adverse environmental effect of these plastics is limited compared to PVC (polyvinyl chloride). The use of PVC should be avoided because dioxins (extremely toxic substances) can be formed if the PVC is burned at temperatures below 600 °C; the production of PVC is also hazardous.

It would be well worth investigating the use of other types of plastics, which can be degraded by the mushroom mycelium. Some degradable plastics are already on the market; they have a higher cost price but if local legislation requires one to shift plastic from organic waste, these bags may be cheaper because of lower labour requirements.
The amount of non-organic waste is reduced in this way. Efficient use of the volume of the bags and the choice for the thinnest quality which gives satisfactory results can reduce the amount of non-renewable material which is necessary to produce the plastic. Some bags for sterilised plastic bag cultivation are very thin and also very cheap. Local resources determine which type of bag can be chosen. 

**Disposal of plastic.** It is quite obvious that non-degradable plastics should be removed before the spent compost is applied to the soil, but quite often the farmers do not care or do not know this. Apart from aesthetic reasons to dispose of litter in a more sophisticated way, plastic suffocates soil life. Plastic waste can easily be reused if it is all made of the same kind of material. Since quite a lot of plastic bags are used in mushroom cultivation, it should be possible to offer homogeneous material for reuse to the original manufacturer. In some cases new plastic bags can be manufactured out of the old ones, depending on the type of plastic. But in all cases it is possible to reuse them. The manufacturer will have to shred the plastic wastes into small pieces and wash them thoroughly.

### 6.1.2 Pesticides

Pesticides have led to pollution, health hazards, and marketing problems. A good farm hygiene can keep many contaminants away. Proper substrate preparation and disposing of cut stalks and spent compost at some distance from the farm all contribute to a clean farm. Sometimes, however, pesticides are necessary to secure the crop. The use of insecticides has to be accompanied by good training on how to use them. Unfortunately, there is not much unbiased information on this subject in developing countries. The manufacturers of pesticides there are mainly interested in selling them and the information on the packaging is often misleading. In developing countries there are fewer restrictions than in the European Community, the USA or Japan. Pesticides may leave residues on the crop when they are sprayed at the wrong time. Eating such mushrooms can be hazardous to health. The public is becoming increasingly aware of environmental problems with pesticides, so if there are any residues found in mushrooms, this will result in a negative effect on sales, too. Another aspect is the health of the farmers. Often they lack the proper equipment to give themselves good protection at the time of application, which results in serious cases of poisoning each year.

It has also happened that farmers apply the pesticides completely according to the rules, but would then wash the containers and throw the water (with a high concentration of the pesticide) in a canal right next to their farms.

Yet another aspect is the effectiveness of the pesticides used. They have to be applied at the proper time, otherwise their effect will be minimal. Some monitoring can help in determining whether it is necessary to apply them at all. A sticky yellow paper in the mushroom house will attract insects and can thus reveal which flies or mosquitoes are present, and in what life stage they are. If they become too numerous, it will be necessary to apply some treatment. If the same chemical is used over and over, the insects may develop a resistance to it. It is better to switch between pesticides that control the same pest. A fungicide which was recommended in the first edition of this Manual, benomyl, has been found to have lost effect against some green moulds, which have
become resistant. Pesticides vary widely in biodegradability. Some are no longer tolerated in Europe, but are still produced for sale in the Third World. Some are very poisonous for a short time, but will quickly degrade. In the appendices a list of the currently used pesticides is given.

6.1.3 Energy
The energy for the heat treatment and/or climate control usually derives from conventional sources, such as wood, coal, gas or oil. The temperature has to be adapted to the source to achieve complete burning. Partial burning typically gives more smoke and carbon monoxide, is more polluting, and less efficient.

The detrimental effects of using conventional energy sources are well known: coal combustion produces $SO_2$ (acid rain), forests are overexploited because of the need for wood fuel, the use of conventional energy sources in general contributes to the global rise of $CO_2$ concentration (greenhouse effect). A change of climate is likely to affect countries in tropical regions more than those in temperate regions. The predicted changes on a regional scale are: drought, flooding, and an elevated temperature, etc.

If a large-scale project is planned, it would thus be well worth considering alternative (renewable) energy sources. Particularly in tropical countries, it should be possible to use solar energy for heating water. This would not lead to any pollution and the maintenance cost could be lower, too. Especially the technique of immersing the substrate in hot water has great potential for solar energy. The initial investment and lack of examples so far have frustrated the actual use of solar energy on a larger scale.

Another source that is rarely used is geothermal energy. There is one Pleurotus farm operating with this energy source in Utah (USA) and an Agaricus farm in Iceland. Hot water is pumped through large radiators with attached fans to heat rooms, or steam can be used in sterilising or pasteurising the substrate. Many developing countries have hot springs or steam geysers. The use of this energy would be beneficial, because it brings operation cost down and is far less polluting than conventional energy sources.

In the Netherlands, several growers nowadays utilise cold and heat storage in underground aquifers: layers of sand with water of ca. 10 °C (cold storage) and 16 °C (heat storage). A heat pump can use the energy in the water efficiently. Actually, this is a kind of sustainable energy too, as summer heat is stored for winter use and vice versa.

Another example of an alternative energy source is described by Dr. Martinez-Carrera, who designed a farm in Mexico operating on biogas. Consult the section on pasteurised substrates for more information.

6.1.4 Utilisation of spent substrate
Spent mushroom substrate can be used in the following ways:
- as soil fertilisers or conditioners,
- as an energy source: either burned for heat (for example the drying oven) or partially combusted to produce charcoal,
- to grow a successive crop of mushrooms,
- as animal fodder.

**Soil conditioner and fertiliser:** All substrates can be used as soil conditioners and/or soil fertilisers. Spent compost from *Volvariella* is not very active and can serve mainly
as a soil conditioner, as it improves the structure of the soil. Most fermented substrates enrich the soil considerably. The mushroom mycelium has decreased the high carbon/nitrogen (C/N) ratio in the original substrate materials. It has been shown that Agaricus compost, compared to poultry manure, gives better results in cultivating cabbage, cauliflower, beans and celery.

Wood-inhabiting fungi generally have a much higher C/N ratio than compost-inhabiting mushrooms. Sometimes it is better to compost the spent substrate before application to the soil. Otherwise it might draw valuable nitrogen from the soil. Care has to be taken that no toxic elements are present in the spent substrate. Wood shavings may contain high levels of copper, arsenie, or chromium. The use of mushroom compost as a fertiliser is common practice. It is easy to find farmers who are willing to clean the mushroom house if they can get the compost. Small-scale farmers usually apply the compost to their own fields. A possible danger of applying the compost near the growing area is that contaminants of the old crop can easily infect the fresh substrate. Heavily infested substrates have to be burned or applied to the soil at a considerable distance from the farm.

Spent compost from sugar cane bagasse for Pleurotus production is nowadays accepted by local nurseries in Puerto Rico. The spent substrate is further composted for four to eight weeks covered under plastic. Then it is dried, bagged and distributed to the nursery owners.

Spent compost from plastic bags should be applied to the soil without plastic. This sounds rather obvious, but lack of environmental awareness in combination with the introduction of non-biodegradable materials is currently leading to serious waste problems. The extension service or technical assistants of a farm should therefore pay attention to inform the users of the spent compost about this. It may well be possible to collect the used plastic and return it to the bag factory.

Energy can be obtained from the spent substrates of most wood-degrading fungi. Both wood logs and sawdust bags can be used for heating (simple) steam boilers or drying ovens. Dry the substrate first, then a much higher efficiency can be obtained. Dried substrates can also be used in cooking. Burning the spent compost and then using it as a fertiliser is also a widely practised custom. By keeping a constant high temperature, smoke (as a result of incomplete combustion) can be minimised. Old wood logs can be reused to produce charcoal. Special ovens have been developed for this purpose in Japan. Oxygen supply has to be limited to obtain a good quality charcoal.

**A successive crop of mushrooms:** Few reports deal with actual commercial applications. In Taiwan it used to be common practice to grow Agaricus in winter and to use the spent compost for Volvariella production. The mushroom house is emptied and the spent compost is mixed with cotton waste. It is fermented for two to four days and then the beds are filled again. The substrate is pasteurised in the beds and the rest of the procedure is similar to that when using fresh cotton waste and rice straw. High yields are obtained in this way. Prof. Quimio of the University of the Philippines at Los Banos performed research on the use of Volvariella compost. Directly after the first flush (the second representing only 20 to 40% of the total yield) the rice straw bundles were chopped into pieces of 3 to 6 cm. These were mixed with 20% rice bran, given a heat treatment and inoculated with Pleurotus. A biological efficiency of 60 to 100% was
obtained, which can be compared to the efficiency when using fresh rice straw. When using spent compost for a successive crop, it is important to give the new mixture a proper heat treatment. Otherwise the present contaminants will spread easily through the new substrate during spawn run. **Animal feed:** Although quite a few researchers have investigated feeding ruminants, swine or poultry with spent substrate from oyster mushroom cultivation, the actual commercial application of this practice is limited. The principle is that the fungi degrade the lignolytic components of the substrate, making this more digestible to animals. Moreover, the fungi enrich the substrate with protein. Moulds such as *Aspergillus flavus* however produce highly toxic substances (aflatoxin). If there is contamination by moulds, the substrate cannot be used. If this application of spent substrate is considered, care should be taken to remove all bags which show the slightest sign of contamination.

The transport from mushroom farm to animal farm should be performed in such a way that the substrate does not lose its quality. The process of preparing the animal fodder may imply drying to prevent microbial growth. In The Netherlands the cut stalks were sometimes used in preparing cattle fodder, but cut stalks cannot be compared to spent compost. This is, however, also a waste material from mushroom growing. It can safely be fed to animals, but care should be taken that the stalks are still fresh enough. Because of their high water content stalks (like mushrooms) deteriorate easily.

### 6.2 The Dutch MBT program

This program (translated: environmentally friendly cultivation) offers growers the possibility to label their product with the MBT logo. They have to comply with a number of fixed regulations and a number of voluntary measures. They also have to register a number of parameters, such as the use of chemicals on a daily basis, administration of the purchase of chemicals, and monitoring of energy and water consumption. Furthermore they have to give an auditor access to all relevant data.

The fixed regulations are:

- Filters for the fresh air: use at least Eurovent 3 (not for oyster mushrooms and Shiitake)
- Fly-catching lights or fly signal plates in every growing room
- Cooking out of the substrate at the end of the crop for at least 8 hours at 70 °C (not for oyster mushrooms and Shiitake)
- Reused packaging has to be cleaned before arrival on the farm

**Voluntary measures:** the employed system credits points for every voluntary measure; at least 12 points should be scored to comply with the MBT system, of which at least 6 points should be scored by points under the section Pest management. The system monitors the following aspects:

- Fly-catching lights in the corridor
- Fly-catching lights and fly signal plates in every growing room
- Empty corridor, with only the equipment necessary for cultivation
- Filters on outgoing air (at least Eurovent 3)
- Eurovent 5 spore filter on incoming air
• Limited number of chemical treatments
• No formalin used on the casing soil
• Low chlorinated water use during the cultivation, no other chlorine use
• The temperature difference of cooling water between in- and outlet should be at least 2 °C
• Incoming air is preconditioned by heat exchange with the soil
• Cold- and heat storage system in aquifers
• At least 67% of the ventilation units are frequently controlled
• The grower separates his waste in paper/cardboard, organic waste and residual waste.

6.3 British Columbia certified organic management standards for mushrooms

The standards for biological cultivation differ from place to place; in general the regulations (e.g. to use straw from biologically cultivated wheat only) pose several problems to the producers of substrate. Often the compost is of less quality and more expensive. The consumers have to pay a higher price to compensate for the higher costs. The following regulations stem from British Columbia.

6.1. Building Requirements
6.1.1. Required:
• Isolation of organic and conventional operations in rooms physically separated from each other by excluding walls or in separate buildings
• As much consideration as possible should be given to minimise negative environmental impact when choosing construction materials.
6.1.3. Regulated:
Copper chromium arsenate-treated lumber installed before January 2002 does not need to be removed. Copper chromium arsenate-treated lumber cannot be in contact with soil used to grow food crops (boxed beds).
6.1.4. Prohibited:
Structures using treated lumber will require a 36-month transition period from installation, treatment, or purchase. Use of new, CCA-treated wood on certified organic land will result in returning the land to a T1 status.

6.2. Source of Propagation Materials (Spawn)
In mushroom production, reproductive material called spawn is usually put on a carbohydrate nutrient source to multiply before inoculation of the growing substrate.
6.2.1. Recommended:
Production of spawn on farm.
6.2.2. Allowed:
Spawn from a certified organic operation.
6.2.3. Regulated:
Spawn from conventional sources must be documented to have been produced without the use of prohibited materials.
6.3. Pest Control
6.3.1. Required:
Hygienic production is fundamental to the success of an organic mushroom operation. Proper pasteurising and sterilising of tools and media are important to avoid disease infestation and spread.
6.3.2. Allowed:
Biological control agents e.g. diseases, parasites and predators
Mechanical traps and exclusion techniques
Pheromone traps
Soaps (see Section 13, Materials List)
Herbal preparations
Solutions of pureed insects
Pyrethrums (see Section 13, Materials List)
6.3.3. Regulated:
Diatomaceous earth
6.3.4. Prohibited:
Synthetically compounded insecticides
Anti-coagulant rodenticides

6.4. Disease Control
6.4.1. Required:
Filtration of air and water in mixed operations carried out in the same building. This is to prevent cross contamination.
6.4.2. Allowed:
Pasteurising or sterilising of equipment and media.
Herbal or other plant-derived controls.
Regulation of atmospheric gas ratios e.g. CO₂/O₂.
Biological control agents e.g. pathogenic bacteria and fungi.
6.4.3. Prohibited:
Synthetic chemical disease control methods.

6.5. Sanitation Practices
6.5.1. Allowed:
Hydrogen peroxide, Steam and hot water, Alcohol, Ultraviolet light.
6.5.2. Regulated:
Bleach (preferred to other synthetic disinfectants), Hydrated lime, Copper sulphate, Iodine
Lye (potassium or sodium hydroxide).
6.5.3. Prohibited:
Formaldehyde, synthetic fumigants and fungicides, Methyl bromide.
Treatment of buildings or growing containers with a prohibited material 12 months prior to use for organic production.

6.6. Growing Media
6.6.1. Required:
Proper sterilisation or pasteurisation of media ingredients and equipment.
Animal manure: must be composted before use.
6.6.2. Allowed:
Certified organic cereals and grains and straw. Compost (care should be taken to ensure that temperatures of 57 °C are reached), Wood logs as a substrate e.g. alder, Gypsum, Limestone, Peat.
Natural hormones e.g. auxins, cytokinins, gibberellins.

6.6.3. Regulated:
Agar may be used in initial spawn propagation
Sawdust: must be documented to be free of prohibited materials and may require tests for heavy metals.
Straw-non-organic – must be documented to have been grown without any prohibited materials.

6.6.4. Prohibited:
Spent mushroom compost from conventional sources.
7 Feasibility: costs and returns

All mushroom growers try to achieve the best economical results from their farms. These do not depend solely on impressive yields, but also on the costs incurred in achieving them. Cost calculation is therefore important to answer the following questions concerning the enterprise:

How to calculate the income derived from the mushroom business?
What is the efficiency of the production facilities which are used on the farm?
What is the best production plan?

This chapter provides a basic model to calculate the profitability of a mushroom-growing project. First, the cost of the substrate has to be determined; an easy task if it is available in the market, but more complex if it has to be prepared. The investment in substrate production facilities and the operating cost of substrate production has to be determined to obtain the price of the substrate. Subsequently, the operating cost of cropping, harvesting and climate control has to be calculated. Finally the total cost of mushroom growing can be calculated and compared with market opportunities.

This chapter is divided into the following sections:

- feasibility studies
- investment in substrate production facilities,
- operating cost of substrate production,
- investments in growing rooms,
- determining the number of growing rooms,
- growing cycle period,
- operating costs of mushroom growing,
- profitability of total operation,
- financing the enterprise,
- prices,
- yields,
- example: cost pricing in Ireland.

7.1 Feasibility studies

Before a grower switches to another mushroom species or if a completely new mushroom growing unit is planned, a feasibility study can help in decision making. Performing a financial feasibility study means that one has to evaluate each step in the cultivation process and predict the costs involved. These costs are then compared to the market situation. The entrepreneur (and often also the bank) then decide whether to invest time and capital in the mushroom venture.

During a feasibility study, choices have to be made on how the selected mushroom is
grown exactly. Other choices include the number of flushes, the growing cycle period, and the number and size of the growing rooms. The expected costs can only be predicted if all these factors are known.

It is wise to compare figures from other companies, but be careful with their interpretation. The yield of White button mushrooms ranges from 30 kg/m² to only 3 kg/m², depending on the optimisation of the successive stages. Both a too optimistic and a too pessimistic assumption of the yield affect the feasibility study negatively.

### 7.2 Investment in substrate production facilities

It depends on the chosen technology what kind of equipment is necessary to produce the substrate. The chapters on the specific cultivation methods give more detailed information. The capacity of the equipment should be considered carefully. When planning a site, future expansions have to be taken into account.

For each investment a depreciation time has to be fixed. An easy way to calculate the depreciation costs per year is to divide the cost per item by their expected depreciation period (linear depreciation).

<table>
<thead>
<tr>
<th>Process</th>
<th>Possible facilities</th>
<th>Depreciation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing the substrate</td>
<td>Different types of mixers</td>
<td>5 years</td>
</tr>
<tr>
<td>Filling bags</td>
<td>Filling machines</td>
<td>5 years</td>
</tr>
<tr>
<td>Heat treatment: energy supply</td>
<td>e.g. solar boiler, steam boiler,</td>
<td>10 years</td>
</tr>
<tr>
<td></td>
<td>biogas installation</td>
<td></td>
</tr>
<tr>
<td>Heat treatment: room</td>
<td>Autoclaves, tunnels, tanks</td>
<td>10 years</td>
</tr>
<tr>
<td>Spawning</td>
<td>Filters</td>
<td>3 years</td>
</tr>
<tr>
<td></td>
<td>Filter housing</td>
<td>5 years</td>
</tr>
<tr>
<td></td>
<td>Clean room</td>
<td>10 years</td>
</tr>
<tr>
<td>Heat treatment climate control</td>
<td>Installation between energy source</td>
<td>10 years</td>
</tr>
<tr>
<td></td>
<td>and heat treatment rooms/autoclaves</td>
<td></td>
</tr>
<tr>
<td>Means of transport</td>
<td>Small trucks, caterpillar</td>
<td>4-6 years</td>
</tr>
<tr>
<td>Small equipment</td>
<td>Scales, sealer etc.</td>
<td>2 years</td>
</tr>
</tbody>
</table>

The investment in substrate production facilities equals the sum of the prices of the above mentioned equipment and facilities. The depreciation equals the sum of investments divided by their specific depreciation periods. The cost of acquiring property has to be added to the investment. If it is hired, the housing costs should be combined with the operating costs in the next paragraph.

### 7.3 Operating cost of substrate production

The costs to produce substrate depend on a number of factors, which are shown in the table. Maximal efficiency will be reached when clever designs of the farm, machines
and equipment minimise the need for handling. The most difficult factor to predict is the amount of labour involved. Different people will accomplish the same job in different periods, which may range from 50% (for the very efficient) to 200% of the average.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depreciation of substrate production facilities</td>
<td>Calculation is treated in the previous paragraph.</td>
</tr>
<tr>
<td>Frequency and amount of substrate production</td>
<td>The more often facilities are used for production, the more efficient. On the other hand, depreciation periods of machines should be shortened to account for increased wear.</td>
</tr>
<tr>
<td>Substrate ingredients</td>
<td>The exact formulation (e.g. sawdust, corn flour) has to be defined after trials; include the transportation costs.</td>
</tr>
<tr>
<td>Substrate containers</td>
<td>The price of plastic bags, trays, etc. varies enormously. Check several types for breakage, leakage and heat treatment durability. Alternatively, bulk processing may be possible, which reduces the costs of packaging the substrate.</td>
</tr>
<tr>
<td>Spawn</td>
<td>The amount of spawn needed is given in the specific paragraphs on the cultivation; total cost should include the cost of transportation of the spawn.</td>
</tr>
<tr>
<td>Energy</td>
<td>For heat treatment and optionally: for controlling the climate in grow rooms.</td>
</tr>
<tr>
<td>Labour</td>
<td>Mixing and moistening substrate ingredients</td>
</tr>
<tr>
<td></td>
<td>Filling substrate in containers if not treated in bulk</td>
</tr>
<tr>
<td></td>
<td>Spawning</td>
</tr>
<tr>
<td></td>
<td>Monitoring spawn run</td>
</tr>
<tr>
<td></td>
<td>Transport</td>
</tr>
</tbody>
</table>

Figures can be estimated by simulating the work flow or even better by a pilot scale plant. The result of the calculations should be the cost price per ton of substrate. This price has to be used in one of the following paragraphs on the operating cost of growing mushrooms.

### 7.4 Investments in growing rooms

The capacity of growing rooms ranges from a few m² to 500 m². Large rooms have the advantage that a single climate control unit for each room is cheaper than more separate units for smaller rooms. A possible disadvantage (apart from the higher initial investment) is that the production of mushrooms in a small number of rooms fluctuates in time, depending on the flushes. Usually rows of similarly dimensioned mushroom houses are built in case of year-round production. If mushroom growing is performed in specific seasons only, simple constructions can be erected. The investment in growing rooms depends very much on
choices like building sheds or brick buildings, and to what extent climate control systems are installed. Consider the costs of the following factors:

- building costs (consult the chapter *Mushroom farms*),
- permits and consultancy,
- climate control (consult the relevant chapter),
- shelves,
- installation of electricity, steam installation etc.,
- number of growing rooms. The result of the calculations is: the necessary investment and the yearly depreciation of the growing rooms.

### 7.4.1 Determining the number of growing rooms

The number of rooms is primarily determined by two factors:

1. desired production volume and
2. growing cycle period.

**The desired production volume** is determined by the market survey. If selling 1000 kg of Shiitake a week is feasible, the number of growing rooms can be calculated if the growing cycle period, the yield, and the capacity of the growing room is known.  

*Example*: how many rooms have to be built for growing 1000 kg of Shiitake weekly?  

Assuming:
- each growing room has a growing surface of 250 m²  
- 50 kg substrate fit on 1 m²  
- 8 weeks spawn run, 12 weeks cropping (both in the same room)  
- the yield is assumed to be 14% of the substrate weight.

Each room produces 14% of 12500 kg every 20 weeks. 12 rooms thus produce 54600 kg mushrooms a year.

**The growing cycle period** is the time between filling and emptying the growing rooms. For continuous production (and labour) it is essential that each week (or every 2-4 weeks) another room is filled with substrate. The harvest will then be distributed evenly in time, too. This will make the product more easy to market then would be the case with a highly fluctuating supply. Consult an example of a growing cycle in the chapter *Cultivation on fermented substrates*.

Financial resources and the capacity of the substrate production may limit the number of rooms to be built at the beginning of mushroom growing. The optimum can later be reached with additional investments, depending on the profitability of the company.

### 7.5 Operating costs of mushroom growing

Quantify the following factors when determining the operating costs of mushroom growing:

- depreciation on investment in growing rooms,
- cost of substrate,
- energy and water,
- labour: monitoring climate control, filling and emptying, removing or opening plastic bags, casing, spraying, picking,
- pesticides,
• packaging,
• transport to the market.

7.6 Profitability of total operation

The exact profitability depends on a large number of factors, like how much money has to be loaned from the bank against which interest rates, is the property owned, is it hired or should it be bought, do family members work in the company, against which wages, etc. It is thus almost impossible to provide a general model for calculating profitability. The following general steps should be performed:

• Calculate the total revenues by multiplying the harvest by the average price
• Deduct the operating costs for mushroom growing (see previous paragraph)
• Make a correction for actual depreciation and interest
• Determine the profitability.

Costs can be calculated from a business management perspective or for tax purposes. Generally speaking, tax costing does not consider one’s own capital and working hours of the grower to be costs. From a business management perspective, anyone who puts his capital in the company is losing interest. Depreciation is determined by historic prices for tax costing. The business management model considers the future costs of replacement as determining factor.

7.7 Prices

A modest estimate of the expected price should be used to calculate the feasibility of a mushroom project. Practice will reveal whether the estimation was realistic. Compared to most vegetables, mushrooms are generally expensive. Retail prices for White button mushrooms are about 5 US$, for fresh Shiitake 10-20 US$ per kg in Western countries. The prices of mushrooms vary from species to species and from country to country. Oyster mushrooms are rather expensive in Europe, whilst they belong to the cheapest mushrooms in China. The opposite is true for White button mushrooms: they are the cheapest species in Europe, and rather expensive in China. The prices of exotic mushrooms fluctuate significantly, from season to season. December is the month with the best prices. In Japan the prices of fresh Shiitake are higher in summer and winter, when no Shiitake can be cultivated outdoors.

Sometimes an extra investment in climate control will soon be earned back by the higher profits that mushrooms bring off season.

When comparing figures on mushroom prices, be sure to compare the right figures. The retail price is of course much higher than the wholesale price. Each grower has to find the most profitable outlet. In developing countries, farmers often sell their products directly to the end consumers. The advantage is that the prices are higher, the main disadvantage is that it takes a considerable time to bring the mushrooms to the market and to sell them. Especially if only limited amounts are produced per farmer, it can be more profitable to form a marketing cooperative.

Mushroom journals generally show the current prices of White button mushrooms of various grades. It may also be worthwhile to check some Internet resources for actual
prices of speciality mushrooms. To summarise the above, the price of mushrooms depends on:
- regional preferences,
- the species and quality,
- the season,
- whether they are sold fresh or conserved,
- whether they are sold for wholesale distribution or to end consumers.
The following chapter Marketing shows how a market survey can be performed, which should give fairly accurate figures.

7.8 Yields

Calculating the revenues of mushroom cultivation depends on the amount of mushrooms produced within a certain time on a certain amount of substrate. The period of time before harvesting depends on the particular mushroom, cultivation method and temperature. These can be found in the growing instructions of the specific mushrooms. Exact figures on yields can also be found in those paragraphs.

Several different methods to measure the yield are commonly used. The most accurate (in a scientific sense) is dry weight of mushrooms versus dry weight of substrate. It requires, however, that both the substrate and the mushrooms have to be dried in order to compare yields. A representative sample of the fresh substrate is dried in an oven under a relatively low temperature. The fresh mushrooms are dried in the same way. More convenient for growers who prepare their substrate from dry raw materials is the so-called biological efficiency: wet weight of the mushrooms versus (air) dry weight of the substrate materials. It is less accurate than the previous method because the percentage of water in the mushrooms and the starting substrate is variable. It has one advantage: a grower can easily determine the expected yield from a given amount of dry organic waste material. Yet another commonly used measure is the wet weight of the mushroom versus the wet weight of the substrate. This method is easiest for growers who buy prepared substrate.

7.8.1 Example

100 kg dry sawdust will make 225 kg of moistened substrate. Suppose 50 kg of fresh mushrooms can be harvested with a moisture content of 90%. The yield can be calculated as follows:
The sawdust should be oven-dried and 100 kg would turn out to be, for example, 90 kg.
The yield (dry weight/dry weight): 5 kg/90 kg x 100% = 5.5%
The biological efficiency: 50 kg/100 kg x 100% = 50%
The yield (wet weight/wet weight): 50kg/225kg x 100% = 22%

7.8.2 Yield expressed in kg/m²

When mushrooms are grown in beds, it is convenient to express the yields in kg/m². Much depends, of course, on how much substrate is utilised per m². In White button mushroom production in The Netherlands 100 to 120 kg is filled per m². In China,
where the compost is less optimal, only 50 to 60 kg is used. The yield per m² is 30 kg in The Netherlands, compared to 3 to 6 kg in China.

7.8.3 Comparing yields
Differences in yields between companies can be due to all factors which are involved in mushroom growing: from the quality of the spawn and substrate to climate control during cropping. In general, the higher the yields, the higher the investment in controlled growing rooms.

Often results from different countries cannot be compared because the products differ. One market may accept longer stems than the other. Oyster mushrooms in Europe have rather short stems, year round a 12-15% yield (cut fresh mushrooms versus wet substrate) is not uncommon. In China 20% yields are reported: actually the yield is similar but the Chinese eat the stems, which account for almost 50% of the weight, too.

A modest estimate of the expected yield should be used to calculate the feasibility of a mushroom project. A number of mushroom ventures has ceased because the planned efficiency could not be reached.

7.8.4 Quality or quantity
Harvesting many kilos always sounds nice, but it can be more profitable to harvest less kilos of a higher quality. The price of first grade *Agaricus* may be twice the price for *Agaricus* of grade 3. Therefore harvesting 20 kg grade 1 would bring more profit than 30 kg grade III (presuming the other costs remain the same).

The quality is determined by the wholesalers and in the end by the consumers. They decide whether the product meets their demands in taste, size, colour, freshness, healthiness, etc. And if it does not, they will cease buying it.

With many mushroom species, harvesting the fruit bodies before their biological maturity will increase their shelf life. The yield may be a little less, but when the product lasts longer, a higher price can be obtained. More information can be found in the chapter Post harvest handling.

Another quality aspect is certified biological cultivation: a niche market where higher prices are paid for ecologically sound cultivation practices. In most western countries the market share of biological food products is between 1–10%.

7.8.5 Example: cost pricing in Ireland
In this paragraph a fairly typical cost of production in € (Euro) is worked out for white button mushroom production in Ireland. The figures stem from TEAGASC and were collected in 2002. The general assumptions are:

- Five shed unit
- Phase two compost
- 10 week growing cycle/26 crops per year
- Yield: 500lbs/tonne
- Achieving an average price of 86 cent or 54p sterling

Two situations are compared: in column A standard filling with 20 tonnes per shed, column B an increased throughput of 24 tonnes per shed (staging). The calculated cost in situation A is 75 cents/lb mushrooms without allowing for repay-
ments, depreciation or any re-investment. The figure for insurance, accountant, maintenance, car expenses, phone, tractor, protective clothing, rent-to-kill contracts, etc. in this example is €300 per crop or €7800 per annum.
In situation B, allowing for increased costs for casing peat, stacking and casing, chemicals and emptying, the production costs decrease 73.8 cent. The margin (ca. €9000) also increases considerably. This opens the possibility of an extra investment of €940 per tunnel for staging to carry an extra 4 tonnes and paid off over 2 years.

<table>
<thead>
<tr>
<th>Mushroom yield (lbs per tonne compost)</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Compost (tonnes)</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>2 Compost Cost per tonne (€)</td>
<td>122</td>
<td>24</td>
</tr>
<tr>
<td>3 Volume of mushroom produced (lbs)</td>
<td>10000</td>
<td>12000</td>
</tr>
<tr>
<td>4 Mushroom price – pence per lb (€)</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>5 Total value mushrooms sold</td>
<td>8600</td>
<td>10320</td>
</tr>
</tbody>
</table>

**Direct Costs**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Compost</td>
<td>2440</td>
<td>2928</td>
</tr>
<tr>
<td>7 Casing</td>
<td>350</td>
<td>400</td>
</tr>
<tr>
<td>8 Oil</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>9 Electricity</td>
<td>190</td>
<td>190</td>
</tr>
<tr>
<td>10 Chemicals</td>
<td>190</td>
<td>210</td>
</tr>
<tr>
<td>11 Packaging</td>
<td>0.1 cent</td>
<td>1000</td>
</tr>
<tr>
<td><strong>Casual Labour:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) Filling and stacking</td>
<td>190</td>
<td>220</td>
</tr>
<tr>
<td>(B) Casing</td>
<td>190</td>
<td>220</td>
</tr>
<tr>
<td>(C) Compost emptying</td>
<td>350</td>
<td>400</td>
</tr>
<tr>
<td>(D) Wash out</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Picking</td>
<td>0.18 cent</td>
<td>1800</td>
</tr>
<tr>
<td><strong>Total variable (direct) costs</strong></td>
<td>6950</td>
<td>8178</td>
</tr>
<tr>
<td>(A) Administration (phone and insurance)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(B) Maintenance</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(C) Transport</td>
<td>0.025 cent</td>
<td>250</td>
</tr>
<tr>
<td>(D) Miscellaneous (car + tractor + accountant)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(E) Staging</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td><strong>Total Costs.</strong></td>
<td>7500</td>
<td>8868</td>
</tr>
<tr>
<td><strong>Production cost per lb</strong></td>
<td>0.75</td>
<td>0.739</td>
</tr>
<tr>
<td><strong>Total Net Margin */Crop (5-16)</strong>.</td>
<td>1100</td>
<td>1452</td>
</tr>
<tr>
<td>19 No of crops</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>20 Margin in Euro</td>
<td>28600</td>
<td>37752</td>
</tr>
</tbody>
</table>

* Total costs and Net Margin are before the deduction of loan interest costs and depreciation.

Source: TEAGASC, 2002
8 Mushroom markets and marketing

This chapter discusses marketing aspects of mushroom products. What marketing strategy is best for your company? What product do you sell at which price to what type of customer? An important question is: Where is value added to a product and at what cost?

As this book is intended for worldwide use, it treats marketing aspects and recent trends in a global way. It cannot deal with specific regional markets and problems, like the current situation in Western Europe where the supermarkets dominate the market. Or that the markets for e.g. oyster mushrooms still have to be developed in many parts of Africa.

Marketing theory usually considers the following four ‘P’s’:

- price
- product (including quality and packaging)
- physical distribution
- promotion.

In addition to those factors, a market survey, a case study on Portabella, trade names and recent developments in the food market are discussed.

8.1 Market survey

The general purpose of a market survey is to determine the size of the market and what price the target groups are willing to pay. When considering producing for the local market, the following questions have to be answered:

- which mushrooms are currently being sold?
- what is their origin, are they gathered in the wild, or cultivated locally or abroad?
- what is their price and availability (note that high prices are often caused by limited production and that extra production lowers the price)
- which mushrooms are/will be appreciated most?
- are there any places which

![Shop display with many types of mushrooms.](image)
lack mushrooms, although there is a demand (e.g. supermarkets, restaurants, large hotels, vegetable wholesalers, market place?
• who are the current distributors and is co-operation possible?
• are there any target groups which can be approached separately, like overseas Chinese, Japanese or other specific ethnic groups?
Before investing in mushroom farms, it is wise to test the market with imported mushrooms. The imported mushroom quality should of course be identical to the chosen cultivated mushroom. Often people’s answers to a questionnaire are more positive than their actual behaviour when the products are offered for real. A question whether they are willing to sign a contract for regular deliveries will show how serious parties are. Sometimes a new product is sold quite well in the beginning, but after a number of weeks sales may drop considerably. By testing the market with imported mushrooms for some months, valuable market information can be obtained.
The market survey should determine:
• the type and amounts of mushrooms to be produced
• which target group to aim at
• the price that can be obtained
• the distribution structure.

8.2 The marketing factor price

The goal of marketing is of course to sell products at the best price. The price that customers are willing to pay, depends on several factors: while price and reliable delivery are very important for professional buyers (wholesalers, restaurants, food industry), for consumers convenience may prevail. Small-scale growers may deliver directly to restaurants and greengrocers, and receive a higher price in return for the time they spend on marketing and distributing. For larger growers this strategy doesn’t work: they need distributors to assure them all their mushrooms are sold. In an ideal situation (from the standpoint of the producers), growers co-operate and prevent the market from being flooded with mushrooms, thus assuring a good price. Unfortunately, the market in most economies develops in exactly the opposite direction. Supermarkets and retailers fuse and merge, and their buying power is becoming bigger and bigger. With many (relatively) small producers, it is difficult to close the ranks and maintain a strong position. Competition between producers (sometimes from other countries) may be fierce, leaving little space to obtain good prices. Price elasticity (how does the market volume respond to price fluctuations) is affected by the situation that supermarkets tend to keep the prices stable all year round.
Especially in bulk markets like White button mushrooms, the price development is towards or even below the cost price.
Mushrooms are still costly in comparison with other vegetables. If the income level in a specific country is relatively low, the mushrooms will mainly be consumed by the upper class and the tourists, or will have to be exported. The presence of a mushroom industry, however, should lead to increased economic development for at least the producers of the crop.
8.2.1 Price information
Some mushroom journals mention the prices in their countries, but usually the information is restricted to different grades of Agaricus. Prices of dried mushrooms are more stable because the product is conserved; even then the price may go up if the harvest of wild mushrooms like Boletus edulis was limited. Recent prices of many different mushrooms can be found in the Mushroom Growers Newsletter (see appendix for address details).

8.2.2 Prices of wild mushrooms
The prices of wild mushrooms fluctuate more than those of cultivated mushrooms, as supply is much more variable. The highest prices are fetched in the winter season, when demand is high (Christmas) and production is low. As explained in the chapter Biology, these mushrooms (with the exception of Morels) cannot be cultivated apart from their host trees. The flavour of some of these species surpasses that of most cultivated mushrooms; especially dried porcini and Trompette de la mort and fresh Chanterelle, Morels and Truffles have a very distinct flavour.

Variability of retail prices of fresh wild mushroom species

<table>
<thead>
<tr>
<th>Species</th>
<th>Vernacular names</th>
<th>Price range US$/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boletus edulis</td>
<td>Cépe (F), porcini (I)</td>
<td>10 – 30</td>
</tr>
<tr>
<td>Cantharellus cibarius</td>
<td>Chanterelle, Girolle (F)</td>
<td>5 – 35</td>
</tr>
<tr>
<td>Cantharellus tubaeformis</td>
<td>Chanterelle gris (F)</td>
<td>8 – 25</td>
</tr>
<tr>
<td>Craterellus cornucopioides</td>
<td>Trompette de la mort (F)</td>
<td>20 – 30</td>
</tr>
<tr>
<td>Hydnum repandum</td>
<td>Hedgehog (E), Pied de mouton (F)</td>
<td>10 – 25</td>
</tr>
<tr>
<td>Morchella sp.</td>
<td>Morel (E), Morille (F)</td>
<td>50 – 200</td>
</tr>
<tr>
<td>Tuber melanosporum</td>
<td>Truffe (F), Truffle (E)</td>
<td>1000 – 2500</td>
</tr>
</tbody>
</table>

8.3 Product
Product life cycle is a term which is rarely heard in agriculture, whilst it is very common in the food industry. Growers used to pay more attention to the growing process and cost price reduction; innovations to add value to their products were rarely considered. Integration of mushroom producers with food industries could lead to a variety of convenience products, which incorporate mushrooms. In this way, producers can add more value to their product. On the other hand, a large number of new food products fail to become firmly established on the market; the investment in product innovation and promotion is not always rewarded with a good return during the product life cycle. A relatively simple existing product ‘innovation’ is combining small amounts of wild mushrooms with cultivated mushrooms; this leads to an appealing product with a better profit margin. The taste of a pasta sauce containing mainly White button mushrooms is greatly enhanced with only a small amount of dried ‘porcini’ (Boletus edulis). Another mushroom product is sliced fresh mushrooms, although the slicing affects the shelf life and the food value of the product negatively. The market of convenience food stuff is expected to keep growing; ready-made meals, ingredients for stir-fried dishes may all contain mushrooms. Up to now, these innovations often originated from other
sectors than the mushroom producers. Apart from convenience, there is also a niche market for special and experimental cooking. On the following three pages an overview is given of marketability aspects of most cultivated mushrooms; it is up to growers and entrepreneurs to use the specific characteristics of these mushrooms to provide the community with valuable products.

8.3.1 Portabella: a case study of product development
The first cultivated *Agaricus bisporus* were grown in France; these were brown- or creme-coloured mushrooms. The brown mushrooms were large in size and rather scaly. Kligman (1927) reports that a single cluster of white was found, which formed spontaneously out of the coloured mushrooms. This cluster was used to make spawn and all the white strains derive from this single cluster.

The production of the button mushroom (*Agaricus bisporus*) in its modern form developed after the second World War. Many consumers were not familiar with 'toadstools' and thus marketers advised to pick the mushrooms at a very young (in fact immature) stage. The buttons also kept longer than fully mature mushrooms. Whole generations of consumers have never actually seen mature *Agaricus*!

Mushrooms with opened caps were considered grade III and sold at a very low price. The employees of the farms often took these mushrooms home and found them to taste even better than the young mushrooms. In the 80's growers 'rediscovered' the traditional large brown scaly mushrooms and called them portobello, later portabella. This made-up Italian sounding name was since then used for mushrooms which were previously unsalable.
<table>
<thead>
<tr>
<th>Species (synonyms)</th>
<th>Colloquial names</th>
<th>Flavour and texture</th>
<th>Appearance</th>
<th>Specific product features</th>
<th>Marketability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>Cultivated mushroom, White button mushroom, Champignon de Paris (F)</td>
<td>Nice texture and fine (relatively weak) flavour</td>
<td>In small buttons (large, medium or small)</td>
<td>Sold fresh, in brine, canned and freeze-dried</td>
<td>A well accepted mushroom; strong competition worldwide; local suppliers can add value by supplying fresh mushrooms to the local market</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>Portobello, Portabella for the open caps; Crimini for the young brown buttons</td>
<td>Nice flavour, actually somewhat stronger than the white buttons</td>
<td>Buttons (Crimini) or open fruit bodies (Portobello), sometimes scaly</td>
<td>Sold fresh</td>
<td>The market for brown and off white is smaller compared to the market for white buttons. The niche markets however provide opportunities for higher profits. Cultivated for its medicinal properties, this mushroom is now grown on a large scale in Brazil and China. Japanese scientists triggered its cultivation and Japan is still a large consumer for this mushroom. Rubbery texture is unfamiliar to the western market. Market seems limited to oriental communities and some ethnic groups in e.g. Tanzania.</td>
</tr>
<tr>
<td>Agaricus blazei</td>
<td>Himematsutake</td>
<td>Distinct almond-like taste, solid texture</td>
<td>Brown from compostlike substrates, often off white when grown on sterilised substrate; similar to Portobello</td>
<td>Mostly sold in a conserved state (dried, powder, extract, or capsules)</td>
<td>See above; the price of the Hirneola is somewhat higher and several cooks prefer the smaller mushrooms.</td>
</tr>
<tr>
<td>Auricularia auricula</td>
<td>Mouse ear, Black wood ear (Hei Mu Er) in Chinese</td>
<td>Weak taste, mainly used because of its rubbery texture; without the sweet taste of Tremella</td>
<td>Ear-like appearance, black when dried, brownish in a fresh state. Thickness is also considered a quality aspect</td>
<td>Most mushrooms are sold in a dry state; there is no taste or texture difference between the dried and fresh product</td>
<td>Most mushrooms are sold in a dry state; there is no taste or texture difference between the dried and fresh product</td>
</tr>
<tr>
<td></td>
<td>(Hirneola auricula-judae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auricularia polytricha</td>
<td>Woolly wood ear (Mao Mu Er) in Chinese</td>
<td>Weak taste, mainly used because of its rubbery texture; without the sweet taste of Tremella</td>
<td>Large (up to 20 cm) rose to pink coloured ears in a fresh state; Velvet-like lower side which distinguishes the species from Hirneola auricula judae</td>
<td>Most mushrooms are sold in a dry state; there is no taste or texture difference between the dried and fresh product</td>
<td></td>
</tr>
<tr>
<td>Coprinus comatus</td>
<td>Inkcap, Shaggy Mane</td>
<td>Nice taste and texture when harvested in a young state</td>
<td>Young mushrooms look like Asparagus-tops</td>
<td>Limited production only with direct outlet to specialty restaurants. Even cooled young mushrooms spoil within four to five days due to liquefaction of the caps</td>
<td>Marketability seems limited to bunched or canned product, as the fresh product has a very short shelf life. Taste, yields and texture offer no problems in marketing.</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>Enoki (Japanese) also used in the USA; official name is Velvet stem Collybia; in Chinese: Gold Needle mushroom</td>
<td>Nice taste and substantial 'bite', though not as rubbery as the Auricularia</td>
<td>Beautiful bouquets of mushrooms are harvested from substrate containers which force the mushrooms to form long stems, hence their Chinese name</td>
<td>Sold fresh or canned. Production is still limited to Japan, China and Taiwan and a few farms in the USA and Denmark</td>
<td>Very suitable mushroom for deluxe western market or Asian communities. Its attractive appearance calls for use as edible decorations.</td>
</tr>
<tr>
<td>Species (synonyms)</td>
<td>Colloquial names</td>
<td>Flavour and texture</td>
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<td>Specific product features</td>
<td>Marketability</td>
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</tr>
<tr>
<td><em>Ganoderma tsugae, G. lucidum</em></td>
<td>Ling Zhi (Chinese); Reishi or Mannetake (Japanese); Ganoderma</td>
<td>Woody mushroom unsuitable for direct consumption; extracts or teas with Ganoderma are extremely bitter</td>
<td>Nice appearance: shiny (<em>G. lucidum</em>) red brown cap and stipe with white to yellow pores</td>
<td>Sold as tea, capsules or in the form of dried mushrooms. Sometimes sold in combination with Ginseng</td>
<td>This much used mushroom in ancient China is an important drug in traditional Chinese medicine.</td>
</tr>
<tr>
<td><em>Hericium erinaceus</em></td>
<td>Lion's mane (USA); Monkey Head mushroom (Hou You Gu) in China; Yamabushi-taki (Japan); Hedgehog mushroom (Por pom blanc (France)</td>
<td>Nice texture, relatively light mushroom with slightly bitter taste</td>
<td>Nice appearance: white spiny clump with spines pointed downward</td>
<td>Sold fresh, dried and in brine</td>
<td>Despite its slight bitterness it is liked by the few people who tried it. A challenge for marketers.</td>
</tr>
<tr>
<td><em>Lentinula edodes</em> (<em>Lentinus edodes</em>)</td>
<td>Shiitake (Japan, USA, Europe); Xiang Gu (fragrant mushroom) or Dong Gu (winter mushroom) in Chinese</td>
<td>Specific garlic-like taste, which becomes much stronger after drying</td>
<td>Nice red brown caps, white gills. The colour darkens when dried. Many different grades are distinguished in Japan, the thick capped winter mushrooms with cracked caps being the most expensive grade</td>
<td>Usually dried or fresh. A special product from China are Po Ku stir-fried shiitake (in oil); subsequently canned. Normal canning results in awful-tasting mushrooms</td>
<td>Ready markets exist for the dried mushrooms within Asian communities; however, competition from mainland China is fierce.</td>
</tr>
<tr>
<td><em>Pholiota nameko</em></td>
<td>Nameko (Japanese) also used in USA and Europe</td>
<td>Solid crunchy texture after cooking, typical</td>
<td>Typically slimy mushroom, attractive shiny orange brown caps</td>
<td>Fresh or canned</td>
<td>The slimy caps have been reported to cause awkward feelings in the USA; in Europe the mushroom is sold without problems for its nice taste and appearance. Production is still limited. Promising marketing potential because of nice texture and flavour. Good keeping ability. Can be grown at high temperatures. An interesting mushroom for deluxe restaurants and hotels because of its beautiful appearance. The colour fades when cooked.</td>
</tr>
<tr>
<td><em>Pleurotus abalonus</em> synonyms: <em>P. corticus, P. dryinus</em></td>
<td>Abalone mushroom</td>
<td>Nice fleshy texture</td>
<td>Large dark-coloured fruit bodies</td>
<td>Fresh or canned (one of the very few Oyster mushrooms which can be canned) Fresh, best sold in separately packed clumps (often many mushrooms arise from a single stem) to decrease risk of breakage</td>
<td>Promising marketing potential because of nice texture and flavour. Good keeping ability. Can be grown at high temperatures.</td>
</tr>
<tr>
<td><em>Pleurotus cornucopiae</em> synonyms: <em>Pleurotus citrinopileatus</em></td>
<td>Golden or Yellow Oyster mushroom</td>
<td>Specific taste, somewhere in between cucumber and melon, fragile</td>
<td>Bright yellow varieties (<em>P. citrinopileatus</em>) and porcelain glazed coloured</td>
<td>Fresh or canned (one of the very few Oyster mushroom which can be canned)</td>
<td>Promising marketing potential because of nice texture and flavour. Good keeping ability. Can be grown at high temperatures.</td>
</tr>
<tr>
<td><em>Pleurotus cystidiosus</em> synonyms: <em>P. corticus, P. dryinus</em></td>
<td>Abalone mushroom</td>
<td>Nice fleshy texture</td>
<td>Large mushrooms, whitish</td>
<td>Fresh or canned (one of the very few Oyster mushroom which can be canned)</td>
<td>Promising marketing potential because of nice texture and flavour. Good keeping ability. Can be grown at high temperatures.</td>
</tr>
<tr>
<td>Species (synonyms)</td>
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</tr>
<tr>
<td><em>Pleurotus djamar</em> synonyms: <em>P. fiabellatus,</em> <em>P. salmoneo-stramineus,</em> <em>P. ostreatoroseatus</em></td>
<td>Pink Oyster mushroom</td>
<td>Leathery texture, specific taste, slightly buttery</td>
<td>Beautiful pink fruit bodies, growing in clusters</td>
<td>Fresh</td>
<td>Cost price higher than other oyster mushrooms due to lower yields. Marketed as exotic oysters in the UK in combination with yellow Oyster mushrooms.</td>
</tr>
<tr>
<td><em>Pleurotus eryngii</em></td>
<td>King Oyster mushroom</td>
<td>Very nice flavour and taste, the thick stem can also be eaten</td>
<td>Bulbous thick stems and relatively small caps</td>
<td>Fresh</td>
<td>Cost price higher compared to <em>P. ostrearatu</em> due to more complex cultivation methods.</td>
</tr>
<tr>
<td><em>Pleurotus ostrearatus</em> synonyms: <em>Pleurotus florida</em></td>
<td>Winter Oyster mushroom</td>
<td>Nice texture, typical <em>Pleurotus</em> fragrance</td>
<td>Grey to blue grey fruit bodies</td>
<td>Fresh</td>
<td>The most cultivated Oyster is often marketed with short stems; if customers can be persuaded to accept longer stems both yields and keeping abilities improve.</td>
</tr>
<tr>
<td><em>Pleurotus pulmonarius</em></td>
<td>Summer Oyster mushroom, Phoenix Oyster mushroom</td>
<td>See above species</td>
<td>Brown or grey mushrooms, sometimes with typical stripes near the margin, growing one by one from the substrate</td>
<td>Fresh</td>
<td>Similar to <em>P. ostrearatu</em>.</td>
</tr>
<tr>
<td><em>Stropharia rugoso-annulata</em></td>
<td>King Stropharia Gartenriese (German) Strophaire (French)</td>
<td>Caps have a specific nut-like scent and taste, stems taste similar to Asparagus</td>
<td>Appealing mushrooms with white stems and red brown caps; form resembles <em>Boletus</em> species but Stropharia is a gilled species</td>
<td>Fresh</td>
<td>The resemblance to <em>Boletus</em> can be used in marketing. Mushrooms should be harvested young as the older mushrooms generate millions of purple black spores and are more brittle than opened <em>Agaricus</em>. The market has not been explored yet for this species for lack of steady supply.</td>
</tr>
<tr>
<td><em>Tremella fuciformis</em></td>
<td>White Jelly Fungus, Silver Ear, (Yin Er), or White Wood Ear (Bai Mu Er) in Chinese</td>
<td>Rather weak sweet taste with rubbery texture</td>
<td>Nice flowerlike appearance</td>
<td>Usually sold in a dried state</td>
<td>The fruit bodies seem less suited for the western market, unless processed in convenience food products.</td>
</tr>
<tr>
<td><em>Volvariella volvacea</em></td>
<td>Paddy Straw mushroom Rice Straw mushroom Tsao Gu (Straw Mushroom) in Chinese Fukurotate (Japanese)</td>
<td>Fresh mushrooms have a texture which is liked much by Asians, the canned mushrooms have lost much of their taste</td>
<td>Typically harvested at a very young egg-like stage Usually sold canned, in brine or fresh. Rarely sold dried (egg-like young fruit bodies cut longitudinally in halves)</td>
<td></td>
<td>Fresh mushrooms have a very limited shelf life and cannot be kept under cool conditions. The mushrooms seem less suited for the western market because of their unfamiliar texture. Acceptability differs from region to region in Africa.</td>
</tr>
</tbody>
</table>
Portabella fetch higher prices than 'normal' white buttons, but the yield is somewhat lower and Portabella’s cannot be cultivated solely: the grower has to pick ‘crimini’, the young brown buttons, and must leave a limited number of young fruit bodies. Climatic conditions have to be adapted to let the mushrooms form scales. All in all, it has shown to be a successful niche product with a good profit margin.

8.4 Physical distribution

A feasibility study or business case should contain a section on distribution aspects: how do customers get in touch with your product. The choice for a specific target group determines how to deal with distributing aspects:

1. consumers (e.g. through farmers markets)
2. restaurants, greengrocers, local supermarkets
3. local wholesalers
4. importers abroad

If the complete production capacity is sold to a single company (which can be the case when selling to wholesalers), the farm is very dependent on this company. It is safer (but less efficient) to have more customers.

8.4.1 Distribution to private consumers

Selling small quantities of mushrooms to private consumers takes considerable time; unless the capacity of the farm (e.g. in developing countries) is very limited (less than say 25 kg a day) it will be difficult to sell the complete production to consumers only. It can, however, be interesting to sell part of the production at (farmers) markets, because relatively high prices are paid for premium fresh products. Moreover, selling at these kinds of markets is promotion for the mushrooms and the grower gets in contact with the consumers. Firsthand information e.g. from specific ethnic groups may lead to unexpected business deals. A grower in the USA met at such a market Vietnamese who were especially fond of a dark-coloured oyster mushroom, while the rest of the market demanded light-coloured strains. The grower managed to find a specific outlet for dark-coloured mushrooms and as his customers also liked the tougher stem parts, he could cut the stems at a greater length thus increasing his yield. He could sell these mushrooms with a good profit margin later to Vietnamese-owned stores, which catered for this specific group. Another way to sell to consumers is treated in the paragraph promotion: to combine farm visits, lectures and sales.

8.4.2 Distribution to restaurants, greengrocers and local supermarkets

Distribution to professional users in the neighbourhood is feasible and less time-consuming compared to selling to private households. With 40–50 restaurants or greengrocers with a weekly demand of 5 kg of mushrooms on average, small mushroom farms can sell their complete production locally. Ideally co-operation with a non-competing supplier (e.g. poultry, or fish) can be achieved to save time in delivering the goods.

The advantage here again is the direct contact with the market and the relative ease to promote new species or innovations. Personally I have found that restaurants were
willing to pay high prices for Shaggy Mane and even for grade 3 opened *Agaricus* because of the personal contact and the story behind the products (this was before the Portabella’s were on the market). The advantages for the restaurants are the freshness and speciality of the products, combined with delivery at their place. Ideally a flexible delivery service can be set up as an extra service to the professional users. It does take at least one day a week to administrate, deliver and keep in touch with the professional users.

8.4.3 *Distribution to wholesalers*

The added value of wholesalers is their distribution network and their ability to match supply and demand. Of course it is not in their interest to be transparent to the growers what they bill their customers, nor what they pay colleague growers for their mushrooms. The wholesalers in their turn are sometimes sandwiched between large buying organisations and the growers; it is easier for them to demand lower prices from their suppliers than to charge higher prices to their customers in a surplus market. A different situation arises when the marketing organisation is owned by the growers and the wholesalers profits return to the individual growers.

8.4.4 *Importers abroad*

Mushrooms and mushroom products can be exported if the quality and price meet the requirements of the buyer(s). Exporting mushrooms from developing countries earn foreign currencies, while this agricultural product does not claim any arable land. Quite a few factors have to be considered. In many developing countries, export is restricted to companies with an export license. They have to follow strict sanitary rules to obtain and keep the license. In case of exporting fresh mushrooms, the conditions during the transport have to be controlled carefully to keep the quality of the product. The quantities should be no less than 500 kg per shipment and preferably higher to cover incurring costs. Only producers nearby international airports or close to borders with a good road infrastructure can export fresh mushrooms, as the time between picking and delivery should preferably be less than two days. Often, preserved mushrooms are exported: in cans, brine or dried. Canned mushrooms are often shipped in 20 feet or 40 feet sea containers; dried mushrooms could be sent in smaller crates.

*How to find an importer?*

The main importing regions are the USA, the European Community and Japan. You can try to get in touch with importers in various ways:

1. Use the Internet to get in touch with mushroom traders; by now at least 95% of the traders have a website. When searching on the Internet, consider that traders communicate most easily in their own language; some of the websites are only in one language. If you wish to export mushrooms to Germany your search results for ‘Pilze’ or ‘Austernpilze’ will give much better results than for ‘(oyster) mushrooms’.

2. Ask the embassies or consulates of the countries you wish to export to, for a list of
importers. Assemble information about possible restrictions (import quota, sanitary rules) for target countries.

3. Participate in international agricultural fairs and exhibits, such as those in Paris or Cologne. Alternatively, you could send information material and products to your Ministry of Agriculture, if it organises stands at a fair. The most efficient but expensive way is a visit to these fairs. To make most of the visit, it is recommended that you make appointments to meet importers.

A workable strategy for export to the European Community is to focus on one country first; the product may be repacked before shipping to other countries.

Quality and packaging
A steady supply, a good and constant quality and a reasonable price are the most important aspects of export. Exporters should be well aware of the demand for a consistently high quality product. The employees of the exporting company may have opinions about quality that differ much from those of the importing company. The presence of larvae of insects is not considered problematic in many African countries (don’t they provide extra protein?) but it makes mushrooms difficult to sell in Western communities.

It is thus very important to follow descriptions in a Letter of Credit (the document which arranges financial and technical matters between seller and buyer, like quality, mode of shipping, packaging, when payments are due etc.).

It will be impossible to export goods which do not meet the specifications of the importing countries. Ask the Council for Agriculture of the European Community in Brussels or the Food and Drug Administration in the USA and Canada to provide you with the specifications for imported goods. These specifications give the different quality grades, drained weight, and some requirements concerning packing and labelling cans.

Quality control can be performed by quality watchers; the importer is thus ensured that the shipment has the right quality. Large international trading companies have their own quality watchers; other parties can make use of companies like ISR or SGS. Preferably, a long-term relationship based on trust develops between buyer and seller, and certification costs can be eliminated.

Other regulations concern the environmental impact of packaging. Supermarkets in The Netherlands e.g. have to decrease the waste caused by packaging. Paying more attention to the way products were packed, delivered some remarkable examples that economy and ecology meet when less material is used for the same functionality. In Germany a system has been set up, where producers have to pay for the waste treatment of the packaging of their products (Grüne Punkt).

As labour is very expensive, the use of easy-to-open crates lowers operating costs in supermarkets.

There is more to packaging than following official guidelines: do they catch the eye of the consumers? Is the form and label of the package appealing to the customers? For example, glass containers from China are out-of-date by European standards and therefore not suitable for the western market. In comparison with tin cans, glass is more expensive, and because of its weight, also more expensive to transport. Furthermore, breakage during transport is higher compared to cans. In this case it seems wiser to
transport mushrooms in brine and fill them in glass in Europe. However, new packages are being developed, which combine the transparency of glass with the weight of tin cans. Plastics can be used to contain half preserved ('pasteurised') foodstuff with a shelf life of three months.

8.5 Promotion

Promotion can be regarded as the communication of the factors price, product and availability (physical distribution). An effective promotion campaign must first of all identify its targets; it depends on the chosen strategy for distribution to which group a message is sent out. Effective and efficient promotion can be as simple as contacting restaurants by phone whether they want to try a specific new mushroom.

A common way of promoting mushrooms to the general public is to provide recipes on the packaging. Extra value can be added when nutritional value and medicinal aspects are printed on the packaging.

Mass communication with advertising in newspapers or on television is too expensive for an individual grower, unless he controls the market. Farmers co-operatives may charge a fee per m² of growing surface or a portion of the spawn price can be withheld for this purpose. The farmers’ organisation may organise the promotional process and print recipes, leaflets etc. The growers can distribute them to their customers, or the central organisation can send them out to the target groups.

Another promotion activity is the organisation of excursions to farms for groups; ideally the grower can handle 40–50 visitors at a time. E.g. half of the group in a room with an exhibition or video explaining the cultivation of mushrooms, the other half of the group can visit the growing rooms together with the grower. At this occasion, the farmer can also sell a (small) part of his production to the visitors, and charges a fee for each visitor.

Effective though time-consuming are cooking demonstrations in supermarkets, restaurants or at a market. Especially in rural areas cooking demonstrations work well. Mind the social situation: don’t aim at the men if the women take care of the cooking.

Case study: Arunyik Mushroom Centre

In Bangkok a mushroom growing company started a remarkable project: the Arunyik Mushroom Centre. It has everything on sale that local mushroom growers need: cultures, spawn, substrates, chemicals for disinfection and equipment like sterilisation units and steam boilers, suitable for the local situation. In addition, the Centre sells books, products from fungi like foodstuffs, mushroom sauce, tea, medicines and of course several types of fresh mushrooms. The centre has different souvenirs for sale, and a monthly newsletter for their members. Next to the shop is an exhibition and a food shop. The visitors can see the whole process of mushroom cultivation. However, the food shop opens at irregular times, depending on the workload at the farm and the convenience of the staff involved.

The company started with limited means. This model could be followed by companies in other countries. They may serve to promote mushrooms and at the same time teach
visitors about the beneficial aspects of mushroom cultivation. No arable land is needed, agricultural wastes are converted into food and the remaining substrate serves as a soil conditioner. Many tourists find it fascinating to learn more about these subjects. A project like this requires a good staff, which can deal both with the general public as well as their members. The Buddhist staff under the leadership of Mr. Thaithatgoon has shown to be able to withstand economic hardships.

8.5.1 Trade names
Large companies (can) invest heavily in brands. In relation to another agricultural market, that of pineapples, it is interesting to note that Del Monte and Dole have managed to establish strong trade names. They invested not only in their brand, but also in quality, reliability and market communication. In relation to mushrooms, this dominance is felt less than in the above example. This may change however with the ongoing concentration in mushroom production. Green Giant is an example of a trade name which markets most if not all the production of the Dieng Djaja farm in Indonesia (see the case study in paragraph 9.6)

8.6 Latin America: an emerging mushroom market

By prof. Dr. D. Martinez-Carrera

Although mushroom cultivation, introduced to Latin America in 1933 from Europe, initially faced serious problems of basic knowledge, working capital, technical assistance, strain or spawn availability, and marketing strategies, several factors have made possible a remarkable development during the last 70 years:
- Cultural heterogeneity and broad ecological diversity have encouraged an increasing consumption of mushrooms as human food, tonic, or medicine.
- The development and adaptation of local technologies have increased commercial production by mushroom growers, involving training of mushroom farm workers.
- Mushroom cultivation has evolved and diversified into large-scale mushroom enterprises and small-scale rural production systems.
- Large private and public investments have been made in many countries in recent years.
- Regional research groups are now actively developing basic and applied scientific programmes, as well as promoting the exchange of technical information.

*Agaricus*, the common cultivated mushroom, and *Pleurotus*, the oyster mushroom, are the most important cultivated mushrooms in an estimated proportion of about 95% and 5%, respectively. *Lentinula*, the Shiitake mushroom, is commercially grown but its impact on the total yearly production is still very low (<0.5%). Commercial cultivation of these mushrooms has started and become established in temperate regions, at high altitudes (>1,300 m), where initial capital investment is low, no sophisticated environmental control is required for growing rooms, and various substrates are easily available. Several mushroom growers have recently started small-scale or experimental production of specialty mushrooms for the world market, such as reishi (*Ganoderma*),
wood ears (*Auricularia*), pioppino (*Agrocybe*), and enokitake (*Flammulina*). Diverse compost formulations are prepared for *Agaricus* production using regional raw materials. Most formulations are based on straw, regional grasses, corn stubble, sugar cane bagasse, horse or chicken manure, gypsum, and a variety of available supplements. Most farms of different countries make or import machinery for specific parts of the cultivation process. A variety of growing systems are used for *Agaricus* cultivation, such as wooden shelves, trays, plastic bags, and recently Dutch shelves. Black soil, peat, coconut husks, rice hulls, and palm fibre are used as casing material. Apart from commercial strains of *A. bisporus* (Lange) Imbach, other species have recently been brought into cultivation. This is the case for a tropical strain from the *A. bitorquis* (Quél.) Sacc. complex, capable of fruiting at 28 °C. Another introduced *Agaricus* strain producing brownish fruit bodies is becoming popular, and it is marketed under the name “portabella” or “portobello”.

Large-scale substrate preparation for the cultivation of oyster mushrooms involves an outdoor aerobic fermentation for a few days, and then pasteurisation in bulk with steam for several hours. Cultivation on a small scale is carried out using rustic pasteurisation methods by immersion in hot water. Main substrates used are straw, coffee pulp, corn stubble, cotton waste, tequila bagasse, as well as other regional agricultural by-products. Oyster mushrooms are cultivated using a variety of systems, such as cylindrical containers, plastic bags in shelves, stacked plastic bags with or without a central support, plastic columns, plastic bags in wooden shelves, and plastic bags laid down on the floor. Most growing rooms are simple, without sophisticated controls for temperature or relative humidity, and they have normally been adapted from other commercial activities to mushroom cultivation. Main cultivated species are European or American strains of *P. ostreatus* (Jacq. ex Fr.) Kumm.; *P. ostreatus* strain “Florida”; *P. sajor-caju* (Fr.) Singer; and occasionally pink varieties. Several strains are capable of growing under subtropical conditions.

Commercial strains of Shiitake (*Lentinula edodes* (Berk.) Pegler) from Japan, China, and South Asia are normally used to cultivate this mushroom in polypropylene plastic bags on a small scale. Growing substrates are prepared with a variety of formulations, based on hardwood sawdust, sawdust from fast-growing tropical trees, and other organic materials as supplements. Several attempts have also been made to produce Shiitake mushrooms commercially using wood logs.

Annual production has increased steadily in Latin America since 1945. During the period 1965-1974, mushroom production in this region increased 1,425%, from 400 to 6,100 tonnes/year. A smaller increase of 693.2% was reported during 1975-1995, from 6,300 to 49,975 tonnes/year. The estimated commercial mushroom production during
the period 1995–2001 increased 32%, from 49,975 to 65,951 tonnes/year, i.e. at a rate of about 5% per year. Due to this development during the last 30 years, Latin America, at present, produces about 1.3% from the total world commercial production of cultivated mushrooms.

Most production is commercialised in the fresh market, and only a small proportion is processed for further distribution. Many countries have high levels of imports to satisfy the local demand of fresh and processed mushrooms. It has been estimated that there is still a low mushroom consumption per capita in Latin America, being about 125 g per year. However, despite the lack of organised marketing strategies, there is a positive tendency towards higher mushroom consumption in most countries, such as Mexico where per capita consumption increased 275% during 1990–2001, from 0.112 kg to 0.421 kg.

Leading countries are Mexico (58.6%), Chile (17.6%), and Brazil (10.6%) accounting for 86.8% of total mushroom production. Mushroom cultivation brings about social, economic, and ecological benefits to Latin America. The total economic value for 2001 reached more than 167 million dollars per year, and about 34 thousand people work, directly or indirectly, for this activity. Around 656,796 tonnes of by-products from agriculture and forestry are recycled every year as substrates for mushroom cultivation. Globalisation is opening up new opportunities and bringing new challenges for the Latin American mushroom industry. This is the case of Mexico whose exports have increased irregularly since 1994, when free trade agreements began to be established with other countries, mainly from North America, Europe, and South Asia. Total exports in the year 2000 reached 1,602 tonnes, whereas a significant reduction to 345 tonnes was recorded for 2001. However, total imports, mainly of processed mushrooms, increased regularly at a higher rate reaching more than 6,531 and 5,109 tonnes in the years 2000 and 2001, respectively. Those countries whose mushroom industry is not yet well established will find it difficult to cope with these new competitive circumstances, as well as other external economic factors.

In a globalised world, the Latin American mushroom industry is trying to become more competitive and efficient, and is focusing on:

1) Expansion of commercial mushroom cultivation towards subtropical areas;
2) Further exchange of scientific/technical information;
3) Technology transfer;
4) Organisation of growers;
5) Marketing strategies to promote domestic consumption of fresh and processed mushrooms;
6) Regulations for local spawn makers;
7) Higher quality standards for fresh and processed mushrooms;
8) Regulations for health risks within farms;
9) Environmental regulations and a further implementation in mushroom farms of food quality systems and certification.

Considering present trends of mushroom cultivation in Latin America, its economic significance for the year 2007 has been estimated to be more than 251 million dollars. This means an overall mushroom production of 98 thousand tonnes, and a per capita mushroom consumption of 187 g. In this way, Latin America will certainly have a further contribution worldwide to mushroom production and marketing, sustainable agriculture, and rural development.

References

8.7 Marketing issues in the USA for Shiitake: the Chinese factor

By Lou Hsu

Even though Shiitake consumption in the USA is still relatively low compared to white button mushrooms, the production is even lower. This is caused by cheap imports from China, at a price lower than the local production cost. In some regions where restaurants favour guaranteed organically grown products, USA growers will have an advantage. Imported Chinese Shiitake mushrooms are not organic and need to endure a 30 day total trip from collection in China to wholeselling in the USA. Some ingenious methods have been developed to reduce the cost of using refrigerated containers, or sending by air. Shiitake with low humidity have a prolonged shelf life. Mushrooms are thus grown under very low moisture conditions under natural conditions outdoors. After collection, the mushroom moisture content is further reduced by drying them slightly under the sun. A special Shiitake strain was developed for this process by tissue culture over tissue culture of good strong mushrooms fruiting under dry conditions. The low moisture mushrooms are shipped in refrigerated containers in the summer, and non-refrigerated containers in the winter. These are used to increase “fresh” mushroom shelf life. The resulting mushrooms are not as soft as ‘normal’ fresh Shiitake, but have a longer shelf life, especially making the trip in winter. The quality does suffer because the mushrooms become very chewy, but they look good.

By understanding the competitors (Chinese exporters) the American growers can educate the USA public of the differences. There are people who prefer quality; others buy low-priced goods. The American public prefers fresh Shiitake and specialty mushrooms, but many Oriental people prefer dehydrated mushrooms, and rehydrate them before
cooking. It is questionable if the low moisture mushrooms will ever enjoy “fresh” status. The strategy of the USA growers is to grow more expensive but organically certified mushrooms. The fast ‘brown outside bag’ process (refer to chapter Shiitake cultivation on substrates) is favoured by most large growers. Many Shiitake growers in the USA have switched to organic certification and/or increased oyster mushroom production. Others have introduced specialty mushrooms such as the King Oyster (*Pleurotus eryngii*), maitake or *Grifola frondosa*, and *Agaricus blazei*.

### 8.8 Future market trends

When economies develop and markets mature, some trends can be seen worldwide:

- supermarkets and wholesalers demand year-round supply, which triggers the development of full climate control. This is currently happening in China, where production used to follow the seasons, but since 2000 several companies started fully climate-controlled operations for continuous production
- large buyers tend to concentrate and in the current situation of overproduction, the price growers get for their products is determined by the cost price and not by the price consumers are willing to pay
- niche markets offer more potential for a reasonable profit margin, but even here the supremacy of the supermarkets is felt
- market share of convenience products will grow
- growers are forced to make extra costs (e.g. Eurep/GAP certification) and have to deal with the rising costs (energy, labour); however, they are not in the position to demand a higher price for their products.

Occasionally, growers manage to establish a different outlet (see the paragraph on physical distribution).

A recent study by Rabobank (2002) predicts the forming of large agro-industrial clusters in Europe and the disappearance of smaller growers. The role of the auctions is thought to diminish, which makes price transparency small.
This chapter is written for extension and government officials who are considering to implement mushroom projects in their country. It contains the following paragraphs:

- determination of objectives of the mushroom project (social, local, financial),
- how to organise the various stages,
- research facilities,
- organisation of support,
- mushrooms and rural sustainable development in Mexico,
- case study of the largest mushroom farm in the world in Indonesia,
- case study: organisation of support and spawn in Fujian province, China,
- extension work in Shiitake mushroom cultivation in Thailand,
- case study: increased output from Polish mushroom farms.

### 9.1 Determination of mushroom project objectives

The objectives of any project have to be formulated first before further activities are undertaken. Possible qualitative objectives are:

- to reduce poverty in a specific region, e.g. mountainous countryside,
- to develop activities from which women benefit in a season when little other work is available,
- to contribute to sustainable agriculture by reusing agricultural wastes,
- to export mushroom products which bring in foreign currency,
- to improve the diet with relatively protein-rich food,

In order to be able to evaluate the project plans objectively at a later stage, these plans should also contain quantitative objectives, like:

- how many families/villages are expected to enrol in the project?
- what financial results are expected?
- how many tonnes of mushrooms will be produced?
- how many mushroom growing rooms will be built?

The next step is to consider who will be engaged in the various steps of mushroom cultivation, like spawn and substrate production.

### 9.2 How to organise the various stages

The following stages of mushroom cultivation have to be taken care of:

- providing cultures,
- producing spawn,
- producing substrate,
- growing mushrooms,
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- collecting mushrooms,
- optionally: conservation, e.g. canning,
- marketing.

These stages are essential to mushroom growing. The government could also plan facilities for research and extension, and financial services like credit or buy-back arrangements.

If mushroom growing in the public sector is completely new to a country, it is wise to cooperate in the beginning with local universities with microbiology laboratories. These could supply the initial amounts of spawn needed for experiments and a model farm. The choice which stages have to be performed by which parties depends strongly on the objectives of the mushroom project and specific situations. The most secure way is to provide individual farmers with a mushroom house, high quality full-grown substrate and buy-back arrangements. This may be impossible because of limited financial resources. Then the delivery of high quality substrate to farmers who should take care of building their mushroom farms themselves can be arranged. In some situations this may also be impossible, because of insufficient infrastructure or the lack of means of transportation. In that case the farmers should be supplied with a good quality spawn. In some cases almost all the stages are performed within the same company. The case study from Dieng Dijaya in Indonesia (see below) shows that only the cultures are provided by another (foreign) spawn company. The mushroom company is capable of performing all the other steps itself.

In Poland a project was implemented to increase the yield per individual grower. In this case three compost (substrate) producing facilities were built and the growers obtained a high quality compost. Spawn was provided by separate companies to the compost yards.

9.3 Research facilities

The government and the mushroom industry may jointly finance research facilities. Financing through the sector can be achieved if central spawn or substrate producers are willing to pay a percentage of sales to the research station. This station should perform experiments to continually improve cultivation techniques. Research might be directed towards new strains with higher yields, or which are more resistant to pests and diseases, but also towards decreasing the environmental stress, associated with the mushroom growing process.

In order to deliver optimal results, the research station should constantly be aware of the problems of farmers and try to predict what kind of long-term research could be beneficial to the sector. There is often a troublesome relationship between farmers and research stations. In part this is due to the farmers’ desire for results on the very short term conflicting with the research station having to plan more on a longer term. Farmers often complain that the research station doesn’t help them with their problems, and the station complains that farmers do not follow their guidelines. Extension services should fill the gap between both, but this is rarely the case in developing countries for lack of funds and infrastructure. The following fields should be profitable for investigation by local research stations:
development of new substrate production techniques: (e.g. the semi-bulk pasteurisation method developed by the Fujian Institute of Light Industry, described as a case study in the chapter *Cultivation on a fermented substrate*, or the way coffee pulp is rendered suitable by immersing it in hot water in Mexico, as described in the chapter *Cultivation on pasteurised substrates*)

development of new strains and species: at present few strains are suited for cultivation under tropical conditions. Strains can also be selected for high yields, good quality, good keeping abilities, etc.

testing different substrates: organic materials differ from location to location. Even the same material, e.g. wheat straw, differs from country to country, depending on the climate, soil, fertilisers used and processing methods. The research station should find out which substrates give the best results.

determining optimal growth conditions for various species: The farmers should receive clear instructions on how to cultivate the different kinds of mushrooms. The research station should have the facilities to test which conditions suit the different species best.

optimising climate control systems or substrate treatment with appropriate technology: Financial resources are always limited, especially in developing countries. The techniques should be adapted to fit the local situation. Locally available materials are much cheaper than imported climate control systems.

pest and disease control

performing regular cost/benefit analyses for the sector: These analyses will reveal which factors deserve most attention from a commercial point of view. The research station could also investigate other applications of mushrooms, e.g. as enzyme producers, for soil bioremediation, or for dyeing fabrics.

9.4 Organisation of support

In the ideal situation the extension service can support or initiate mushroom growing activities by:

- setting up model farms,
- supplying reliable information to the target group,
- organising reliable spawn production or high quality mother cultures or mother spawn for small-scale spawn manufacturers,
- solving the cultivation problems of the farmers, which may include pest and disease management, increasing the yields, monitoring the crop, substrate preparation etc.,
- organising marketing aspects of the product, like grading, quality control, contracts with canning factories, promotion,
- organising financial aspects like credit,
- taking social aspects of the implementation into account: for example, who is reached by the extension service, does the scale fit the local tradition, are the farmers really interested in the project or is it seen as imposed from above?

This ideal situation rarely exists in developing countries, nor is it perfect in developed countries. Sometimes the extension workers are poorly paid and will try to get a commission, for instance from a pesticide company. They will then advise the farmers to
spray heavily. Often they do not look at mushroom growing from the farmer’s point of view, but only try to improve their own situation. Much depends on the personality of the extension officers. If they are really interested in improving the farmer’s position, they still have to cope with the farmer’s limited resources. Farmers will notice when an officer really cares about their lives and will listen better to such a person. On the other hand, the extension officers should listen to the real problems of the farmers and try to solve them or to redirect the problems to the research station. The government can support good extension services not just by funding them, but also by evaluating them through the eyes of the target group: the farmers. Another way is to offer extension officers an incentive if they do their job well. This can be reached if they organise sales and receive a percentage of it. Another way would be to make arrangements between the grower and the extension officer: the higher the yield, the more the extension officer gets paid.

9.5 Mushrooms and rural sustainable development in Mexico

By prof. Dr. D. Martinez-Carrera

More than 50 ethnic groups or “mestizo” peasants which are settled in different regions across this country, form diverse rural communities. Many communities have been there for centuries, and have developed strong cultural traditions and systems of adaptation, not just to manage their regional natural resources in a sustainable way, but also to take advantage of technology transfer programmes. Social and applied research work is being carried out to show that wild and cultivated edible mushrooms can promote sustainable development in rural communities. Strategies based on continuous research and assessment have been developed to achieve this.

9.5.1 Development of the Rural Household System (RHS) model

To understand the context in which mushroom gathering and/or cultivation can be carried out in a rural community of indigenous people and/or peasants, a model called the rural household system (RHS) was developed. The RHS is a concept to assess the rural household unit and its affairs as a system. This model of analysis shows how Mexican communities, associated to primary activities within rural regions, can be divided in different types of RHSs. Most RHSs have developed subsistence strategies to cope with their own daily living under conditions of poverty. These sustainable strategies are complex processes involving social, ecological, and economical factors. The RHS interacts directly or indirectly with community, regional, and/or global systems. RHSs are normally integrated by three main subsystems:
1. Family members (organisation, life circumstances);
2. Conventional agricultural activities (crop plants, livestock);
3. Extra-agricultural activities [utilisation of the forest (firewood, medicinal plants, various timber and non-timber products), gathering of wild edible mushrooms, mushroom cultivation, the making of produces (coal, bricks), trading, labouring].

One or several family members from most RHSs are capable of gathering or cultivating edible mushrooms as an extra-agricultural activity for:
1. obtaining monetary (money provided by the main RHS activities) income,
2. complementary (money provided by supplementary RHS activities) income, or
3. potential (not monetary but satisfying RHS needs, such as food crops cultivated for own consumption) incomes to satisfy their basic household needs.

The model permits to assess:
1. Socio-economic conditions of the community;
2. The level of organisation of RHSs in the community;
3. The potential capacity of a RHS to perform the gathering of wild mushrooms and/or the cultivation of edible mushrooms, as well as their processing;
4. The relative importance that these activities (mushroom gathering, cultivation, and processing) may reach within the RHS, community, and/or region; and
5. The potential level of RHSs for undergoing extension work and/or technology transfer.

9.5.2 Strategy for wild mushrooms
The traditional management of wild edible mushrooms by Mexican rural communities follows different strategies, depending on the ecosystem management philosophy in the developed regions. Traditional gatherers in Mexico belong to a local culture, normally represented by indigenous people and/or peasants. They make use of remote communal forest regions lacking good facilities and infrastructure. Community organisation permits the use and management of timber and non-timber forest products by RHSs. These gatherers actually live within forest regions, and their knowledge is passed on from one generation to another. They are mainly devoted to primary activi-
ties (conventional agricultural and extra-agricultural), and there is a division of labour within the RHS. Men and their sons are normally involved in mushroom gathering, while women are usually devoted to household activities, such as cooking, selecting, cleaning, and marketing of wild mushrooms. This situation, however, appears to have recently been changing, as most adult men in rural communities are moving or emigrating towards urban areas or foreign countries, looking for better opportunities. Women are thus getting more involved in agricultural and extra-agricultural activities (e.g., mushroom gathering) performed outside their house.

Mushroom gathering is mainly associated with other gathering activities. Rural communities traditionally use more than 112 different species of wild mushrooms from their communal fields and forests during the rainy season. The average period devoted to mushroom gathering by RHSs is 31.5 days per year. An adult peasant can pick about 6.3 kg of wild mushrooms in a typical journey (8 hours; 21-26 km in a day, on foot). These data indicate that the ecological impact of mushroom gathering is also highly heterogeneous, as Mexican gatherers traditionally visit 2-10 different places in a day, picking around 0.5-6.0 kg at each site when mushrooms are found. It has been estimated that mushroom gathering represents up to 19.2% of the overall income obtained from agricultural and extra-agricultural activities.

Two critical factors for the future of traditional mushroom gathering are:

1. To avoid over-exploitation and forest decline, and
2. To keep the overall activity sustainable in a globalised world.

Social, economic, and land tenure systems prevent rural communities and government authorities from establishing conventional measures for mushroom conservation (e.g., to define forest reserves, to close forest regions to the public, the control of picking, and the legal protection of edible species gathered). In addition, the quality of the harvested fruit bodies is a serious limitation for the marketing of wild mushrooms, as they are normally bruised, unclean, partly broken, and irregular in size. The stalks and caps are often associated with larval damage from insects; and they undergo rapid deterioration after picking. Many wild mushrooms collected are unfortunately lost before marketing, as commercial quality standards are not reached.
Therefore, alternative strategies are being developed for the traditional management, processing, and marketing of wild edible mushrooms in rural communities. These strategies are based on:

1. The establishment of management and conservation principles through community organisation;
2. Training to improve the whole traditional process of mushroom gathering considering the complex social, economic, and ecological factors involved; and
3. The transfer of processing technologies (freezing, drying, canning, controlled atmosphere packaging) to avoid high mushroom losses and to increase RHs's incomes (monetary, complementary, potential).

Studies are being carried out in central Mexico, where matsutake mushrooms [*Tricholoma magnivelare* (Peck)Redhead] are gathered commercially by differing rural communities since the middle of the 1980's. Several advantages of processing wild edible mushrooms are:

1. Fruit body quality is standardised;
2. Urban consumer’s reluctance to eat wild mushrooms is diminished, as they will trust mushrooms, processed according to international regulations more than fresh wild mushrooms;
3. Mushrooms are available throughout the year;
4. Good recipes may highlight certain culinary, nutritional, and medicinal properties of mushrooms;
5. The market will be bigger because the retail prices can be lowered;
6. Still, the value added to wild mushrooms is increased for those who harvest and process them, as they can operate more efficiently and also process second grade mushrooms;
7. National and foreign private enterprises can be involved in commercialisation;
8. Management and conservation practices can be established to regulate commercial picking and to avoid forest decline and over-exploitation;
9. Jobs and profits can be generated within rural communities.

However, several strategic issues have to be considered, too:

- A correct and reliable identification of wild edible mushrooms. The local traditional knowledge available in rural regions is important to achieve this goal;
- Mushrooms may lose their natural taste and aroma during processing;
- Several wild mushroom species are preferred fresh by consumers from some countries;
- Financial and technical assistance is required to reach high quality standards of the processed product;
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- Long-term ecological research is going to be established for assessing the impact of commercial harvesting on natural mushroom production, and to find possible methods for increasing natural yields.

9.5.3 Strategy for cultivated mushrooms

Since 1989, intensive basic, applied, and social research work has permitted exploring a new approach of mushroom cultivation: rural production for satisfying regional needs. This was originally carried out with a targeted and representative social group in Cuetzalan, Puebla, Mexico. This led to the development of a model for technology transfer which brought about the possibility to incorporate mushroom biotechnology in rural development. It has now been shown that the following technologies of mushroom cultivation and marketing can be carried out under rural conditions:

1. Spawn preparation,
2. Mushroom production,
3. Processing (canning).

Financial data demonstrated that the model is applicable and viable, and has an impact on local mushroom consumption.

The model of rural production consists of a central farm(s) cultivating and/or processing mushrooms commercially for the local and/or regional market. This farm(s) should have the capacity to produce and distribute high-quality spawn for mushroom production in rustic farms or for domestic cultivation. Spent substrates are composted (natural composting or vermi-composting) and recycled as organic fertiliser or soil conditioner in the fields.

This model is being implemented in selected regions, considering four critical factors:

1. financial and technical support,
2. community organisation,
3. local tradition to consume wild edible mushrooms,
4. availability of regional agricultural by-products.

Thus mushroom biotechnology brings about social (improvement of local diet; participation of peasant women in the production process; organisation), economic (incomes, labour opportunities), and ecological (efficient use and recycling of agricultural and forestry by-products) advantages when incorporated to rural development. Mushroom cultivation at commercial, community, and own consumption levels, is now being integrated into the development of improved systems of traditional agriculture, without affecting the environment and social values of indigenous and peasant communities. In general, the rural production of edible mushrooms is performed...
through rustic cultivation methods on a large or small scale. Mushrooms are sold directly in the local fresh market or even processed further to reach international markets.

Fundamental trends of rural cultivation show that mushroom biotechnology can be adopted and adapted to the RHS needs, keeping a relative balance with other agricultural and extra-agricultural activities. Mushroom cultivation provides incomes, labour opportunities, and food to the RHS, encouraging household members to remain in the community. Three types of RHSs have been defined on the basis of the level of integration of mushroom cultivation within the RHS: 1) Constant grower (CG): mushroom cultivation is established as the main extra-agricultural activity, leading to the reduction or displacement of less profitable or less important productive activities; 2) Frequent grower (FG): mushroom cultivation is integrated proportionally to the rest of agricultural and extra-agricultural activities; and 3) Occasional grower (OG): conventional agricultural and extra-agricultural activities remain, and mushroom cultivation is carried out occasionally. According to this typology, three regions have also been identified for RHSs located around the main urban centres of mushroom consumption: 1) Near the market, 2) Intermediate distance from the market, and 3) Far from the market. Rural mushroom cultivation is therefore evolving directly associated to main mushroom markets. Accordingly, those RHSs operating as CGs are normally located near the market, FGs are at an intermediate distance from the market, and OGs are far from the market. Depending on this location, RHSs take advantage differentially from the social, economic, and ecological aspects of mushroom cultivation. Governmental programmes for rural development are driving this differential impact through the financial support to RHSs for developing projects of mushroom production and processing which highlight the economic impact (CGs, FGs), or for social projects against poorness in remote regions (OGs). At present, domestic markets are centralised and demand fresh mushrooms as the main commercial product which is highly perishable. Most RHSs in the country can only carry out mushroom production on a low scale for the fresh market due to their limited socio-economic conditions and capacity for marketing. Their impact is mainly observed within the community system (Fig. 6). RHSs will gradually have a further impact on the regional and global systems.

9.5.4 Prospects in a global context

Future development of wild mushroom gathering and rural mushroom cultivation in Mexico is being promoted thoroughly by marketing strategies oriented towards a significant increase in the national consumption of fresh and processed mushrooms (cooling, vacuum cooling, freezing, controlled atmosphere storage, canning, drying). High standards of mushroom quality will then be required by the market. These factors will lead RHSs to evolve dynamically and economically, mainly those located far from the main mushroom markets, thus promoting rural sustainable development.

References


9.6 **Case study: Dieng Djaja, Indonesia**

One of the biggest mushroom producers in the world can be found at the Dieng plateau in central Java, Indonesia. It produces and cans 40 tonnes of Rice straw mushrooms and 100 tonnes of White button mushrooms a day. The producer developed its own method to prepare the compost, using sugarcane bagasse and chicken manure. The company is nowadays of tremendous importance to the region because it provides not only work to farmers directly engaged in mushroom growing, but also offers local farmers the possibility to grow other crops, which can be fertilised with the spent compost from the mushroom growing process. Without fertiliser, these crops would be impossible to grow on the plateau, which is very unfertile and poor in organic matter due to the volcanic nature of the region.

Many problems had to be solved before the company grew to its present size, employing 10,000 people directly and a multiple of that figure indirectly.

**Specific situation:** In the beginning there was no electricity, almost no roads, and no water supply. The main advantage of the plateau is that the temperatures are relatively low compared to other locations in Indonesia. On the other hand, all the substrate ingredients and raw materials for canning have (had) to be transported by trucks to the plateau. The roads are winding through the mountains and often it takes more than an hour to progress only five km! Logistics are also important in the transport of the employees to the farm. Each day 2000 people have to be picked up from the villages surrounding the company and brought back at the end of the day.

Another disadvantage is that it is very hard to find a flat piece of land. A lot of excavation work had to be performed before growing houses and composting yards could be built. A suitable casing soil (peat) is not readily available. Therefore soil from Lake Ambarawa is used, but this has a relatively high nutrient content and its water-holding capacity is much lower than that of peat. The local population is in general poorly educated and has little idea on what hygiene really means. Despite all these problems, the company managed to grow to its present size in twenty years.

**Organisation:** The spawned substrate is partly sold to contract growers and partly used in the facilities of the mother company. The contract growers tend the mushrooms
and harvest them when they are still rather small, at most 3.3 cm to meet the strict requirements of the main buyer, Green Giant. They then sell the mushrooms back to the mother company which cans the mushrooms. The company arranges the marketing of the canned goods and trains the growers on how to take care of their farms. The contract is set up in such a way that the farmer owns his farm after seven years. He can still obtain the compost and the casing material and deliver his product back to the mother company after that period. Although his farm is small (producing 20,000 kilos a year) compared to high tech farms in the Western hemisphere, it is a profitable business in the region.

Social aspects: Many people in the area would like to become a contract grower, because this would obviously lead to a steady income and a nice house. The wages of contract growers are thrice as high compared to people who have a paid job. Farmers who wish to enrol are thoroughly screened. They may not have more than two children and they should be fit to work. The population in Indonesia is growing at a rapid pace and Java is already one of the most crowded islands in the world. The government thus promotes two children families with slogans like DUA ANAK CUKUP, meaning: two children are enough. The ordinary people often add a line: TIGA BOLEH, meaning: but three is also allowed. Rich people can afford to have more wives (and thus more children) on this Islamic island. Spending the money is not easy in this remote area, therefore some contract farmers wanted to take an extra wife. The company decided, however, that they should pay back the loan first and only then marry once more.

9.6.1 Case study: Agaricus production in Fujian province, China
One of the main producers of Agaricus in the world is the province of Fujian in China. This is an interesting area to study, because the production is achieved by tens of thousands of small farmers on the countryside with limited resources.

The starting point for the spawn is the Fujian Institute of Light Industry. The cultures are tested here for the presence of dieback disease (a virus) and when found to be free of viruses, they are dispatched to the branch stations. These will multiply the cultures and sell them to local spawn manufacturers. These in turn will provide the thousands and thousands of growers with spawn. Selling other cultures or spawn than provided by the provincial institute is illegal. In the past the source of spawn was uncertain and growers would like to grow high yielding varieties most. The canning factories (usually located near the branch stations), however, need consistent quality.

Therefore only high quality cultures which have a reasonable yield are distributed. The farmers construct relatively simple growing rooms, and prepare the compost as described in the case study in the chapter Cultivation on fermented substrate. The farm-
ers harvest the White button mushrooms and sell them to the collecting stations for canning.
Knowledge is also transferred according to the organisational structure shown in the figure. The production per m² is still rather low, but the production will rise rapidly with increasing technology transfer and more suitable strains. The Fujian Institute of Light Industry is constantly performing breeding experiments and adapting locally available techniques.

9.7 Increased output from Polish mushroom farms

White button mushrooms have been cultivated in Poland from 1900 onwards. Before World War II, the spawn originated from France and was imported via Germany. After the war mushroom growing was stimulated by the government by funding a spawn factory and publishing a handbook for mushroom growing. Mushroom growers have always been private companies in this communist country, but the taxation level has not prevented the mushroom sector from developing. Since 1961 mushrooms were exported, first to Austria and later to Germany. From 1981 onwards, the Dutch processors discovered the quality and low price of the Polish mushrooms. Export rose from 10% of the volume to 80%. The golden era for the mushroom sector had started. In 1991, however, the World Bank imposed strict conditions on Poland, which resulted in a sharp increase in the price of energy and other commodities. The processing industry turned out to be unprofitable. Fortunately the domestic market then accepted a reasonable price for the fresh mushrooms. Still, production dropped from 95,000 tonnes in 1990 to 65,000 tonnes in 1992 due to increased production costs.

9.7.1 Strategy for reconstruction of the mushroom industry

The Department of Edible Fungi at the Institute of Vegetable Crops initiated a strategy to reconstruct the mushroom industry. The strategy was worked out with a group of farmers and started with a strength/weakness analysis:

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weak points</th>
</tr>
</thead>
<tbody>
<tr>
<td>a strong tradition in the cultivation and production of edible mushrooms</td>
<td>relatively low yields per tonne of compost</td>
</tr>
<tr>
<td>abundant supply of raw materials</td>
<td>lack of sufficient quantity of high quality compost</td>
</tr>
<tr>
<td>available energy sources (coal)</td>
<td>incorrect pasteurisation by the farmers led to infected substrates (with a.o. nematodes)</td>
</tr>
<tr>
<td>low labour costs compared to adjacent European countries like Germany</td>
<td>low profitability due to increased production costs and low yields</td>
</tr>
<tr>
<td>mushrooms were usually produced seasonally</td>
<td></td>
</tr>
<tr>
<td>existing infrastructure with mushroom growing rooms</td>
<td></td>
</tr>
</tbody>
</table>
From the analysis it was concluded that the main problem was the quality of the compost, which was difficult to prepare because of limited resources of the farmers. By performing this step in tunnels, the process could be optimised. Filling the beds properly was also difficult in the old situation. By using compressed rectangular blocks with spawned compost, which has been produced under optimal conditions, the main problems could be solved.

In April 1992 the production of the compost blocks started at three locations: in the western, southern and central part of Poland. The size of the produced blocks was established in such a way that the existing shelves, which ranged from 1.2 to 1.6 metre width, could all be used. A room with 200 m² of growing surface can be filled in 16 hours. The growers remove the upper part of the plastic and replace it with paper. After 14 days the blocks are ready for casing.

**Extension:** Intensive training courses were set up for the growers to adopt the new technology before the first blocks were produced. Good growers imported compost blocks to gather information about yields and how to handle the blocks. The increase in price also had to be discussed with the growers. They used to pay US$ 30 for a tonne of green compost, the price of a tonne of spawned compost in blocks was US$ 77.5. The increase in price was due to compost loss during the heat treatment (27%), the heat treatment itself, spawn, plastic, and labour costs. The growers didn’t have to pasteurise their growing rooms any more, which would increase their lifetime. The yield would increase from 15 to 25 kg per m² and the variable cost per kg of mushroom decreased from US$ 0.71 to US$ 0.42. Therefore the profitability of the private mushroom farms increased considerably. The investment in compost block production units with tunnels was valued at about US$ 700.000. This investment has created additional production worth US$9,000,000 annually.

This case study shows that the improvement of a single, crucial step can considerably increase the total output.
Ten years later, the mushroom industry in Poland has developed enormously. Dutch mushroom growers invested heavily in tunnels and production centres and brought much expertise to the country. Polish mushrooms have a high market share in one of the biggest markets, Germany. The picking costs are dramatically lower compared to other European countries. The quality is not always as consistent compared to the products from the Dutch mushroom industry, however.
10 Practical aspects of spawn production

Basically, spawn production is nothing more than putting mycelium of the desired mushroom in suitable sterilised substrates under aseptic conditions. The starting culture can be made from a fresh and healthy fruit body or obtained from a type collection. From this starting culture more agar cultures are made. These serve to inoculate larger containers (like bottles) with spawn and these can be used to inoculate the final spawn substrate. However, in practice, spawn producing is not that simple. Suitable strains have to be developed and tested first, which takes several years. Then they have to be maintained under strict conditions to avoid degeneration. Furthermore, the production room has to be kept meticulously clean to avoid any contamination. When the mycelium has colonised the spawn substrate, it has to be kept and delivered under cooled conditions. If high quality spawn of the desired strain can be obtained at a reasonable price, it is wiser to concentrate on the mushroom growing process. If not, spawn has to be produced or multiplied at the farm.

This chapter discusses in detail how spawn is produced. It contains the following paragraphs:

- Requirements: sterilisation units, clean rooms, incubation rooms
- Cultures: preparation of media, tissue cultures, sub-culturing
- Spawn containers: different types of bags, glass bottles, plastic bottles
- Preparation of spawn substrates: mother spawn, preparation of final spawn, grain spawn, wooden stick spawn, sawdust spawn, Volvariella spawn substrate
- Quality control: contamination vectors, case studies: Malaysia and Fujian province, China
- Figures on a small-scale spawn laboratory
10.1 Requirements

The minimal requirements for a spawn production unit are:

- a sterilisation unit (pressure cooker, autoclave),
- sterile environment: inoculation box, laminar flow cabinet or clean room,
- incubation rooms,
- laboratory equipment like petri dishes, test tubes, scales, alcohol, flame. The raw materials include:
- elements for media preparation,
- substrate material (grain, wooden sticks, sawdust, or even oil palm fruit fibre),
- spawn containers.

10.1.1 The sterilisation process

The goal of sterilisation units is obviously to sterilise substrate materials. Grain, sawdust, or compost contain large numbers of contaminants. A single grain kernel may contain an estimated 100,000 bacteria, 25,000 fungi and more than 400,000 actinomycetes. Each single one of these contaminants is capable of spoiling substrates which have not been properly sterilised. Two groups of organisms are most common in spoiled spawn: bacteria and lower fungi. Most spores of fungi are effectively killed at temperatures around 100 °C. Their occurrence indicates unhygienic practices rather than poor sterilisation. Bacteria are more resistant to heat. Especially the endospore-forming bacteria survive treatments below 121 °C. This means that sterilisation has to be performed under an overpressure of at least one atmosphere.

Theoretically, 15 minutes at 121 °C should be sufficient to kill all organisms. It takes time for the steam to heat the inner core of substrates to the desired temperature, depending on the way the sterilisation unit is filled and on the heat capacity and conductivity of the substrate. The initial spore count in the substrate further determines the sterilisation period: substrates with lots of contaminants (like compost) need a longer sterilisation time than agar media from almost sterile ingredients. The volume of the spawn containers is another factor which determines the sterilisation period. The discussed sterilisation units all use steam to sterilise substrates. Other ways to sterilise substrates, like gamma-radiation, or microwave radiation are rarely used in substrate preparation and are therefore not discussed in this book. Depending on the scale of operations, either pressure cookers, laboratory autoclaves or industrial autoclaves are used.

10.2 Sterilisation units

10.2.1 Pressure cookers

The cheapest option is to obtain one or more large pressure cookers. Select pressure cookers which maintain the pressure if the final temperature is reached. The most simple pressure cookers blow out steam when the pressure is too high; often the pressure inside will drop below 1 atmosphere overpressure, causing media to boil. Petri dishes with agar media may become a mess if this type of pressure cooker is used. The pressure cookers should be delivered with an inside rack; this will effectively ensure a more even temperature distribution inside the pressure cooker. The heat source is ei-
ther external (gas burners) or built-in (electric). The advantage of pressure cookers with thermostatically controlled electric heating elements is that they allow for precise temperature regulation. The main disadvantage of pressure cookers in general is their limited capacity. Even a large pressure cooker cannot contain much more than 10 litres of spawn at a time. If four batches a day are run, only 200 litres of spawn can be produced per week with a single unit.

10.2.2 Laboratory autoclaves
Laboratory autoclaves are appropriate if limited amounts of spawn are required. Autoclaves for laboratory use can process 100 to 300 litres of substrate in one batch. Hospital autoclaves belong to this kind of autoclave; they are rather expensive if bought new because they can operate under higher pressures than needed for mushroom cultivation. They are usually heated by a built-in electric heating element. This means installation is simpler than for large industrial autoclaves, which have to be heated by a separate steam boiler. There are two types of laboratory autoclaves: horizontal or vertical. Vertical are more comfortable, because they can be filled easier. They usually have only one door, which means that both sterilised and fresh (dirty) material have to be moved within the same room. This increases the risk of contamination. Still, one door autoclaves can perform well if hygienic conditions in the spawn production unit are considered carefully. The best option is to have an autoclave with two doors. In that case, the autoclave is filled at the ‘dirty’ side, and the opposite door is opened in a clean room after sterilisation.

10.2.3 Industrial autoclaves
If several tonnes of spawn (or sterilised substrate) have to be produced each week, industrial autoclaves (retorts) are the best option. Industrial autoclaves range from about 1 metre diameter and 2 metres long to 2 metres diameter and 12 metres long. They are typically constructed from steel pipe. There is no need for stainless steel, because this would dramatically increase the price. Carbon steel is sufficient. At times retorts can be obtained cheaply from an old canning industry. If secondhand retorts are made of stainless steel and can be purchased at a reasonable price they are very nice to work with. New autoclaves are rather expensive. Ideally they are fitted with two doors to decrease the risk of contamination. All modern spawn manufacturers use autoclaves with doors at both ends. Check the following options when purchasing an autoclave:
- is the capacity sufficient for the desired production?
- does it comply with local legislation concerning steam under high pressure?
does it have steam spreader pipes to distribute the steam evenly? (necessary in autoclaves larger than 1.5 metres)
what kind of doors are preferred? Quick opening but more expensive spider doors, or wing-nut knock-off doors, which can be cheaply fitted on steel pipe?
does it have two doors? and if so,
does it have a one way valve to allow for the drawing in of clean air from the clean room only?
does it have a drain valve for drawing off condensate?
what material is it made of? Has anti-corrosive paint been applied if it is not made out of stainless steel?
does it have safety blow out valves?
does the autoclave have a valve on top to vent cold air?
does it have ports for exhausts, inputs and sensors?
The steam is provided by a steam boiler. The capacity of the boiler should be large enough to heat the contents of the retort quickly. A boiler with a slightly higher capacity may be able to provide two autoclaves with steam. The highest energy input can be expected in the beginning of the sterilisation process, after a while only some steam is necessary to keep the pressure. Another autoclave can then be heated with steam from the same boiler. The complete installation (boiler, pipes and autoclave) should be tested regularly for safety; leakages in the system have led to explosions due to the high pressure involved. At least one Chinese mushroom scientist has been killed by operating an unsafe autoclave.

10.3 Clean environments
A clean environment is absolutely essential to spawn production. Especially the stages at which the containers with sterilised media are opened have to be performed under aseptic conditions. The air carries numerous contaminants, which easily infect the sterilised media. It is hard to imagine the number of particles in the air. Typical clean desert air values are 200,000 particles per cubic foot (3,800,000/m³). In industrialised areas these values raise tenfold. A single smoker will raise the number of particles indoors a hundred times. Not all of these particles are hazardous to mushroom cultures; for instance those consisting of clay, silica or decayed biological material. The spores of various micro-organisms, however, pose a threat to sterile media. Most of them fall in the size range of 0.4 to 20 micron. They also adhere to larger particles in the air. Only viruses are much smaller, 0.05 micron, but these usually stick to larger particles.
It is almost impossible to create a completely sterile environment. As long as the degree of contamination is below a certain level, the sterility of the environment is adequate. The spawn producers in industrialised countries tolerate very low levels of contamina-
tion, whereas a reasonable fall out percentage for tropical countries (with limited resources and a high infection pressure) is 5%. Three ways to create a clean inoculation area are described here:

- simple inoculation cabinets for small-scale production;
- laminar flow cabinets for medium-scale production;
- clean rooms if more funds are available and a higher output is expected.

### 10.3.1 Inoculation cabinets

These simple inoculation cabinets are widely used all over South-East Asia. They can be constructed cheaply from locally available materials. The front glass can be opened to fill the cabinet with the sterilised media. The inside is disinfected by a mist of 70% ethyl alcohol and 10% chlorox solution. Another way to sterilise is to use potassium permanganate in a formaldehyde solution. The gases will thoroughly sterilise the interior of the cabinet. Take care with these chemicals: formaldehyde e.g. is poisonous and may cause cancer (at a temperature of above 10 °C and sufficient fresh air, it will degrade within 12 hours).

Ultraviolet (UV) lights can be used in addition to the chemicals. The short wavelength radiation of UV light kills most spores. It is, however, difficult to place the lights in such a position that no shadows are cast in a room. Put them out as soon as you start to work, otherwise in the long term the UV light may cause skin cancer. It is not sufficient to sterilise only the inside of the cabinet. The direct environment should also be kept absolutely clean. The floor and the walls should preferably be of cement.

### 10.3.2 Laminar flow cabinets

A laminar flow system consists of a fan, a duct, filters and the laminar flow hood. Laminar flow has the advantage that contaminants can spread in only one direction. In turbulent airflow it is possible that spores move in different directions, causing more contamination. A laminar flow cabinet will be sufficient in most cases. The filter size must match the high pressure fan. The ventilators are rated by the producers according to the volume of air they can blow through materials of specified resistance. About 0.45 m/s air velocity is considered best for a good laminar flow. The fan should be able to push sufficient air through the filter to reach the required air velocity. In fact, the capacity of the fan should be a little higher to account for pressure losses and higher static pressure of the filter when it becomes loaded with particles. HEPA (High Efficiency Particulate Air) filters are discarded when their resistance doubles. If a pre-filter
is installed, the lifetime of the HEPA filter is generally as long as that of the complete clean room. Try to obtain furnace filters as pre-filters: they are generally inexpensive and easily available in many sizes in Western countries. A simple cloth can also serve as a pre-filter.

The initial resistance of HEPA filters is high, so the pressure will rise significantly in front of the filter. The filter has to fit the housing very well, otherwise impure air is blown into the transmitted air stream through imperfectly sealed seams.

The efficiency of a filter is a measure of how well a filter removes particles of a given size. If a filter is rated 99.99% for 0.3 micron particles, then only one out of every 10000 particles of that size will pass the filter. This is the typical value for HEPA filters. There are even more efficient filters, like ULPA (Ultra Low Penetration Air): 99.9999%, VHSL: 99.99995% and MEGA: 99.999995%.

HEPA is generally sufficient but some laminar flow cabinets contain ULPA filters. The filters are the heart of any laminar flow system, but other factors have to be considered too: the people and their hygiene, the construction of the ducts and filters in such a way that no spurious air can be sucked in. Laminar flow cabinets can also be bought ready-made. The advantage is that tests have been carried out to detect leaks, so it will definitely provide clean air. The disadvantage is that the price of it is much higher than a self-made laminar flow cabinet.

10.3.3 Clean rooms
If large quantities of spawn or substrate have to be inoculated, then one may choose to build a complete clean room, with either an open plenum or an individually designed system of ducts. A laminar flow ceiling can effectively keep contamination low: spores from workers’ shoes stay close to the floor and are swept outside, without coming into contact with the sterilised media. The atmosphere in this kind of room is generally exchanged 10 to 20 times per hour. The overpressure will keep contaminants out, especially if an anteroom has been constructed to act as a barrier. Consult the supplier of filters for specific designs. It is more difficult to obtain a real laminar flow in a room than in a cabinet. Non-laminar flow clean rooms will do also, if the cleanest air is guided along the work place. The air velocities in non-laminar flow clean rooms should stay between 0.15 and 0.45 m/s. Lower velocities allow contaminants to spread, higher velocities allow contaminants to accumulate in areas where turbulent air causes dead air pockets.
10.3.4 Incubation rooms
The inoculated spawn substrates have to be incubated for about two weeks for grain spawn to two months for sawdust spawn. Small amounts of spawn, slants or petri dishes can be kept at a constant temperature in an incubator. These cabinets hold a constant temperature and the more expensive types regulate CO₂ as well. Their main advantage is that they can be well controlled. When larger amounts of spawn have to be produced, incubation rooms have to be set up.

10.3.5 Design of incubation rooms
The interior of an incubation room should consist of non-biodegradable materials. It should be easy to clean, with smooth surfaces all over the place. If paints are used, non-mildewing enamels are preferred. Do not use paints which contain fungicides, as these are usually very toxic. A constant temperature is essential to incubation, so the rooms should be insulated well.
Shelves should be designed in such a way that the floor beneath them can be cleaned easily. They are typically made of galvanised iron or Formica. If large spawn containers are used, the shelves should be open to dissipate the generated heat more easily. Alternatively, the spawn containers can be placed on carts. In that case the carts are simply pushed from the clean room (where they are filled) to the incubation room. As soon as the mycelium has grown all over the spawn substrate, the spawn containers are checked and packed. The spawn is then kept in a chilled room until shipment. The size of the room of course depends on the desired capacity. The capacity of the incubation room is determined by the spawn run period and the available surface (of the shelves or carts). Around 60 kilos (90 litres) of spawn can be placed per m² if the bags have to be placed apart. The size and type of bags/bottles determine how much spawn substrate exactly can be placed per m².
For example: if eight shelves of each 5 m² are available, then 450 litres of sawdust spawn (with an incubation period of eight weeks) can be put in the incubation room each week. If grain spawn with an incubation period of two weeks is produced, 1800 litres of substrate can be put in the same incubation room each week.

10.3.6 Climatic conditions in incubation rooms
Temperature: The main climatic factor which has to be considered during incubation is temperature. Inside large spawn containers (e.g. 5 or 10 litres) the temperature will be higher than room temperature. The thermostat has to be set a few degrees below the temperature of optimal mycelial growth then. If small containers are used, e.g. one litre bottles or bags, then the temperature in the room will be almost identical to the temperature inside the spawn containers.
A constant temperature will prevent condensation water on petri dish lids and inside spawn containers. The condensate might carry otherwise dormant spores of contaminants to the nutrient-rich media and thus cause spoilage. Especially bacteria love condensates.
CO₂ levels: There should be some ventilation to avoid ambient CO₂ levels exceeding 1000 ppm. If the room is completely filled with inoculated substrates, the mycelia will produce large amounts of CO₂. Inside the bags/bottles, the CO₂ level can be as high as
20,000 - 40,000 ppm. The CO₂ level should not exceed 50,000 ppm as mycelial growth lessens then. **Humidity:** Humidity inside the spawn containers should be high enough to prevent the top layer of the substrate from drying out. This factor is more affected by the design of the spawn containers than by humidity in the room. The room humidity should be low (30-50% relative humidity) to avoid moist conditions, in which spores of contaminants could germinate.

10.4 Cultures

The first steps in spawn production are always performed on artificial media. These should contain sufficient nutrients for the mushrooms to grow, like saccharides and a solidifying agent (agar or gelatine). The mycelium grows on the two-dimensional surface of the medium, and will later be used to inoculate larger amounts of (three dimensional) substrates like grain. The following containers for culture media are normally used: test tubes or petri dishes (or in some developing countries flat whiskey bottles). Test tubes are fine to store strains and for use as starter cultures for the inoculation of petri dishes. They are usually called slants, because they are laid in a slanted position after sterilization to increase the surface area of the medium. Petri dishes (or flat whiskey bottles) have a greater surface area for the mycelium to grow on and are in use for the next step in spawn production. They are also much used to evaluate mycelial growth speed and to perform scientific experiments on a laboratory scale. The complete procedure involves the preparation of the medium, filling the test tubes or petri dishes and sterilising them.

10.4.1 Preparation of media

Many media have been developed which support mycelial growth of fungi. Tissue cultures of mycorrhizal mushrooms require very specific media. Cultivated mushrooms are mainly saprophytic and require less specific media. Most species grow on the following media:

1. **PDA: potato dextrose agar extract medium.** Ingredients: 200 g diced potato, 20 g agar powder, 20 g dextrose or ordinary white cane sugar, 1 litre water. Wash and weigh the potatoes and cut them in small pieces. Boil them for about 15 to 20 minutes until they are soft. Remove the potatoes and add water to the broth to make exactly 1 litre. Add the dextrose and the agar. Be sure to add the right amount of sugar and agar, otherwise the medium will become either too...
soft or too hard. Stir occasionally and heat gently until the agar has melted. The agar should be hot when poured into the test tubes or bottles, otherwise it will become lumpy. Fill the test tubes for about one fourth. Then the tubes or bottles are sealed with cotton plugs.

2. **Rice bran decoction medium.** For culture preservation the preceding recipe for PDA is commonly used, but for multiplying cultures the following recipe is cheaper and easier to prepare. It is in use for *Volvariella, Pleurotus* and *Auricularia* in the Philippines. Ingredients: 200 g rice bran, 1 litre water, 20 g getatine. Boil the rice bran for about 10 minutes in the water. Filter, save the broth and melt the gelatine and pour into bottles and sterilise.

3. **Wheat agar.** Ingredients: 32 g wheat kernels, 1 litre water, 20 g agar. Boil the wheat kernels in 1 litre of the water for 2 hours. Filter the broth after 24 hours and add water to exactly 1 litre. Add the agar to solidify the broth. Fill test tubes or petri dishes and sterilise for 30 minutes.

4. **Malt agar 2%.** Ingredients: 0.4 litres brewery malt solution, 0.8 litres water, 15 g agar. This commonly used medium in culture collections contains the vitamin B complex and various saccharides. It is made by diluting brewery malt with water to a 10% sugar solution (measured with a Brix saccharose meter: level 10). Mix the malt solution with the water, bring to a boil and add the agar. Adjust the pH value to 7 with KOH (potassium hydroxide). Fill the test tubes/petri dishes and sterilise for 20-30 minutes at 121 °C.

5. **Oatmeal agar.** Ingredients: oatmeal/lakes (30 gr), 1 litre water, 15 g agar. A nutrient-poor medium can be prepared by wrapping oatmeal flakes in a cloth and hanging them in a pan to simmer for 2 hours. Squeeze and filter through a cloth. Use the agar per litre to solidify the broth. Do not sterilise under high pressure, otherwise the agar will not solidify. Keep the test tubes for 30 minutes at 102 °C at 0.1 atmosphere.

The use of distilled water is recommended in many books, but is not necessary. In case of scientific experiments it is of course advisable to control each ingredient carefully, but when just multiplying cultures there is no need to use absolutely pure water. The same applies to the kind of agar used: agar which is sold in Chinese shops for consumption works fine and is much cheaper than laboratory agar. It is also possible to buy ready-made PDA or malt extract agar. How to prepare these should be written on the package. It is more expensive, but labour-saving, to buy the ready-made powder. A further advantage is the constant quality of the powder, whereas a self-produced product may vary depending on the ingredients. Any surplus of media can be stored in a freezer for several months; it should be sterilised before use. In all cases it is possible to add antibiotics to suppress the growth of bacteria. This should not be performed as a routine measure: if lots of contaminants occur, then hygienic measures should be checked instead of trying to fight the bacteria with chemicals. Adding broad-spectrum antibiotics like gentamycin or tetracyclin can be helpful, however, when isolates from wild mushrooms are made. Note that gentamycin can be autoclaved without losing its antibiotic properties; tetracyclin and a range of other antibiotics have to be added after the sterilisation process. The tetracyclin is added in a fixed ratio to ethanol; soon after the sterilisation process, the solution is added to the substrate under sterile conditions.
10.4.2 Preparation of slants

After the medium is filled in test tubes, these have to be sterilised before they can be used. The most commonly used sterilisation units in small-scale laboratories are pressure cookers. Water has to be poured into the cooker to the level of the rack. The bottles/slants are placed in the racks with a plastic covering to prevent water from wetting the cotton plugs. Then the lid is firmly closed. The air vent should be open at the beginning to allow the air to escape. This will take five to ten minutes from the moment of boiling and steam escape. The air vent is closed and a pressure gauge shows the pressure rise. Sterilise under pressure for 20-30 minutes.

Let the contents of the pressure cooker cool to below 90°C. If the pressure cooker is opened when still under pressure, the media will start to boil and will be absorbed by the cotton plugs. This would increase the risk of infection. Air will normally be sucked in by the cooling pressure cooker. Some types can hold a vacuum, with other types extra precautions are necessary to prevent incoming air from contaminating the media. A paper cloth saturated with alcohol can be placed over the place where air is sucked in. Alternatively, the pressure cooker can be allowed to cool in front of a clean air flow from a laminar flow cabinet.

To increase the surface area, the test tubes or bottles are put in an inclined position when the agar is still fluid. Take care that the agar does not touch the cotton plug, otherwise it might become contaminated. Do not put the test tubes upside down until the agar has solidified, otherwise a small portion of the agar may solidify at the other side of the slant or too close to the plug. Note that agar will have difficulties in solidifying acidic media if sterilised.

10.4.3 Preparation of petri dishes

For glass petri dishes, the procedure is similar to the preparation of slants. Each petri dish should be filled with about 3-5 mm of medium and closed. When the agar has solidified, the dishes are stacked. Each stack can be put in a heat-resistant plastic bag, which is closed with a rubber band. A large pressure cooker can hold 3 stacks of 10 cm diameter petri dishes. Take care the dishes stay in a horizontal position during sterilisation; otherwise the media will leak out and cause a mess.

Plastic petri dishes should be sterile (they are usually sterilised by gamma radiation) and packed in airtight plastic. The medium should be sterilised in flasks, covered with a plug. After sterilisation, it is poured under aseptic conditions (under a laminar flow) in the dishes. The package of petri dishes should only be opened in front of the laminar flow; all the dishes in the package should be used. Plastic petri dishes cannot be sterilised in a pressure cooker because they would melt.
10.4.4 Tissue cultures
Young and vigorous mycelium can be obtained from a young fruit body (preferably in button stage) in the following way, using:
- scalpel,
- alcohol,
- sterilised agar slants, petri dishes or bottles with agar,
- flame (non-smoking),
- clean table to work on, or preferably a laminar flow cabinet or inoculation box.
Wash the mushroom thoroughly. Dip the scalpel in alcohol, then flame it until red-hot. Let it cool down for 10 seconds. Now break the mushroom lengthwise (do not cut it with a knife, since contaminants from the surface can stick to the blade). Do not touch the inner surface with your hands. Use the heated scalpel to take a small piece (2x2 mm² is sufficient) of the inner tissue out. Take care that no outside surface tissue is included. Open the test tube/petri dish. (When using test tubes: heat the mouth of the tube in the flame to kill unwanted spores.) Then put the scalpel with the tissue in the middle of the agar. Immediately replace the plug. Inoculate at least three, but preferably more, cultures.
(Note on Auricularia: Cut the ear-like fruit body along the edge with heated and cooled scissors, scrape off some of the inner tissue with a heated and cooled scalpel, transfer to agar surface in the same way as described before.) Incubate the newly inoculated slants or petri dishes at 25 °C for ten days. Volvariella cultures should be incubated at 35 °C. Within three to four days mycelium will cover the tissue and branch out on the agar. If no growth occurs on the agar, check the following:
- Suitability of the type of medium for this kind of mushroom.
- Proper preparation of the medium (maybe the pH is incorrect).
- Possibly the mushroom was too old. Try a fresher specimen.
- Possibly the scalpel did not cool down before taking the tissue sample, thereby overheating the mycelium.
The mycelium should be white (except for some special mushrooms like Morchella) and grow out from the tissue. If yellow, blue, green or grey mycelia form on other places on the surface, then these are fungal contaminants. A creamy, shiny growth often indicates bacterial contamination. The culture might be saved if the mycelium is not attached to the contaminant by cutting it out and transferring it to a new slant. Take care not to touch the contaminated area with the scalpel. Never try to cut away contaminants: merely by moving parts of agar with infections, numerous spores will spread all over the surface of the dish.
10.4.5 Sub-culturing

Once a pure culture of the desired mushroom is obtained, it has to be multiplied. Inoculate more test tubes using the methods discussed. Note the number of transfers: label the originally isolated cultures T1, the next tubes T2 (isolated from T1), T3 (isolated from T2), etc. It is not possible to keep on transferring the cultures on agar forever. The mycelium will degenerate after a certain number of transfers. It is advisable not to transfer more than eight times (T8).

1. Sterilise the scalpel red-hot in the flame.
2. Take the plugs out of the test tubes (meanwhile the scalpel will cool down).
3. Keep the mouth of both test tubes above the flame.
4. Cut a small square 5x5 mm² from the ‘mother’ test tube culture.
5. Put the square in the middle of the agar of the new test tube.
6. Keep the mouths of the test tubes above the flame for three seconds.
7. Put the plugs back in the test tubes.
8. Sterilise the scalpel again for the next transfer.

A similar procedure has to be followed when inoculating petri dishes. A small piece of 5 x 5 mm² is laid in the middle of each new petri dish. Inoculated petri dishes should be wrapped in kitchen plastic (or the professional Parafilm™, which is more expensive) to avoid contaminants creeping in along the edges. Often dormant spores cannot be seen on the petri dish or slant, but establish themselves quickly when transferred to grain. Covering the dishes with plastic is therefore absolutely necessary to prevent contaminants.

10.5 Spawn containers

The containers for both mother and final spawn vary from place to place. Locally available materials and substrate treatment determine the choice.

10.5.1 Requirements

Spawn containers should be made out of heat resistant material, if the substrate is filled in the containers before a heat treatment: mostly used are glass, polypropylene (PP).
When gamma-radiation as sterilisation method is used, PE bags are chosen, as these do not disintegrate like PP does. When heated, some types of bags release substances that frustrate mycelial growth. If mycelial growth in bottles is much faster than in bags, this could be the reason. The spawn containers have to be tested whether they can stand the temperature inside the sterilisation unit. If higher pressures are used than 1 atmosphere overpressure, the temperature will be higher than 121 °C. PP bags sometimes crack easily after having been submitted to the sterilisation process. Avoid bags with seams: these tend to split after the heat treatment. The exchange of metabolic gases like CO₂ with ambient air has to be ensured; unwanted spores, however, have to be kept outside the containers.

The balance between amount of substrate and air filter capacity (or lid permeability) has to be considered. If too much air is exchanged, the spawn near the lid or filter is likely to dry out. This can be easily seen in bottles, when mycelial growth is even in most of the substrate but much slower near the upper surface.

Often, glass or heat-resistant plastic bottles are used for the mother spawn. Wide-mouthed jars, milk bottles and dextrose bottles can also be used. Dextrose bottles are ideal, because they can be obtained for free from hospitals and they have air outlets that can easily be plugged with cotton wool. They are well suited for mother spawn, because the narrow opening will make it easier to transfer the mother spawn aseptically to the final spawn. They can be used for final spawn too, but if the mycelium has grown the spawn material into one big clump, the bottles have to be broken to get it out. If the spawn is still rather young it can be removed using chopsticks.

Polypropylene bags with filters or cotton plugs to allow aeration are much in use for the final spawn (both sawdust and grain). Their size varies from 2.5 to 15 litres for grain spawn. For sawdust usually 1.8 to 8 litre bags are used. Keep in mind that it takes more time to sterilise large containers. The heat conductivity of the spawn substrate is generally poor.
10.6 Mother spawn

Mother spawn can be used to inoculate either grain spawn or a second generation of mother spawn, using, for example, wooden sticks. These sticks, in turn, can be used to inoculate the final spawn. Some spawn manufacturers soak the sticks in a nutrient-rich solution before sterilising. These sticks can be kept in a refrigerator for at least six months without losing vigour.

The sticks can also be used as final spawn. If they have sharp ends they can perforate plastic bags and reduce spawning labour (also refer to Preparation of wooden stick spawn).

In simple laboratories, grain mother spawn should not be used to inoculate another generation of grain mother spawn because the risk of contamination and degeneration will become too great. Liquid spawn is used by a number of sophisticated spawn producers to inoculate the final grain spawn. This kind of mother spawn is further discussed in the case study on exotic mushroom cultivation in Finland in paragraph 22.8; liquid spawn can easily become spoiled with bacteria. Consult a recent publication by Stamets for more information on this inoculation technique (Growing gourmet and medicinal mushrooms).

10.6.1 Preparation of grain spawn

The main advantage of grain is that it is very nutritious for fungi and forms kernels easily. The kernels can easily be dispersed in the substrate. The main disadvantage is that it provides an optimal substrate for other organisms, too. The chances of contamination are therefore much higher compared to sawdust or wooden stick spawn.

Kinds of grain. Different kinds of grains can be used like wheat, rye, millet, rice or sorghum. Some growers prefer millet spawn as millet can be sterilised for a longer period without breaking apart. Wheat grain cannot be sterilised that long, thus more endospores may survive the heat treatment.
Quality. A good quality of the grain is very important. It should contain only few broken kernels, few bacterial endospores, little extraneous debris and be recently harvested.

Preparation. The moisture content of the grain should be around 50%. If it is higher, mycelial growth may be faster but the danger of wet spot bacteria is also greater. If it is drier than 35% mycelial growth will be rather slow.

There are basically three ways to prepare the grain, both with advantages and disadvantages. The first one is most used for operations on a larger scale.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Boil grain first, drain, then fill in containers and sterilise</td>
<td>Uniform moisture content and consistency are assured</td>
<td>More handling than methods 2 &amp; 3</td>
</tr>
<tr>
<td>2. Put dry grain in containers, add recommended amount of water, allow to sit overnight, then sterilise</td>
<td>One step method</td>
<td>Danger of uneven moisture distribution with exploded kernels</td>
</tr>
<tr>
<td>3. Put dry grain in containers, add hot water in excess, drain after 8 hours, sterilise</td>
<td>Easy method</td>
<td>Grain may stay too dry</td>
</tr>
<tr>
<td></td>
<td>Less danger of exploded grain kernels</td>
<td>Time-consuming procedure</td>
</tr>
<tr>
<td></td>
<td>Uniform distribution of moisture</td>
<td></td>
</tr>
</tbody>
</table>

Several additives can be mixed with the grain after the excess water has been drained. Vermiculite prevents the grain from getting sticky, chalk or ground limestone ($\text{CaCO}_3$), and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) have a positive effect on the structure of the substrate and stabilise pH.

Grain spawn formulas. Grain in small containers can be moistened to a higher content than grain in 15 litre bags.

The following recipes adjust for the size of the container. (The concentration of additives can be up to six times higher than in these recipes, e.g. 4500 grams wheat, 100 grams gypsum, 25 grams chalk. The amounts of additives vary between producers; the formulas below do not use any chalk. Run tests to select a favourite recipe for the mushroom at hand):

1 litre containers:
200 g rye, 200 ml water, 1 g gypsum (50% moisture)

2 litre containers:
480 g rye, 400 ml water, 2 g gypsum (45% moisture)

4 litre containers:
800 g rye, 600 ml water, 4 g gypsum (43% moisture)

10 litre containers:
2200 g rye, 1500 ml water, 8 g gypsum (40% moisture)

75 litre containers:
3300 g rye, 2000 ml water, 12 g gypsum (38% moisture)
The final moisture content depends on the original moisture content of the grain; the above formulas give an indication of the gradual decline in moisture content when using larger containers.

(Adapted from Stamets, Growing gourmet and medicinal mushrooms)

**Sterilisation.** Sterilise the spawn containers in an autoclave. It depends on the autoclave, the way the spawn containers are packed (dense or loose) and the size of the containers for how long. Two hours are usually sufficient for 500 g containers; 3 kg bags have to be sterilised for about three to four hours. Shake the bottles when taking them out of the autoclave. This will improve moisture uniformity and keep the kernels at the bottom from sticking together. The spawn containers can be jammed against soft but sturdy objects to ease shaking. A bald car tire or a padded chair are good tools to facilitate shaking. These should be kept absolutely clean.

![Transferring several squares of mycelium from a petri dish to sterilised grain.](image1)

![Sealing grain spawn bags under sterile conditions (courtesy L. Hsu).](image2)

**Inoculation.** After the temperature in the center of the container has dropped to below the maximum mycelial growth temperature, the spawn containers can be inoculated. Use at least one (for 250 ml bottles) or two (for bigger bottles) squares of 10 x 10 mm² from the full-grown agar of the mother culture for each bottle. More pieces of agar can be used to speed up colonisation. Use a clean room for this work, since the grain can be contaminated very easily. It is best to lay the bottles horizontally to decrease the chance of contamination when using an inoculation cabinet. All equipment should be sterilised before use.

**Incubation.** Incubate the bottles until the mycelium has grown all over the substrate. The temperature should be close to the optimal temperature for mycelial growth (consult the table in the chapter Technical details: an overview). The humidity in the incubation room should be low (30-50%) to prevent any moulds from developing on ceiling or walls. Shake once (after eight days) or twice during the incubation period (or every three or four days) to distribute the mycelium evenly and to prevent kernels from
sticking together. It will take about two weeks for most species to colonise the substrate completely.

**Storage.** Keep the spawn in the refrigerator (except for *Volvariella* and certain strains of *P. djamar* spawn) and only take it out when needed. Grain spawn can spoil in one night at temperatures above 25 °C.

### 10.6.2 Preparation of the final spawn

The choice for a specific spawn substrate depends on the chosen species and the employed cultivation method. The following table shows which spawn substrates are used most frequently.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivation method</th>
<th>Final spawn substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agaricus bisporus, A. bitorquis</em></td>
<td>fermented substrate</td>
<td>grain</td>
</tr>
<tr>
<td><em>Auricularia polytricha, Hirneola auricula-judae</em></td>
<td>wood logs, sterilised sawdust in plastic bags</td>
<td>sawdust or grain</td>
</tr>
<tr>
<td><em>Flammulina velutipes</em></td>
<td>sterilised sawdust in bottles or bags</td>
<td>sawdust</td>
</tr>
<tr>
<td><em>Ganoderma spp.</em></td>
<td>sterilised sawdust in bags</td>
<td>sawdust or grain</td>
</tr>
<tr>
<td><em>Hericium erinaceus</em></td>
<td>sterilised sawdust in bags</td>
<td>sawdust or grain</td>
</tr>
<tr>
<td><em>Lentinula edodes</em></td>
<td>sterilised sawdust in bags</td>
<td>grain, sawdust, liquid spawn</td>
</tr>
<tr>
<td><em>Lentinula edodes</em></td>
<td>wood logs</td>
<td>sawdust, wooden plugs, sawdust plugs</td>
</tr>
<tr>
<td><em>Pleurotus spp.</em></td>
<td>pasteurised or sterilised substrates</td>
<td>mainly grain, occasionally sawdust</td>
</tr>
<tr>
<td><em>Siropharia rugoso-annulata chips</em></td>
<td>pasteurised straw or wood</td>
<td>straw, wood chips</td>
</tr>
<tr>
<td><em>Tremella fuciformis</em></td>
<td>wood logs</td>
<td>sawdust (only <em>Tremella</em>)</td>
</tr>
<tr>
<td><em>Tremella fuciformis</em></td>
<td>sterilised substrates in plastic bags</td>
<td>sawdust (mixed cultures)</td>
</tr>
<tr>
<td><em>Volvariella volvacea</em></td>
<td>pasteurised or raw substrates</td>
<td>used tea leaves, ipil-ipil leaves, rice straw with sawdust, cotton waste</td>
</tr>
</tbody>
</table>

An advantage of grain spawn is its vigour. A disadvantage is that it spoils rapidly and is very nutrient-rich, and thus more susceptible to contamination. Grain spawn is unsuitable for outside use, as it will be consumed by rodents. Grain spawn gives rise to a faster temperature rise in the inoculated substrate than sawdust spawn, which may or may not be desirable.

The advantage of sawdust spawn is that it can be kept at a higher temperature much longer before it spoils. The substrate material is also cheaper than grain. Wood plugs
and wooden sticks decrease labour at spawning, but are usually more expensive. The substrate for *Volvariella* spawn is mainly determined by the available materials. It should provide more aeration than the other substrates.

10.6.3 Grain as final spawn substrate material
Grain is treated the same way as discussed above for the mother spawn. It may be inoculated by grain spawn or wooden sticks.
Final grain spawn is inoculated in the following way with grain mother spawn:
1. Check the mother spawn again for contamination. Fluffy growth, bottles with only partial growth, greasy looking kernels all indicate irregularities. Bacteria often have a specific smell, which is somewhat sour. The presence of yeasts, too, is indicated by a typical smell. Do not use any suspicious bottles. It is better to throw away one bottle at this stage than 20 at a later stage.
2. Break the grain spawn in the jars by slamming them against the palm of the hand or shaking them against a bald tire. (Some spawn manufacturers leave the shaken bottles overnight to check whether the mycelium recovers. If it does not recover within one day, then the bottle is probably infected by bacteria.)
3. When the receiving containers have cooled down, they can be inoculated by someone wearing clean clothes, preferably in a clean room. Take the lid or plug of the bottle containing mother spawn and remove the plug or lid of the receiving container with the other hand. Pour one-fiftieth to one-tenth of the mother spawn con-
tainer in the receiving container (if they have the same size). Another method places both mother spawn and receiving containers horizontally and a spoon is used to inoculate. The risk of contaminants dropping in is lowered when the containers are placed horizontally.

10.6.4 Preparation of sawdust spawn
The method is similar to preparing sterilised substrate for wood-degrading species. Check the chapter on the cultivation on sterilised substrate (21) for more details.
Preparation. The sawdust should be free of splinters or bigger pieces of wood when plastic bags are used as spawn containers. These splinters might damage the bags allowing contaminants to enter after heat treatment of the substrate. The sawdust has to be stacked on a heap and moistened. By keeping the heap moist, the sawdust will soften. This will ease the absorption of water. The optimum moisture content is around 60%. Do the squeeze test to determine whether the substrate is moist enough. The exact moisture content can be determined by weighing a representative sample before and after it has been in an oven for one hour. Apply the supplements (like 20% rice bran compared to 80% of the dry sawdust) and mix very well. If one component is added in a rather low concentration, then it is better to mix it first with a smaller amount of substrate and only then apply it to the larger heap. Otherwise its distribution probably remains uneven.
Fill the substrate in bags or bottles. Special machines have been designed in Japan and Taiwan to fill bottles and bags. These are similar to the ones used in commercial sawdust plastic bag cultivation. Make a hole by pressing a stick in the substrate. The mycelium can grow faster with such an aeration channel. Put a plug on top of the bottles or bags.
Heat treatment. Sterilise the bags for two hours if they are small (500 g) or longer if they are bigger. Let them cool down, then inoculate with the mother spawn (usually grain). When sawdust spawn has incubated for a long period, the old mycelium on top should not be used for spawning.

10.6.5 Substrate formulations for Volvariella spawn
Volvariella needs more aeration during vegetative growth, therefore the substrates have a different structure. The following substrates can be used. After sterilisation, they can be inoculated with grain mother spawn.
1. Spent tea leaves. Chinese restaurants in particular spend a considerable amount of tea leaves each day. If these are kept apart from other waste materials, they can very well be used for spawn production. Wash the leaves in water, dry them in the sun and store them for later use. To use them the leaves have to be soaked in water for more than two hours, drained and mixed with a buffer, like 2% CaCO₃ to create a suitable pH. Pack the leaves loosely in bottles and sterilise for 30 minutes at 115 ºC under pressure. Inoculate with grain spawn.
2. Sawdust with ipil-ipil leaves. Sawdust alone is packed too tight for Volvariella, but in combination with ipil-ipil (Leucaena leucocephala) leaves it can be used. Use three-fourths sawdust and one-fourth ipil-ipil leaves (weight/weight). Mix the two components, put them in a container and place a weight on top. Add water and let it ferment
for four days. All the materials have to be below the water level. Wash the drained material three times. Drain again and add 5% rice bran. Apply the squeeze test to see whether the mixture is wet enough. Fill the bottles and sterilise for one hour.

3. **Cotton waste or kapok.** Cotton waste from the textile industry can be used both for spawn production and as a bedding material. It brings the highest yields as it contains a lot of cellulose. Use card waste for spawn preparation. It contains some debris, like seeds, that will allow for better aeration. Soak the cotton in water for some hours, add 2% CaCO$_3$ and fill the bottles. Sometimes the mixture will become too tight. Use rice straw, tea leaves or other material to improve aeration. Fill PP plastic bags or bottles with the substrate and sterilise.

4. **Rice straw.** Chop the straw in pieces of 2 to 3 cm and soak them in water for about one day. Drain the excess water and fill the bottles with straw. For each 500 ml bottle 5cc of a 2% sucrose and 2% peptone solution should be added.

**10.7 Spawn quality**

10.7.1 Quality control

**Contaminations:** if an infection occurs in a spawn laboratory, the spawn maker should carefully analyse how the contaminant entered the substrate. Both the stage at which infection occurred and the pathway (vector) through which the infection entered are important. Stamets (1993) distinguishes six vectors of contamination:

- the spawnmaker,
- the air,
- the media,
- the tools,
- the inoculum,
- mobile contamination units.

The following table shows at which stage which vector can lead to problems.

<table>
<thead>
<tr>
<th>Stage in spawn preparation</th>
<th>Vector of contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>sterilisation</td>
<td>• the media may contain spores of contaminants if insufficiently sterilised</td>
</tr>
<tr>
<td></td>
<td>• unclean air can be sucked in when the sterilisation vessel cools down</td>
</tr>
<tr>
<td>inoculation</td>
<td>• the inoculum seems to consist of the right mycelium only, but may be infected with contaminants</td>
</tr>
<tr>
<td></td>
<td>• the air can introduce unwanted spores during the time the spawn containers are opened for inoculation</td>
</tr>
<tr>
<td></td>
<td>• the spawn maker may touch (by accident) the wrong parts of the spawn container or inoculum, dirty hands or skin flakes may cause contamination in the process of inoculating the spawn containers</td>
</tr>
<tr>
<td></td>
<td>• tools which have not been properly sterilised, like the inoculation loop, scalpels etc. can introduce unwanted spores</td>
</tr>
</tbody>
</table>
### 10.7.2 Different kinds of contaminants

Determining the exact species of contaminant is less important than recognising when and how the infection occurred. Still, the kind of contaminant may give an indication of what went wrong. Unhygienic conditions during inoculation may give rise to a variety of different fungal contaminants.

Contamination by fungi can usually be recognised by the typical colours of their mycelium. At times a distinctive zone can be recognised between inoculated mycelium and contamination. Very common are *Penicillium* and *Aspergillus*. If they are allowed to develop, the infection pressure in the laboratory will become very high. It is advisable to sterilise the contaminated containers and to open and clean them only after sterilisation. Several contaminants may cause skin diseases or allergic alveolitis (a lung allergy). Insufficient sterilisation often leads to outbreaks of bacteria. Bacteria are more difficult to detect than fungi. Some will give the grain spawn a greasy appearance and emit a sour smell. Bacteria develop easily in grain spawn. If not detected in the stage before inoculating the final spawn, all the spawn derived from the contaminated bottle of mother spawn will become useless.

In sawdust spawn, bacteria are even more difficult to detect. If bags inflate within days after inoculation, this can be caused by an outbreak of bacteria which produce large amounts of CO₂.

Quality control in spawn manufacturing means constant inspection of the inoculated containers and keeping to a strict hygienic regime. Quality can be poor for a number of reasons:

- the strain has degenerated during the multiplication process,
- cultures have infected the strain,
- the spawn has not been stored properly (too warm or too cold, or substrate in the containers has dried out),
- the spawn does not consist of mycelium of the desired mushroom only; other organisms are present in the substrate, such as bacteria.

### 10.7.3 Degeneration

Degeneration is at times difficult to trace. Large spawn companies test mother cultures in their own growing rooms before releasing spawn from these cultures on the market. The clearest signs are loss of mycelial growth speed and the formation of cottony sec-
tors in a number of species, e.g. *Agaricus*, *Dictyophora* and others. Containers with cottony sectors have to be taken out of the incubation room for these species. Cottony sectors in *Pleurotus* are no sign of degeneration, however.
The mycelium should be white, except for *Lepista nuda* (purple), *Morchella* (greyish-yellowish), and *Pleurotus abalonus/cystidiosus* (black coremia form on the white mycelium).

**Viruses:** An infection by viruses is difficult to spot. If the mycelial growth rate is reduced and cottony sectors in a normally stringy mycelium appear, then this may be due to either contamination by viruses or a degeneration. Viruses can be detected by scanning electro-micrographs or electrophoresis. Both techniques can only be performed in a microbiological laboratory. Scanning electro-micrographs require very expensive equipment.

**Storage and purity:** Good spawn shows vigorous mycelial growth and contains no other organisms. If it has been stored too long the vigour will become less. Spawn from Oyster mushrooms will become very compact after prolonged storage and is therefore more difficult to apply evenly during spawning.

On the other hand, it is advisable to give the mycelium a resting period between successive transfers. Keeping it in a refrigerator for some months is better than fast, continuous vegetative multiplication. The best way is to start from a fresh mother culture every three months. The fresh mother culture can be obtained from a tissue culture or from culture collections and research stations.

Refrigerated spawn of most cultivated mushrooms can be kept for up to six months after complete colonisation of the spawn substrate. If the spawn is not pure (e.g. contaminated with bacteria) the storage time is much shorter. Even spawn from renowned modern companies has been found to contain numerous bacteria because of insufficient sterilisation. Spawn has to be kept cool (4 to 6 °C). The exceptions are *Volvariella* and some strains of *Pleurotus djamor*. Their mycelium would die at this temperature. For the Rice straw mushroom a keeping temperature of 15 °C is recommended, for the pink oysters a temperature above 12 °C after colonisation of the spawn substrate. The spawn manufacturer should only sell high-quality spawn and inform the growers until what date it can be kept. Opened spawn containers cannot be stored for later use. Do not use half of a bottle because contaminants will spoil the rest of the spawn. Often contaminants (e.g. green moulds like *Trichoderma*) can infect spawn without visible signs of contamination at the beginning of the infection.

10.8 **Spawn quality control in Fujian province, China**

This paragraph focuses on the organisation and quality control of the spawn supply in a special region. The province of Fujian is the biggest producer of *Agaricus* in China. Most of the product is canned and exported. As the White button mushroom industry is responsible for considerable amounts of foreign currency, it is supported by the government. Canning is linked to industry, so the Fujian Research Institute of Light Industry established a branch completely devoted to mushroom growing. Its aim is to:

1. select and breed good strains,
2. organise a network for spawn supply,
3. examine new growing techniques,
4. conduct training courses,
5. provide advice.

**Strains:** The Institute develops strains that suit local conditions. Three types of strains are considered:

- H = for high yielding but low quality,
- G = for good quality,
- HG = for the intermediate strains.

The growers, of course, like to grow the H strains, but the canning factories demand a good quality. New strains have to be tested on at least 1000 m² before they are released for commercial cultivation. The mushrooms from the pilot farm have to be canned, and the tins will be opened at the annual spawn conferences and thoroughly checked. At these conferences the results of the strains of the last season will also be discussed. These spawn conferences are held at different places in the province each year. The quality of the product is especially important, because the customers cannot check the quality at the time of purchase.

The cultures are tested for virus disease by electrophoresis. If the strains meet the standards, their cultures will be multiplied and distributed to the branch stations. The nine branch stations cover the whole province and are usually attached to canning factories. They multiply the cultures again and these are sold to the many spawn makers. Simple and effective methods have been developed to ensure hygienic measures at the level of the spawn makers (check the paragraph on Inoculation cabinets at the beginning of this chapter). These spawn makers in turn will finally produce the spawn for the tens of thousands of small farmers. It should be noted that the countryside of mainland China is very big and that it is difficult to reach all the farmers. Most of them grow only on 200 to 500 m². The growing season is from September to April. During the summer the simple houses will be removed, because the farmers have to grow other crops then.

**10.8.1 Problems in spawn production in Malaysia**

The market for Oyster mushrooms in Malaysia is developing slowly because of the high production costs. The mushrooms are still rather expensive because losses during production are high. The following problems are commonly encountered in Malaysia:

1. Difficulties in obtaining suitable strains: yields are only 20% compared to 40% in other countries (biological efficiency is low).
2. There are no strain preservation facilities available in Malaysia. Strains are kept by tissue culture, which in the end leads to degeneration and loss of strains.
3. There is a lack of technical know-how in maintaining and preserving pure cultures.
4. The contamination rate in producing the spawn is 5% (culture transfer takes place in an inoculation chamber sprayed with 70% ethyl alcohol and 10% chlorox).
5. The contamination rate of the final substrate is definitely too high: 10 to 20%. More suitable strains have now been sent to Malaysia from the addresses in the appendix, and with the information supplied in this book on preserving cultures, degeneration can be avoided to some extent. The contamination rate can be lowered by following the guidelines in the section on clean rooms in this chapter. The contamination rate in the final substrate can be lowered by maintaining strict hygienic measures during and after spawning. Possibly the heat treatment was insufficient.
10.8.2 Figures on a small-scale spawn laboratory

The following figures show how spawn can be produced with minimal investment and little time. If the following scheme is used, 9 kg of grain spawn can be produced within 3.5 hours. Actual labour during this time is only 45 minutes. If more high pressure cookers are used, then the time can be used more effectively. The spawn substrate materials needed are: 4.5 kg wheat, 6 litres of water, 100 g gypsum, 25 g chalk.

Time schedule for spawn production

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time (hours:minutes) since start 0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>weighing the components</td>
<td>0.10</td>
</tr>
<tr>
<td>bring the wheat to boil (starting with hot water)</td>
<td>0.25</td>
</tr>
<tr>
<td>turn off the gas</td>
<td>1.15</td>
</tr>
<tr>
<td>drain excess water</td>
<td>1.35</td>
</tr>
<tr>
<td>mix, add chalk, gypsum and put in bags/bottles</td>
<td>2.05</td>
</tr>
<tr>
<td>Sterilisation</td>
<td></td>
</tr>
<tr>
<td>Heat pressure cooker</td>
<td>2.15</td>
</tr>
<tr>
<td>Pressurise in cooker</td>
<td>2.25</td>
</tr>
<tr>
<td>Sterilise bottles</td>
<td>3.25</td>
</tr>
<tr>
<td>Turn off heating, cool down to release pressure</td>
<td>3.5</td>
</tr>
<tr>
<td>Inoculate next day, when the bottles have cooled down</td>
<td></td>
</tr>
</tbody>
</table>

Equipment

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Investment (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High pressure cookers</td>
<td>550</td>
</tr>
<tr>
<td>Mixing and draining bowls</td>
<td>75</td>
</tr>
<tr>
<td>Large pan</td>
<td>125</td>
</tr>
<tr>
<td>Gas burners</td>
<td>200</td>
</tr>
<tr>
<td>Spawn containers</td>
<td>25</td>
</tr>
<tr>
<td>Additional investments</td>
<td>250</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1225</strong></td>
</tr>
<tr>
<td>Optional: clean room, laminar flow system</td>
<td></td>
</tr>
</tbody>
</table>

Production costs (excluding ca. 25 hours of labour) In US$ per 400 litres of spawn

<table>
<thead>
<tr>
<th>Cost</th>
<th>Cost per 400 litres of spawn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depreciation</td>
<td>10</td>
</tr>
<tr>
<td>Interest</td>
<td>5</td>
</tr>
<tr>
<td>Grain, chalk, gypsum</td>
<td>57</td>
</tr>
<tr>
<td>Energy</td>
<td>5</td>
</tr>
<tr>
<td>Inoculation material</td>
<td>3</td>
</tr>
<tr>
<td>Plastic bags, plugs, PVC rings</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>95</strong></td>
</tr>
</tbody>
</table>
Spawn is usually delivered in litres instead of kilograms. One litre of spawn equals 0.68 to 0.75 kg. For 400 litres of spawn, in The Netherlands, an amount of US$ 660 has to be paid (US$ 1.65 per litre). The costs for self-produced spawn are only 95 US$, thus producing spawn at home seems profitable if labour is cheap. The total production time for 400 litres of spawn is 25 hours, not including the time needed for shaking the bags to get an even colonisation. It is therefore possible to run a farm and at the same time produce the spawn. On the other hand, spawn making should not be performed if a good quality spawn can be obtained at a reasonable price. It is better to focus on a specific part of the mushroom growing process than to try to cover all aspects.

(Information kindly supplied by Onno Harmsen, The Netherlands.)
11 Climate control in general

This chapter gives a general outline of climate control and low input technology options. State-of-the-art installations are described in the following chapters. After an introduction, the following climatic factors are treated:

- ventilation (fresh air and circulation air),
- temperature,
- relative humidity of the air,
- CO₂ level,
- light.

11.1 Introduction

Every mushroom strain has its own set of climatic conditions under which it performs best. The objective of climate control is to create the suitable conditions during the successive phases of mushroom cultivation, such as pasteurisation, spawn run, induction of primordia, growth of mushrooms, picking etc. First one has to determine which climatic conditions have to be met during the successive phases of mushroom growing. Each individual phase (substrate preparation, spawn run, cropping) has its own set of optimal climatic conditions.

One also has to determine whether these conditions have to be met in a single room, or in several different rooms. It may be more cost-effective to have separate rooms for spawn run (with limited climate control, apart from temperature) and use special cropping rooms (with more expensive equipment to control humidity and air flow, too) only for fruiting. The two extremes in regulating climatic conditions are:

- no climate control at all: cultivation in open air,
- complete climate control, where every factor can be controlled without affecting the others.

The second extreme is also rather expensive for developing countries. It is treated here in a very general way, because it shows an optimally equipped climate control. Every grower can decide which parts of equipment can be deleted or replaced by cheap, locally available materials.

11.2 Ventilation

The term ventilation can be misleading: both circulation and fresh air are kinds of ventilation, but they are completely different. Fresh air is outside air, which is blown in to improve climatic conditions inside the growing room. Circulation air is the air inside the growing room which is blown through the growing house (circulation) to smooth out climatic differences in the growing room and to keep evaporation going on. To avoid confusion, this manual will refer to fresh air and circulation air and not use the term ventilation.
11.2.1 Circulation air
Air flow over a mushroom bed will increase the water evaporation rate. If the air flow is non-uniform, some beds will become drier than others. The picture shows how air circulates in a standard Dutch White button mushroom growing house.

11.2.2 Fresh air
Fresh air can be blown in the growing rooms in order to:
- reduce CO₂ levels,
- adjust the desired temperature inside the growing room,
- adjust the desired humidity inside the growing room.
If there is a large difference between outside air conditions and the desired conditions inside the mushroom growing house, the fresh air has to be conditioned. If outside air is relatively cool, then heating it would lead to very dry air, which would have to be humidified before it is blown along the substrate.

11.3 Ventilation systems
Basically, there are two ventilation systems: overpressure and underpressure. The following specifications stem from the Standaardplan Champignonkwekerij, the Dutch specifications for standard mushroom houses. Each mushroom cell measures: width 6 m, length 17.75 m, height 3.8 m. The total growing surface is 200 m².

11.3.1 Underpressure ventilation system
Underpressure ventilation system
1. fresh air inlet
2. self-closing blinds
3. coarse particle filter
4. deflection plate (the hole in this plate should have the same diameter as the fan inlet)
5. mixing fan, 300 mm diameter, capacity 3000 m³ per hour at 120 Pa.
6. rectifier, e.g. honeycomb structure
7. PVC airduct, 380 mm diameter, 35 holes with a diameter of 55 mm every 500 mm, directed downwards, every 1000 mm a 25 mm hole directed towards the ceiling and wall
8. suction fan, capacity 4.500 m³ per hour at 300 Pa.
9. self-closing blinds 10. air outlets
The suction fan (8) sucks the air out of the room. Fresh air will then enter through the
11. CLIMATE CONTROL IN GENERAL

air inlet (1). The mixing fan, 500 mm behind the air inlet, can distribute the incoming air.

**Advantages of underpressure system:** A relatively simple and cheap ventilation system. It is possible to regulate circulation air and fresh air independently.

**Disadvantages:** conditioning of fresh air is performed inside the growing room. Cold outside air might fall and is difficult to mix. There will be more differences in climate than in growing rooms with the overpressure system.

The fans inside the growing room produce a lot of noise and have to be turned off during picking. This may decrease the quality of the picked mushrooms.

### 11.3.2 Overpressure ventilation system

The overpressure ventilation system in modern mushroom growing rooms consists of:

1. fresh air inlet
2. fresh air valve
3. circulation valve
4. air filter
5. air cooling unit
6. heater
7. humidifier
8. fan, capacity 4,500 m³ per hour, 500 Pa counterpressure
9. fresh air channel: 500 x 600 mm
10. circulation air channel: 500 x 600 mm²
11. main air channel: 500 x 600 mm² or 560 x 560 mm²
12. choke valves
13. air channel 400 x 400 mm² or 500 x 300³ mm or PVC duct: 450 mm diameter
14. outlet, with filter
15. self-closing blinds

The air channel (13) contains 32 holes of 55 mm diameter each every 500 mm, which are directed downwards.

Fresh air is sucked into the air channel (9) through the inlet (1). The fresh air is precon-
ditioned by the cooling unit (5), the heater (6) and the humidifier (7). It is then blown into the growing room, creating an overpressure. Circulation air can be mixed with fresh air in any combination, determined by the settings of the valves (2) and (3). If the fresh air valve is completely open, the circulation valve is completely closed.

**Advantages of the overpressure system:** the air which enters the room is already preconditioned. Therefore, mixing cold outside air with warm growing room air doesn’t pose many problems.

Another advantage is that the air-outlet can simply be directed in a separate channel towards a heat-exchanging unit to precondition fresh air. A third advantage is that the fans are outside the growing room, thus causing less noise in the growing room.

**Disadvantages of the overpressure system:** the system is rather expensive, both to install and during use. All the air has to pass the airconditioning units, which increases the required pressure. Therefore a relatively heavy fan is needed, using more electricity. Another disadvantage is the high air velocity in the cell. Especially during the primordia formation phase of *Agaricus* this may cause troubles. Other mushrooms, like Oyster mushrooms, respond even worse to high air velocity.

### 11.3.3 Alternative overpressure ventilation systems

In Oyster mushroom cultivation, CO₂ levels and air velocity are even more important than in White button cultivation. The above described systems may create too much airflow, which is undesirable for Oyster mushrooms. Some growers therefore developed the following technique:

The Oyster mushrooms are grown in plastic bags, which are put in vertical racks. The growing room has a separate ‘ceiling’ which is separated from the rest of the cell by a perforated plastic sheet. Fresh air is blown in the empty space above where it is humidified and heated. In this way, only conditioned air will be flowing slowly through the holes in the plastic ceiling and evenly distribute itself over the racks with the substrates. There is no circulation air, as the Oyster mushrooms are very sensitive to CO₂. The air outlets are close to the ground, between the racks. The bags, however, cannot be stacked as high as in *Agaricus* cultivation (five shelves), because the air quality further down would be insufficient.

### 11.4 Temperature

The mushrooms selected for cultivation at a given site should be able to grow at temperatures close to normal outside air temperatures. Otherwise, expensive heating or cooling systems will be necessary. Three different temperatures are important:

- air temperature outside the growing room,
- air temperature inside the growing room,
the substrate temperature. The substrate temperature is an important parameter in both mycelial growth and fruit body formation. The substrate itself produces heat, the amount of which depends on the activity of microbes (including the mycelium of the cultivated mushroom) in the substrate. It may be necessary to remove excess heat by introducing air of a lower temperature. The cheapest way to control the air temperature inside the growing house is to use outside air to adjust to the desired optimal temperatures. E.g. in the summer fresh air is allowed in during the night, while during the day circulating air assures even climatic conditions in the growing room. If many micro-organisms are present in the substrate (like in compost), the temperature of the substrate is likely to be higher than the ambient air temperature. This can be desirable because it increases evaporation, which is necessary to transport nutrients from the mycelium inside the substrate to the fruit bodies. A high substrate temperature (> 35 °C), however, can trigger the thermophilic microflora. Micro-organisms in this group thrive at temperatures between roughly 30-55 °C. When they start to grow, they produce more heat, which increases the substrate temperature. In this way the mycelium of the cultivated mushroom can be killed.

Substrates usually have a low heat conductivity. The temperature inside the substrate can thus rise significantly above the ambient air temperature. If the substrate temperature is higher than the optimum range for the grown mushroom, yields will decrease accordingly. For this reason layers of compost should not be thicker than about 25 cm, and bags with pasteurised straw not heavier than 25 kilo.

11.4.1 Thermometers
The classical thermometers which measure the temperature-dependent volume of fluids (like mercury or alcohol) are mainly used by small-scale mushroom growers because they are readily available and relatively cheap. Glass thermometers break easily; a metal casing is necessary to prolong their lifetime. Newer types of thermometers measure the change in resistance of platinum coil, due to changes in temperature. This type of thermometer should be calibrated individually, because each coil responds somewhat differently to temperature. They can easily be used for automatic temperature control, because the output is electrical. A third way to determine temperature is by using a thermocouple. Two different metals are welded together at two spots. One spot is held at a fixed temperature (often a mixture of water and ice at 0 °C), the other is placed in the room where the temperature has to be determined. The temperature difference will induce a difference in electric potential be-
tween the two welds, which can be measured by a micro voltmeter or a sensitive galvanometer.

11.4.2 Heating
The growing rooms can be heated with different devices, with specific advantages and disadvantages. For pasteurisation and cooking out, the use of steam is the most efficient option. A heater which preconditions the air before it enters the room is normally used in combination with the overpressure system. Heating pipes can be used in both over- and underpressure systems.

<table>
<thead>
<tr>
<th>Heating system</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>steam</td>
<td>very fine dispersion of heat without the problem of dry air; cook-out of growing room is possible</td>
<td>steam boiler has to run continuously, high operating cost and energy use</td>
</tr>
<tr>
<td>heating pipes along the walls, on both sides of the growing room</td>
<td>lower operating cost</td>
<td>air becomes very dry inside the growing room</td>
</tr>
<tr>
<td>heated air ventilation system</td>
<td>air can be dried to avoid bacterial blotch; more uniform conditions inside the growing room</td>
<td>air may become too dry, extra humidification is necessary; all air has to pass the heater unit, which causes extra flow resistance and thus higher electricity cost</td>
</tr>
<tr>
<td>low temperature gradient system (many plastic tubes radiating either heat or cooling)</td>
<td>can be used for both cooling and heating</td>
<td>innovation technique with unexplored potential</td>
</tr>
</tbody>
</table>

11.4.3 Cooling
In developing countries the usual problem is cooling rather than heating. The cheapest option is to blow fresh air in during the night. This may not be sufficient, however, therefore cooling systems are used. Another approach would be to select strains for their ability to grow at higher temperatures, thus reducing the need for cooling. Two aspects have to be distinguished: the way cooling itself is generated, and the way it is transmitted to the room. Thus the last option can be run with all four below-mentioned cooling generation systems.

<table>
<thead>
<tr>
<th>Cooling system</th>
<th>Principle</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical cooling</td>
<td>air is forced through a cooling unit with freon; the energy in the air is used to evaporate the freon, which cools the air</td>
<td>thorough cooling possible</td>
<td>high operating cost, high investment, regular inspections necessary, environmentally unfriendly</td>
</tr>
</tbody>
</table>
### 11.5 Relative humidity

Air humidity is one of the most important factors in the growing room climate. Relative humidity is the ratio between the amount of water actually present in the air and the amount of water that air of the same temperature can maximally contain. The absolute humidity of air is strongly dependent on the air temperature. The warmer the air, the more water it can contain maximally. This means that air will become relatively drier when it is heated, and become moister when it is cooled. So-called Mollier diagrams have to be obtained with the exact figures for a location at a specific altitude.

#### 11.5.1 Humidity sensors: hygrometers

The least expensive humidity sensor is a hair-hygrometer. This device is based on the fact that a hair (or a bundle of hairs) will become longer with increasing relative humidity. The relative humidity can be directly shown on an analog scale. The main disadvantage of this kind of hygrometer is that it has to be calibrated often. An indirect,
but more exact way to determine the relative humidity employs a psychrometer. Two thermometers, one with a wet and the other with a dry bulb, show different temperatures. By comparing these with the Mollier diagram (or psychrometric scale) the relative humidity can be measured with an accuracy of 2%. Psychrometers can be used to calibrate hygrometers.

11.6 CO₂ level

During spawn run, high CO₂ levels don’t cause problems. In fact, rem elevated levels are favourable for mycelial growth. During fruiting, however, many mushrooms are sensitive to high CO₂ levels, under which they typically develop long stems and small caps. It depends on the species and on the strain what CO₂ concentration can be tolerated. Different strains of the same species (e.g. Pleurotus ostreatus) can differ in sensitivity to elevated CO₂ levels. Less sensitive strains are desirable because less fresh air has to be blown into the growing house. The operating costs will be lower if already conditioned air can be re-circulated, instead of having to condition fresh air. Only if CO₂ levels become higher than the maximum concentration for the specific species, fresh air input has to increase. As long as they are lower, fresh air admittance is determined by temperature or humidity only.

Figures below were derived and adapted from several (sometimes conflicting) sources, a.o. Stamets: Growing gourmet and medicinal mushrooms. The discrepancy in figures can be due to different strains, with varying sensitivity to CO₂ levels.

<table>
<thead>
<tr>
<th>Species</th>
<th>Allowed p.p.m. CO₂ during fruiting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bitorquis</td>
<td>&lt; 3000</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>&lt; 800</td>
</tr>
<tr>
<td>Coprinus comatus</td>
<td>500 – 1000</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>2000 – 4000</td>
</tr>
<tr>
<td>Ganoderma lucidum</td>
<td>20.000 – 40.000 for antler fruit bodies</td>
</tr>
<tr>
<td></td>
<td>&lt; 2000 for normal fruit bodies</td>
</tr>
<tr>
<td>Hericium erinaceus</td>
<td>500-1000</td>
</tr>
<tr>
<td>Lentimula edodes</td>
<td>1000-2000</td>
</tr>
<tr>
<td>Lepista muda</td>
<td>&lt; 1000</td>
</tr>
<tr>
<td>Pholiota nameko</td>
<td>800-1200</td>
</tr>
<tr>
<td>Pleurotus cornucopiae</td>
<td>&lt; 800</td>
</tr>
<tr>
<td>Pleurotus cystidiosus</td>
<td>&lt; 2000</td>
</tr>
<tr>
<td>Pleurotus eryngii</td>
<td>500 – 1000 at primordia formation</td>
</tr>
<tr>
<td></td>
<td>&lt; 2000 during fruit body development</td>
</tr>
<tr>
<td>Pleurotus diamor s.l.</td>
<td>500 – 1500</td>
</tr>
<tr>
<td>Pleurotus ostreatus</td>
<td>550 – 700 (strain dependent)</td>
</tr>
<tr>
<td>Pleurotus pulmonarius</td>
<td>400 – 800</td>
</tr>
<tr>
<td>Stropharia rugoso-annulata</td>
<td>&lt; 1500</td>
</tr>
<tr>
<td>Volvariella volvacea</td>
<td>1000 – 5000</td>
</tr>
</tbody>
</table>

11.6.1 CO₂ meters

The price of CO₂ meters limits their use. A reliable meter from e.g. Siemens costs more than US$ 1000. Only highly sophisticated mushroom farms employ CO₂ monitoring.
equipment. One meter is sufficient to measure the CO₂ levels in all growing rooms if a multiplexer is used. This device ensures that air from different growing rooms is periodically sampled. The air is pumped from the growing rooms to the meter. The commonly used meters have a range of 0-3000 ppm with an accuracy of 100 ppm. The measuring principle is that CO₂ absorbs more infrared light than the other gases in the air. A photo-electric cell measures how much infrared light is absorbed. A calibrated curve then translates the measured light absorption into a CO₂ concentration. This kind of meter has to be calibrated regularly by measuring air with 0 and 2000 ppm CO₂. These certified samples have to be bought.

11.7 Light

Some mushrooms only form normal fruit bodies if they receive sufficient light. The following table shows which mushrooms need light to fruit:

<table>
<thead>
<tr>
<th>Species</th>
<th>Light Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>no light requirements</td>
</tr>
<tr>
<td>Agaricus bitorquis</td>
<td>no light requirements</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>under the absence of light it produces the typical light-coloured 'Golden needles'; under natural conditions the cap turns yellow with a brown umbo</td>
</tr>
<tr>
<td>Lentinula edodes</td>
<td>8 hour cycle</td>
</tr>
<tr>
<td>Pleurotus sp.</td>
<td>8 hour cycle</td>
</tr>
<tr>
<td>Tremella fuciformis</td>
<td>no light requirements</td>
</tr>
<tr>
<td>Volvariella volvacea</td>
<td>no light requirements</td>
</tr>
<tr>
<td>Auricularia spp.</td>
<td>500 lux of directly radiated light</td>
</tr>
</tbody>
</table>

For other species the light requirements are not known to the author. The symptoms of light-deficiency are similar to those of high CO₂ levels: long stems and small caps. In the case of Pleurotus the absence of light can lead to coral-like structures with many expanded stems. Strains within the same species show different spectral response. In general a normal strip light provides the right spectrum for most mushrooms. As a rule of thumb, one should be able to read a newspaper in the entire growing room. An 8 hour per day cycle is usually sufficient.
12 Simple mushroom farms

The previous chapter described how the climate can be controlled in closed rooms. Usually a number of these rooms together with other facilities are combined in a mushroom farm. The building of state-of-the-art mushroom farms with modern technology is discussed in the next chapter. This chapter discusses some elementary aspects of mushroom farms:
- selecting a site for the mushroom farm,
- indoor versus outdoor cultivation,
- one room for all phases or each phase its own room,
- standardisation of mushroom farms,
- farm layout.

The second part of this chapter describes a number of simple mushroom farms from all over the world:
- cultivation sites for Shiitake on wood logs,
- intercropping with other vegetables,
- plastic sheds,
- cultivation of various mushrooms in a closed mushroom house, lined with plastic inside (from Taiwan),
- simple mushroom houses (from the Philippines),
- a concrete barn (from Malaysia) and its advantages over attap barns,
- a design for Volvariella production (from Puerto Rico).

12.1 Selecting a site for the mushroom farm

The following factors should be kept in mind when selecting a site for a mushroom farm:
- distance to market,
- availability of substrate material,
- transportation of both product and substrate materials,
- outside temperature has to be close to the desired range of the cultivated mushroom to avoid expensive cooling or heating (e.g. in mountains instead of lowlands),
- no sources which pollute the air with biological contaminants (e.g. spores of green moulds) such as waste dumps, compost piles, sawing mills etc. should be near the mushroom farm,
- desired capacity of the farm,
- possibility to enlarge the farm in the future,
- ready availability of clean water.

The mushroom farm should provide suitable climatic conditions, as discussed in the
previous chapter. The examples in this chapter show how cost-effective structures can be set up. It is also possible to adapt existing structures, such as defence tunnels, bunkers, caves, chicken houses, old milk factories, slaughter houses etc. Some successful mushroom cultivation operations take place in old defence tunnels, both in China and in Cuba. Many mushrooms need light during the fruiting stage; these are therefore more difficult to grow in natural caves. *Agaricus* and *Coprinus comatus* can be cultivated without light.

12.1.1 Indoor versus outdoor cultivation
Mushroom cultivation is usually divided into indoor and outdoor growing techniques. Actually this is just a gradual distinction. Outdoor cultivation requires low investment, but there is little control over environmental factors. When more factors can be regulated, indoor cultivation is approached: the mushroom house becomes increasingly expensive with increasing control over the climate. Stability of yield and spreading of production is possible when the mushroom house is air-conditioned, but this is rather expensive. Outside cultivation is very cheap, but depends on natural conditions and is therefore more prone to crop failure. Resources of the farmers, availability of technology, price of product and costs of growing determine the investment. There are numerous publications dealing with completely air-conditioned mushroom houses, but relatively few on simple, cost-effective constructions. A number of these are described in this chapter.

12.1.2 One room for all phases or each phase its own room
There are basically two concepts in the way substrate and climate are treated:
1. leave the substrate in the same place and change the surrounding conditions;
2. move the substrate from one room to another, each designed specifically to meet the demands of the specific phase.

1. *Agaricus* cultivation before 1980 in The Netherlands on shelves, where growers used to pasteurise and spawn the substrate themselves, is developed around the first view. The substrate is left in the shelves for subsequent phases. Each growing room must be facilitated with equipment to handle all climatic conditions which occur during the cultivation process. This can mean rather high building costs but it requires little handling during the growth cycle.

2. The technology of growing *Flammulina* in Taiwan and Japan is clearly developed around the second view: substrate is moved from sterilisation chambers to spawn run rooms to fruiting, controlling and harvesting rooms. Another example of this concept is described in the case study on the *Volvariella* farm by Mignucci in Puerto Rico. In each room the climate regulation equipment is optimised for the specific phase of mushroom growing. The disadvantage of these kinds of systems is the constant flow of substrates through the farm from room to room. A clever design and the use of trays can still lead to efficient operations.

For any given mushroom species both technologies can be applied successfully. A number of *Agaricus* farms is built according to the second concept. The substrate is moved around in these farms in trays. In some cases only spawn run is performed in a separate room, but cropping and picking in the same room.
### 12.1.3 Farm layout

Before one can start to plan the layout, the processes which will have to be performed at the mushroom farm have to be listed. Whether or not an inoculation room is required, for example, depends on whether growers prepare their own substrate or buy already inoculated substrate. Furthermore, the farm layout should provide:

- an efficient flow of substrate materials,
- measures to prevent contamination on the farm,
- efficient use of space.

<table>
<thead>
<tr>
<th>Process</th>
<th>Requirements</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>storage of dry substrate</td>
<td>A dry room, where the ingredients are shielded from outside conditions like rain and (possibly ingredients contaminated) wind.</td>
<td>Depending on the amount of material which has to be kept in stock.</td>
</tr>
<tr>
<td>fermentation</td>
<td>Concrete floor, preferably with a roof. The floor should be inclined (0,5 cm per metre) to collect percolate water. Plenty of water should be available.</td>
<td>The size of the floor depends on the dimensions of the heaps. For Agaricus: the heaps are 2 metres wide with a variable length. Between the heaps at least 1 metre is necessary for turning by machines. If turned by hand, one heap should have additional space of 4 metres on one side. One metre of the heaps will result in about 1,5 tonnes of compost.</td>
</tr>
<tr>
<td>mixing and moistening of substrate ingredients</td>
<td>Concrete floor and/or substrate mixers.</td>
<td>About 24 m² is needed to mix three tonnes of substrate; dimensions of the mixer depend on the amount of material to be mixed per batch.</td>
</tr>
<tr>
<td>pasteurisation by steam</td>
<td>Either a tunnel or in beds Steam boiler with sufficient capacity; for wheat straw substrate: 1.1 kW per tonne.</td>
<td>1 m³ can contain about 400-500 kg of substrate.</td>
</tr>
<tr>
<td>pasteurisation by immersion in hot water</td>
<td>Waterproof containers and means to keep the water at 70 °C.</td>
<td>1 m³ can contain about 400-500 kg of substrate.</td>
</tr>
<tr>
<td>spawning pasteurised substrates</td>
<td>Preferably a closed room with overpressure and filtered air.</td>
<td>Depends on the amount of substrate to be spawned per batch.</td>
</tr>
<tr>
<td>spawning sterilised substrates</td>
<td>Closed room with a laminar flow or a clean room.</td>
<td>If the room has a capacity of the substrate production of one day, the sterilised substrate can cool down in a sterile environment, thus reducing the risk of contamination.</td>
</tr>
<tr>
<td>Process</td>
<td>Requirements</td>
<td>Dimensions</td>
</tr>
<tr>
<td>------------</td>
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<td>------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>spawn run</td>
<td>A room with constant temperature, shielded from insects and airborne spores.</td>
<td>Substrates can be stacked more densely during spawn run than during cropping; up to twice the amount of substrate can be put in a spawn run room compared to a cropping room. The demand for cooling depends much on the used technique, outside temperatures and how much substrate is packed into a room.</td>
</tr>
<tr>
<td>cropping</td>
<td>A room with ventilation, high humidity, and temperature control.</td>
<td>About 50-100 kg of substrate can be placed per m², depending on the type of bag or quality of the substrate involved.</td>
</tr>
<tr>
<td>harvesting</td>
<td>Easy access to the crop (which is easier with tray cultivation than with cultivation in beds).</td>
<td></td>
</tr>
<tr>
<td>storage</td>
<td>Cold room, heat-conductivity of insulated harvested floor: 0.58 W/m²; heat conductivity of ceiling product and walls: 0.25 W/m².</td>
<td>Depends on the way the harvested mushrooms are packed. Between each pallet at least 10 cm of free air should be reserved.</td>
</tr>
</tbody>
</table>

### 12.1.4 Standardisation of mushroom farms

If a project is planned for a larger area it is advisable to standardise the mushroom houses. Individual results can then easily be compared and standardisation is also an advantage if climatic conditions like ventilation have to be considered. The success of mushroom growing in The Netherlands and some years ago in Taiwan is due to a number of factors, amongst which standardisation is one of the most important, along with research and extension services and financial climate. In The Netherlands the high degree of mechanisation was only possible because all growers used similarly-sized growing rooms. Filling, casing, spawning etc. are all carried out by machines which are made for beds with specific dimensions. If every grower had invented his own system mechanisation would not have been possible. Climate control is also strongly affected by the dimensions of the growing rooms. Farmers who built differently dimensioned growing rooms usually encountered problems in obtaining uniform climatic conditions everywhere in the room.

### 12.2 Outdoor cultivation: Wood log production of Shiitake

Outdoor cultivation is very similar to the growth of the Shiitake in nature, the costs are therefore limited. On the other hand, the grower has rather limited control over the environment: actually only rain and sun covers are available. The spawn run time (laying) is divided into two periods that require somewhat different conditions. During temporary spawn run (first stage of spawn run) sometimes a plastic film, bamboo mats or similar material, are used to keep the humidity at the required level. The laying yard
should not be located on a swampy soil or in the reach of ground fogs.

**Shading:** during fruiting, the logs are either raised in a forest with sufficient shading or artificial shading is arranged. Artificial shading can be provided by a black plastic net that allows aeration and rain to pass. A more simple construction in mainland China covers the roof of a very simple wooden structure with leaves.

**Conditions for a permanent yard (second stage of spawn run):**
- Temperature between 18-27 °C
- Humidity 50 to 70%
- Air circulation moderate
- Light: some light is necessary for fruiting, direct sunlight should be avoided to prevent the logs from drying out

**Protection from rain:** when the mushrooms are maturing, they have to be protected from rain, otherwise their quality will be reduced and the mushrooms will be more difficult to dry. Therefore a facility must be available to cover the rows of logs with a plastic film. This facility can be attached to the artificial shading construction or can be a free-standing shed-like construction. Water should be easily available year-round because the logs have to be watered frequently. To prevent the growing area from becoming too swampy, most growers construct drenches to drain the growing area. In case the logs receive a water bath prior to fruiting, some large containers that can be filled with water should be present. To facilitate handling, these may consist of concrete ponds adjacent to the wood log rows. If the temperature is appropriate, but the ambient humidity is too low, then it is advisable to grow the logs in a tent-like construction to keep the right humidity.

### 12.3 Intercropping with vegetables

Intercropping vegetables and mushrooms has the following advantages:
- the same piece of land can yield an extra crop,
- the vegetables provide shade and air humidity for the mushrooms,
the spent mushroom substrate can be used as a fertiliser or soil conditioner. The main disadvantages are:
- insects and airborne contaminants have easy access to the substrate and mushrooms,
- labour-intensive method with low picking yields compared to growing in specially designed rooms.

Intercropping is therefore most suitable in countries with low wages for mushrooms which either grow very fast (Volvariella; refer to chapter 20: Intercropping corn and Volvariella), or which are less susceptible to pests and diseases (like Ganoderma and Auricularia).

In mainland China a method has been developed to grow Auricularia in rows between sugarcane. The sugarcane provides the right humidity and shade for Auricularia to fruit. The distance between the rows of sugarcane is 1 to 1.5 m. The roots of the sugarcane grow in elevated 'dikes'. The sterilised plastic bags with Auricularia substrate are hung on bamboo sticks between the sugarcane, as shown in the figure. Plastic is laid on top of the ditches to increase the humidity. The CO₂ from the Auricularia mycelium is said to stimulate the growth of the sugarcane.

Pest and disease control is difficult to achieve, but as the bags are hung free from the ground, and Auricularia is less susceptible than most other mushrooms (because of its rubbery texture), acceptable results have been reached with this method. How to prepare the bags can be found in the chapter Cultivation on sterilised substrates, in the section on Auricularia.

12.4 Indoor cultivation: considerations for growing rooms design

12.4.1 General shape

The shape of the rooms should allow for:
- good air circulation,
- easy management of substrates.

Most growing rooms are rectangular, with a length to width ratio of about 3:1. Rectangular rooms have the advantage that ducts, running along the length of the room, can uniformly distribute conditioned air throughout the growing room. Growing rooms with curved roofs (like halfround sheds) have the advantage that there will be few dead air pockets. Growing rooms with flat roofs can also function well, if care is taken to avoid condensation.

12.4.2 Insulation

Energy can be saved and the climate can be much better controlled if the growing rooms are well insulated. Agaricus-compost in poorly insulated mushroom houses is difficult to pasteurise; the heat leaks away and the compost doesn't reach the necessary high temperatures, unless high capacity steam boilers are used. A simple insulation
option is to cover the lower part of the growing house with earth. Alternatively, growing rooms in China have been dug in the ground. In fact this is why mushroom growing in caves requires only little energy. Growing in caves however requires more labour, which is the reason why more and more French growers are ‘coming out of the caves’. In some cases (Cuba, China) mushrooms were grown in bomb-proof defence tunnels. Insulation materials range from sandwich panels, consisting of galvanised steel with a plastic coating and poly-urethane inside, to roofs thatched with rice straw. Poly-urethane can also be sprayed against the walls; a disadvantage is that it is rather vulnerable to scratches. Another way of insulation is to use two sheets of plastic with sheets of rock wool or glass wool in between, as is the case with modern sheds.

12.4.3 Climate control
The different ways to control the climate are discussed in the previous chapter. When designing the growing rooms, sufficient room and holes in the walls should be planned for ducts, water pipes, electricity etc.

12.4.4 Floors
Slightly inclined cemented floors provide a smooth surface which can easily be cleaned. The tilted floors allow excess water to drain. A screened basket should collect the coarse debris. The drainage system of different cells should not be connected; otherwise a disease in one growing room can easily spread to other rooms. The floors should also be smooth to facilitate handling and transport of materials. The cement can also be isolated, or the material below it. Sea shells provide a natural isolation material which is sometimes used for this purpose.

12.4.5 Doors, windows and other openings
Doors should close adequately to prevent insects from entering the growing rooms. A double door, with a wire mesh for the second entrance, can help in keeping insects out, too. The same rules apply for windows. The openings through which air is either blown in or out of the rooms should have at least a simple filter or cloth before them. The smell of mushroom mycelium is very attractive to flies.

12.5 Sheds
A simple shed basically consists of half-round bent metal pipes or flexible construction material covered with plastic. The temperature can be controlled to some extent by
shielding the plastic with bamboo mats to provide shade and opening the plastic to keep the temperature down by increasing ventilation; the latter also reduces humidity. Raising the temperature is of course possible by giving the sun free access to the plastic. These kinds of growing areas have the advantage that they are cheap, but climate control is very limited. There is also no good protection against pests and diseases. Modern sheds, as they are used in Ireland, are well insulated (R-value ca. 4). Important aspects are the lifetime of the plastic cover, as degradation of the plastic occurs under the influence of UV light. The connection of the sheds to a corridor is more difficult, compared to a rectangular building.

12.6 Mushroom houses in the Philippines

For indoor mushroom cultivation a concrete building or a metal frame with a plastic cover can be used. A simple construction of locally available materials will do too. The maintenance costs are somewhat higher, but the investment is much lower. In the Philippines a mushroom house for 2000 bags (1.2 kg each) built of straw mats, nipa and/or cogon grasses can be built for 7000 P (approximately US$ 225, 1991, materials only). Only a limited surface area is needed as the mushrooms are grown in shelves. These shelves are usually made out of bamboo, but wood will do, too. For Agaricus and Volvariella cultivation it is necessary that the substrates in the houses can be pasteurised. The size of the houses has to be adapted to the capacity of the steamer. A good size for a Volvariella farm in the Philippines is 4 m wide, 6 m long, 2.5 m high. Under ideal circumstances, the floor is made of cement to facilitate cleaning. The house has to be closed during pasteurisation, so the doors and windows must fit tightly. Two oil drums connected with a pipe can provide enough steam to pasteurise the mushroom house.
12.6.1 Concrete mushroom farms in Malaysia
The traditional mushroom farms in Malaysia are built of attap, but concrete houses have some advantages, like:
1. providing good protection against pests like insects and rodents,
2. relative ease of maintenance,
3. durability.
A disadvantage is that concrete houses are more expensive. In Malaysia a concrete house can be built at a cost of US$ 2600, while an attap barn costs US$ 2110. Ordinary farm houses converted into mushroom houses by lining with a plastic sheet do not provide sufficient aeration and pests will have easy access. The basic structure of the reported mushroom house in Malaysia consists of a wooden roof truss on reinforced cement columns. Dimensions of the growing room are: 17 m long, 7 m wide and 4.5 m high. The walls are constructed of hollow concrete blocks on a sandy soil. The hollow blocks provide considerable insulation during hot sunny days. Polystyrene foam is used to line the asbestos roofing, again to provide good insulation. (Asbestos is hazardous to health and its use should be avoided. Its use is quoted in the original report, however.) Windows in the walls provide the necessary aeration, for instance after chemical fumigation. A 0.6 m wide opening running all along the ridge of the roof and covered by a 0.3 m elevated asbestos roof further increases ventilation. All cracks and seams were sealed during the construction of the farm. The windows and doors are all covered with mosquito nets. The entrance consists of a double door, the inner one with a mosquito net. A farm like this can be built on open farm land, but preferably under trees for extra shade. The racks consist of preserved soft wood. A water spraying installation supports sprayers every 1 m between the racks.
(Reported by C.C. Tong and Z.C. Chen in Mushroom Journal for the Tropics, 1990, vol. 10)

12.7 Ho-type mushroom house in Taiwan
The Ho-type mushroom house was originally designed for Agaricus production by Prof. M.S. Ho of the Taiwan Provincial Farmers Organisation. The house can be used for mushroom cultivation in beds on bulk substrates, for Tremella cultivation on wood logs and for the cultivation of various mushrooms in small sterilised bags.
The lifespan of the houses is determined by the number of heat treatments. These cause the incorporated plastic to age rapidly. The lifespan of mushroom houses in which Agaricus is grown (which requires a heat treatment in the growing room) in Taiwan is generally three to five years. If they are used for other mushrooms, they can last for over ten years, thus reducing the costs of growing. Some of the houses
are covered with a 7 cm layer of rice straw. This effectively shields the house from sunshine and heat penetration.

**Bed cultivation:** Two tiers of bamboo-framed shelves run along the entire length of the house, except for an alley at each end. Total production area is 150 m². There are five shelves to each tier, thus each shelf has a growing surface of 15 m². There is a space of 55 cm between the shelves, the lowest one starts at 13 cm from the ground. The footpath between the tiers, the side walls and the edge of the beds is 0.6 m. A plastic sheet covering has proved to be effective to keep up the temperature during the heat treatment.

The bamboo has to be sterilised before use with a diluted PCP-Na solution for three and one-quarter hours. Perforations have to be made in each node of the bamboo before the treatment. Ventilation is provided by installing a 0.5 or 0.25 horsepower blower. The fresh air is distributed in the house by means of a polyethylene duct with perforations. The air inlet, doors and windows are shielded with a mosquito net to keep insects out. The Ho-type mushroom house is commonly used for *Agaricus* and *Volvariella* production in Taiwan. Composting and substrate preparation are performed outside. Heat treatment, spawning, spawn run and fructification all take place inside.

**Cultivation in small bags:** Usually tiers are constructed from locally available materials. Sometimes only the floor of a mushroom house is used, because the temperature on the floor is lower than in the rest of the house. This depends on the selected strain and local temperatures.

### 12.7.1 Experimental Volvariella farm in Puerto Rico

In Puerto Rico, a design for a *Volvariella* cultivating farm has been developed by Mignucci and others. The design of the farm is such that each cultivation stage is performed in a specific room. This means that the substrates have to be moved to successive rooms. There is more control of climatic conditions than in the simple mushroom houses for *Volvariella* in the Philippines (see above).

The farm is divided into five areas: 1. storage, 2. fermentation, 3. pasteurisation and spawning, 4. vegetative growth, 5. fruiting.

The storage room (1) has a height of 5.9 m. It has a big sliding door with a width of 3.05 m and a height of 3.05 cm. This provides easy access for trucks delivering the substrate materials. All other rooms have a size of 6.1 x 3.7 m².

Both the composting area (2) and the pasteurisation room (3) have cement floors. A steam boiler with a capacity of 250 kg steam/hour (running on liquid gas) is contained in a separate room, between (3) and (4). A pipeline system distributes the steam over rooms (3), (4) and (5), with valves controlling the steam inlet.
Volvarella cultivating farm in Puerto Rico (adapted from Mignucci). Upper drawing shows side view, bottom drawing top view. Notice that substrate streams don't cross to avoid contamination.

The room for vegetative growth (4) is lower than the other rooms, because the cultivation baskets can be stacked at this stage. Substrate temperature can easily be kept at 35 °C without using much energy. The fructification room (5) has fans installed 2.1 m from the floor. It has a translucent roof panel to allow daylight to stimulate fruiting. Side windows further contribute to the light requirements of the straw mushroom. The roofs of rooms 1, 2, 3 and 4 are all opaque. They are inclined to allow quick drainage of water during heavy rains. Rooms (3), (4), and (5) are equipped with a sliding metal door with an inside screen to prevent insects from flying in. Plastic baskets measuring 55.6 x 46.3 x 32.5 cm³ are reported to be used as substrate containers. These can be stacked on wheeled frames, which can easily be moved from room to room. The advantages of this design are the following:

- A continuous production of mushrooms: all stages are performed simultaneously;
- The division in compartments allows for good sanitation measures;
- The structure is designed for low energy and machine input.

A disadvantage is that it requires rather high investments and the design cannot easily be adapted by small farmers. The future will show whether this design will gain popularity.
13 Advanced mushroom farms and tunnels

By Luc Klunder, Gicom BV

13.1 Introduction

As will be mentioned in chapter 17, the main goal for the production of Agaricus mushroom compost is to create a selective and homogeneous substrate. At most modern mushroom farms, the preparation of such compost is done outside the growing room. The composting process is divided in 3 stages or phases before the substrate is filled in the growing rooms. This chapter describes the most commonly used methods of preparing the substrate and modern production facilities.

The diversity in modern mushroom farms is huge. Mainly due to different logistics, climate conditions, marketing methods and history, each region has its own way of producing mushrooms. Therefore this chapter will highlight the most commonly used composting and growing facilities.

The key word with mushroom growing has always been hygiene. This starts already with the selection of raw materials and continues nowadays even beyond the consumer’s kitchen. Although it is often considered an agricultural activity, the impact of little mistakes in setting up, building and managing a farm is devastating.

Apart from factors that are critical to any other business entity, like accessibility, labour, and availability of raw materials, the hygienic aspect is very important. Preferably a farm is set up in such a way that raw materials enter the facility on one road, the compost or mushrooms leave the farm on a totally different road. On the premises the routing of the product should be done in such a way that no material has to go back to previous stages. If possible prevailing wind should be taken into account so the wind will take away the spores instead of blowing spores from one side to another. For those farms that also perform composting, neighbourhood acceptance is another important issue. Wind is a transport medium for odours as well.

When setting up a new facility, it is absolutely prudent to take future expansion into account, regardless of the intended system. Most successful mushroom farms have been expanding over time. Modular systems, with a logical batch size and enough free space around therefore deserve preference, because that is what enables the expansion. Both composting and the growth of mushrooms demand special attention towards the selection of building materials, climate control equipment and machinery. The following conditions determine the choice of specific materials:

- high temperatures combined with high absolute humidity
- large variations in temperature from one room or tunnel to another
- compost, percolate and condensation water that are highly corrosive
- frequent use of water, sometimes under high pressure or with corrosive groundwater
• contact with chemicals and frequent use of disinfectants
• corrosive vapours.
Builders and contractors, who have never been active in building and installing tunnels and growing rooms, often underestimate the problems and the special requirements. It is therefore advisable to seek co-operation with experienced companies. Looking for evidence of quality by visiting existing compost producers or growers, rather than trusting nice brochures, prevents a lot of aggravation, regrets and financial losses.

13.2 Phase I installations

Some 30 years ago it was found that there are several benefits of a phase I system with forced aeration:
• The fermentation becomes more controllable
• The process is more consistent
• The process is more thorough throughout the material and, as a result, it can be speeded up
• Less raw materials are required
• Less influence from weather conditions
• Odours occurring from anaerobic compost are minimised, and – as it became more important due to more awareness of neighbours – odours that are escaping from the installation can be collected and treated.

These benefits – especially those that have environmental effect – have had a deep impact on modern mushroom farms.

The principle of any aerated system is a ventilation system combined with a type of floor in which air can be distributed.

Depending on how far the composting process needs to be enclosed, several systems are distinguished. There are aerated floors, bunkers and tunnels. Furthermore the pre-treatment of the compost can be done outside, partially outside, or fully indoors.

An aerated floor is constructed out of concrete, either a grid floor or a spigot floor. This aeration floor is fed with a ventilator, providing the air.

Once walls are constructed that divide the aerated floor into several separate boxes, the system is referred to as bunkers.

A step further in the evolution of forced aeration starts when the composting process takes place in a totally enclosed box: the tunnel. A tunnel provides the possibility for circulation of the air. Influences from rain, snow, sun or wind are minimised. Most modern tunnels are constructed out of concrete. Dimensions of these tunnels vary in length, width and height, depending on the necessary amount of compost, raw material input and on the aeration system.

With compost temperatures up to 84 °C or 183 °F special attention should be given to pouring the concrete. It should be built strong enough to prevent the occurrence of cracks.

13.2.1 Fully indoors

The term ‘fully indoors’ refers to a completely enclosed building, in which preparation of the compost mix, transport of the compost and the composting process are done inside a building.
13.2.2 **Floor types**

Originally forced aeration of phase I compost was done on grid floors, similar to the floors that are in use for phase II and III composting. Due to a large opening in the floor – 30% opening or more – the composting becomes less flexible and material easily falls through the floor, which makes it necessary to clean the plenum underneath the grids.

In the early 90s the first spigot floors were developed. With this type of floor, a means of very thorough aeration is provided into a reinforced concrete floor. Due to its construction this type of floor can be made strong enough to empty the floor with heavy loaders.

A spigot floor is built up out of pipes with conical shaped nozzles. From one side the pipes are fed with air from a fan, providing the aeration. The number of pipes and nozzles, the distance between pipes and in the longitude between the nozzles determines the matrix of aeration. The diameter of the hole in the top of the spigot is a balance between air resistance, turbulence and air speed. Both the matrix and the diam-
to ventilate the compost, so moisture can be taken out and the compost can be cooled. Several systems can be distinguished. There are floors, bunkers and tunnels with a central fan and there are systems with one fan per tunnel.

**Central fan systems.** In the central fan systems, one fan provides air to a central duct. From this duct, branches provided with a valve distribute the air over various bunkers or tunnels. This is a relatively low-cost system, both in investment and in costs per ton. A benefit is that the central fan runs more constantly, so less start-up time is required. The system is at pressure straight away. Circulation of air is possible, yet air cannot be blown into exactly the same tunnel as where it comes from.

**One fan per bunker or per tunnel.** With this set-up each tunnel has its own fan. When it comes to management, this system might be easier to understand than the central fan system, due to a more direct link between fan movement and aeration of the compost. However, this system is more expensive when it comes to investment and generally consumes more energy.

In a tunnel installation, this system can easily be provided with the possibility of air-circulation. Composting with higher ammonia levels then becomes an option. This seems to have benefits, yet it is still not fully understood.

The choice of a fan is determined by several factors:
- The backpressure
• The volume of air required  
• Aggressiveness of the air (ammonia, moisture and temperature)

In general, backpressure and volume of air trade places. If the resistance that the air gets in ducting, the floor and in the compost, goes up, the air volume that a fan can supply goes down at a certain level of energy consumption.

When it comes to pressure, in general there are 2 systems distinguished; a low pressure system and a high pressure system. The latter gains popularity.

The low pressure system can be found mainly with phase I grid floors. With such a floor, large volumes of air are necessary, and pressure loss is relatively small.

In a spigot floor-type tunnel, resistance needs to be higher for air distribution purposes. Therefore the fan necessary for such a floor is generally capable of supplying higher pressures, up to 7 kPa. This does not mean that the process works with this pressure — if it is necessary to work with such pressures, the compost mix is either too wet or too densely filled. With such fans, it is possible to fill more tons per square metre of tunnel, and slightly more variation in moisture content can be handled. Yet, the latter is only marginal.

Quantities of air vary in practice between 5 m³/hour/ton up to 20 m³/hour/ton, again depending on the raw materials, the possibility of circulation of air, outside climate circumstances and the discipline of the workers creating the blend.

The aggressiveness of air needs to get special attention if air is circulated. With a combination of high levels of ammonia, warm temperatures and high moisture levels the ideal climate is introduced to cause corrosion of steel, even if it is coated, hot dipped galvanised or painted.

13.3 Environment, odour and ammonia

Over the last decades most compost producers and mushroom growers felt the growing pressure of environmentally related topics. Whether initiated by neighbours, law or environmental groups, every compost producer will sooner or later be facing the necessity of adapting the system to one with less odour, ammonia, water or noise emissions. Environmentally sound installations therefore have a better opportunity of staying in business in the long run.

**Water.** Water emission is merely a matter of good management and is very much related to how an installation is built. While implementing a composting farm, serious attention should be given to water flows. Mixing clean, dirty and intermediate water flows in an early stage, is often a cause of an excess of dirty water, especially in those areas where melting snow or heavy rainfall are responsible for a large part of the an-
nual rainfall. Keeping the rainwater, the percolate and the clean water separate as long as possible pays off. Costs for cleaning or discarding the water are rising around the world. Composters who can clean or reuse their water will therefore save costs and gain credit amongst environmental groups and the public.

Also, water is a very important ingredient for each composting process. Although scientists are still searching for what makes one compost different from another, it is known that healthy water creates better compost than dead water. With increasing organic substances in the water and with rising temperatures, water movement and aeration become more important to keep the right flora and fauna in the water and to reduce odour (see below).

**Noise.** Noise pollution is regarded as a problem in densely populated areas. Most of the solutions can be found in the selection of the right building materials. Since most of these come from other industrial processes, it is not specific for the mushroom industry and is therefore left without further illustration.

**Ammonia and odour.** Ammonia and odour control are often considered the problems most difficult to tackle for composters. Although the environmental pressure is highest at phase I installations, some phase II installations and an occasional growing facility face the same problems. At combined farms where both compost is made and mushrooms are grown, negative emotions from neighbours might be greater than their willingness to distinguish between the odour sources of chicken manure, percolate water, phase I, II or III compost and spent mushroom compost.

The most important issue when tackling the odour problem is to start with the source. Anaerobic organic matter is most likely the biggest source of odour. By providing a good aeration system, both percolate water and compost can be held aerobic. Although odour will still be produced, the amount of odour and the intensity of the odour can be reduced tremendously.

Also storage of raw material such as water and (chicken) manure should be kept to a minimum in order to have the smallest creation of odour.

In case these measures at the source are not sufficient, several purifying devices or modules can be applied for odour and ammonia control. The most commonly used modules are described in the paragraphs below.

### 13.3.1 Biofilter

In general a biofilter is an aerated pad where the odorous air is blown through a bed of an organic carbon source, such as wood or root chucks, or spent mushroom compost. If kept moist enough and at the right temperature, bacteria surviving on the carbon source, reduce the odorous compounds in the air. It is therefore critical that the biofilter is proceeded with a water washer and that adding cooling air in the process air flow must be possible.

Floors are constructed either from grids or with spigot pipes. In areas with a high sunlight intensity, covering the biofilter will stretch the lifetime of the biofilter material. Although dead materials are used in some filters – such as polystyrene – the composting process lends itself better for living material. Airflows are never constant either in volume or in odour and ammonia concentration. This makes it harder for bacteria to adapt to the ever-changing habitat. Organic material such as wood or bark carries enough
13.3.2 Acid washer

In most countries around the world ammonia in the exhaust air is not a major problem. Ammonia is not always considered a severe odour and dissipates quickly in fresh air. However, local laws might force the compost to gain back some of the nitrogen or ammonia out of the exhaust air. Moreover, acid washers can be found in combination with biofilters, because biofilters cannot stand the high ammonia rates that normally escape from the phase 1 process.

In the acid washer, normally a tank where process air is slowed down, water with dissolved acid is sprayed over the process air. The pH of this water is kept around pH 4. The acid reacts with ammonia and creates a salt called ammonium sulphate. If the salt in the water reaches a certain concentration, the salt crystallises. Prior to that the salty water needs to be refreshed. This salty water can be reused as a nitrogen fertiliser. Ammonia reductions of 95% or more can be achieved. Important is that the washed air is then washed with neutralising water, so pH becomes 6 to 7 again. Any concrete or steel that comes in contact with non-neutralised water will erode or corrode quickly.

13.3.3 Water washer

As stand alone device, as a next step after an acid washer or as a humidifier in front of a biofilter, a water washer can be built. In the water washer air speed is reduced and water is sprayed over the water. The water washer can enhance the ammonia and odour reduction slightly, yet only limited to the point of saturation. Beyond that a water washer starts returning the am-
monia in the airstream. In case the water is used too long, or not held aerobic, it starts contributing in odour production.

13.3.4 Strawfilter
A special biofilter is the strawfilter. Used mainly at facilities where both odour problems occurred and where fresh straw is used as a raw ingredient for composting. Instead of using wood, the biofilter floor is covered with straw. After several weeks of usage as carbon source for odour-removing bacteria, the straw can be reused in the composting process.

13.3.5 Chimney
The simplest device to minimise odour problems is a chimney. Although in itself it does not lower the odour concentration a lot, it can be found at many composting facilities in combination with an acid washer or a biofilter. The goal is then to bring the exhausting air higher in the atmosphere, where the air dilutes faster. Provided it is built out of stainless steel or other lasting material, costs for a chimney are only made during construction.

13.4 Phase II/III tunnels
By preparing full-grown compost in bulk, land, labour and machinery can be used more efficiently. Phase II and III tunnels can be considered as bioreactors in which selective compost mixed with spawn is left under optimal climate conditions for opti-
mal growth of the mycelium. The spawn run influences the further growth of the mushroom severely.

A good spawn run is only possible if climate conditions in the tunnels are right, the compost is selective and all hygienic precautions are taken care of. In modern mushroom growing, farms can only survive if the production method is right. Mistakes in building tunnels will definitely lead to a loss of quality and quantity of the mushrooms. Therefore hygiene can be considered the most important matter for planning and running phase II and III tunnels.

Dimensions of phase II and III tunnels vary, depending on the required batch. Although exceptions occur, phase II/III tunnels are 4 metres wide and vary in length between 20 and 40 metres. The roof is normally 4-4.5 metres above the grid floor. Older generations of tunnels have a filling capacity starting with 800 kg/m². Modern tunnels are designed for filling capacities of 1.500 kg/m² and go beyond that in practice. This is possible due to improved climate control techniques. Due to raw material loss – approximately 30-40% of the input mass during phase II composting and 7-15% in phase III – the output of the phase II tunnels can be filled in less phase III tunnels. In practice this often is from 3 phase II tunnels into 2 phase III tunnels. A minimum of 4 tunnels is required (at any given moment 1 phase II tunnel and 3 phase III tunnels) in order to use all tunnels for both phases II and III.

The materials available, outside wheather conditions, building requirements, together with the various alternatives for mechanisation, determine in no particular order the most optimum set-up for tunnels.

13.4.1 Foundations and floors

Foundations of tunnels depend largely on the circumstances of the bottom on which is being built. Local requirements make this topic too diverse to discuss for this book.

Careful attention should be given to drainage. Poor drainage in the floor often is the cause of contamination later in time, which results in poor hygiene. Since this is poured in the concrete this can not be modified later – as with most building activities.

Although the spigot floor type has gained much popularity in phase I, this type of floor is less favourable for phase II and especially for phase III. The benefit of being able to empty the tunnels by a loader, instantaneously creates a more severe pressure on hygiene of the installation, especially because the walls need to be constructed out of a stronger material, like concrete, than panels or gas concrete. This does not benefit the isolation value, and therefore enhances changes for condensation in the compost. Hygiene problems occur more easily at phase II and III tunnels with spigot floors in comparison to plenum-type tunnels.

Most tunnels are equipped with a plenum floor, which is covered with concrete grids. A glide and pull net make it possible to empty the tunnel automatically. The floor needs isolation. A slope in the floor of 1-2% with the deepest plenum at the inblow side significantly improves drainage and air distribution in the plenum. In order to have no influence from other tunnels, floors are better poured separately without being in direct contact with neighbouring tunnels. Higher backpressures from the fans make it important to construct the inside of the plenum out of concrete or gas concrete that is covered
by bricks on which the grids can rest. Also the supports of the tunnel winch justify concrete plenum front- and rear end walls.

Sideview from phase II/III tunnels with a fill and spawn hall and an empty hall. The bottom of the plenum should be built with a slope. The gridfloor is level.

A tunnel complex with cavity walls that completely separates one tunnel from another, has the benefit of having less heat- or cold bridges between tunnels. Due to warm air rising up within the cavity, tunnel walls remain dry from the outside, which increases the isolation value.

13.4.2 Steel structure
The steel structure needs to separate the tunnels from one another, and hold the outside cladding. This outside cladding is best if constructed out of isolated panels, so condensation at the inside of the building is minimised. Most steel structures also need to hold the climate control units and fill- and empty machinery. Also snow-, rain- and wind loads need to be considered. The compost in the tunnels create a load downwards and sideways. Therefore a construction company should design the steel structure. Steel structures are made of hot-dipped galvanised steel, finished with stainless steel bolts. This to withstand influences from ammonia, disinfectant, warmth and moist air.

13.4.3 Tunnel cladding
Most modern tunnels are constructed out of gas concrete (also known as autoclaved aerated- or cell concrete) or stainless steel panels with polyurethane filling. Condensation may not drip onto the compost.
Stainless steel panels with polyurethane filling are widely available all over the world. The isolation value of a 8 cm thick stainless steel panel is initially higher compared to a 15 cm gas concrete panel or block. This means that less building space is required. Also stainless steel panels are less easy to damage by rocks or steel in the compost compared to gas concrete. On the other hand, gas concrete is easier to repair, once damage is done. Despite of its light weight, gas concrete can be used as a supporting wall. The cavity between 2 tunnels should be left open at the top,
so natural ventilation can take place, keeping the outside of the cladding dry. Therefore the cavity should never be isolated.

Since gas concrete absorbs both cold and warm air it reacts slower to outside temperature and weather changes compared to stainless steel panels. As in growing rooms, with phase II and III tunnels the gas concrete takes care of a more consistent climate compared with stainless steel panels.

In general it can be said that the lifetime of gas concrete tunnels is considerably longer than stainless steel panels, mainly because the insulation value remains more constant over time. This combination of benefits normally justifies a slightly higher initial investment.

Both gas concrete and stainless steel panels need to be coated with a vapour-tight coating. Gas concrete tunnel walls are supplied with either marine plywood covering of the wall or with a special wallcoating that protects the wall from being damaged by stones or other hard materials that might be in the compost.

13.4.4 Doors

Phase II and III tunnels are constructed with one door for both filling and emptying, and with doors on both sides. Tunnels with one door require less floor surface, due to the lack of an extra hall. In this situation the climate units are normally mounted behind the rear wall in a technical corridor.

Tunnels with doors at both sides have a fill- and spawn hall on one side of the tunnels and an empty hall at the other side. The benefit of this set-up is that spawned compost never goes through the same hall as conditioned compost. Cross contamination changes are therefore reduced. Especially if spawning and emptying needs to be done in a limited time, or when there are too many tunnels in a row, this set-up is advantageous.

The climate units are normally on top of the tunnels. Air is blown underneath the compost through an air hose that crosses the tunnel from the roof to the plenum. This hose also has to be hooked off during filling or emptying.

Doors can be built either with a winch, so doors have hinges and are opened by hanging them over the top, or with a rail system, in which doors are lifted and rolled away in front of other tunnels. The latter option keeps the doors up straight, so gravity gets less chance to reformat the doorframes. Normally doors are built up out of stainless steel panels and have an inspection hatch. Doors need to be closed tightly, so no leakage occurs.
13.4.5 Isolation of the outside building
For isolation purposes – both under hot and cold atmospheric conditions – it is advised to build a roof over the tunnels. This roof needs to be steep enough for rainwater and snow to run off into a gutter where – even in case of a heavy rainfall – no water can run off into or upon the tunnels. Isolation of the outside building is often underestimated. The air above the tunnels can easily become much warmer than outside temperatures, so condensation under the roof construction is to be expected. Isolation with stainless steel panels is therefore a proven method of minimising this. The same applies for the spawn hall and the empty hall.

13.4.6 Ventilation
Phase II and III composting requires 150–200 m³/h per hour, per ton of fresh compost. The tendency towards more kilos of compost per square metre has led to higher pressures.

13.4.7 Cooling
If tunnels are built in an area with warm outside climate conditions (maximum summer temperature above 23 °C or very humid air during the hottest moment of the day) cooling is required for phase III tunnels. Especially if transport or unconditioned storage is involved, or if the cooling capacity in the growing rooms is limited. Cooling the compost is only possible by evaporation of water out of the compost. This always costs tonnes of compost and percentages of moist. Under climate conditions where cooling is required year-round, the cooling coil can be built in the circulation duct. However, in most areas the cooling is only necessary in several months of the year. Then the cooling coil can be built in the fresh air inlet, so less air resistance needs to be overcome. Cooling capacity differs around the world due to outside weather conditions. Fresh inlet air needs to be cooled back to 16 °C in order to be able to cool the compost.

13.5 Phase IV rooms
In production cycles where composting is geographically separated from growing rooms, phase IV rooms are distinguished as an intermediate phase. In this stage the spawn-runned compost is filled in trays and left to grow the mycelium in the top-soil up to the point of buttoning.
These rooms normally are not different from growing rooms, although the dimensions of the room are adapted to a forklift instead of pickers. Hence, the ceiling might be higher and the room might be longer and wider.
Ventilation and cooling capacities need to be higher in terms of floor surface compared to normal growing or picking rooms. Due to the relatively large amount of compost that is in such a room, both ventilation and cooling should be adapted. Cooling capacity to at least 10 °C is a minimum requirement.

13.6 Growing rooms
When the mycelium has colonised the compost, the mushroom houses are filled. Perhaps apart from the Pennsylvania doubles, found at the East Coast of the United States,
there are no standard dimensions for mushroom houses. In the past decades many farms have been specialising in a certain method of harvesting. Also improved machinery and climate control enables farms to adapt to market requirements rather than suppliers’ domination when building a farm. Labour, still a very important issue, combined with regulations regarding labour also has its influences.

The key element of hygiene of course remains. In practice this starts with picking the right location. Preferably not in the surroundings of any other growing facility. Marketing the mushrooms is easiest if production can be managed as evenly as possible. Especially for the fresh market it is desirable to have an even flow in the produced quantity of mushrooms. Depending on what growing schedule is chosen, the number of growing rooms should be determined – for instance a 4, 5, 6 or 7 weeks schedule requires respectively at least 4, 5, 6 or 7 rooms or a multitude of that number.

13.6.1 Foundations and floors
As with tunnels, foundations of rooms largely depend on the circumstances of the bottom on which is built. Consultation from local builders or engineers is therefore advised.

Special attention must be paid to the floors throughout the whole mushroom farm. Whether it concerns a tray farm or a shelf farm, thresholds or other irregularities in the working corridor create daily returning problems for internal transport. This also means that the floors must be well poured to allow smooth operation of revolving doors.

As with tunnels it is advisable to build a drainage system in which different water flows can be kept separated. Since hygiene is such an important aspect, cleaning is a daily returning task. It is therefore advisable to lay gutters, protected by grilles, along the central gangway of each room, and along the working corridor. Each gutter should have a sunken drain trap in the middle. The drain grilles should both be strong and as small as possible, to withstand the use of fork trucks and lorries.

Rainwater drainage should be via PVC pipes with a diameter of at least 10 cm.

Upon a bed of sand, a work floor is poured. Putting some isolation between the sand and work floor is advisable. Upon the work floor a reinforced floor of at least 15 cm is
poured. Special attention should be given to expansion joints. A separately poured floor prevents the floor from cracking. Cracks lead to differences in temperature and are a potential hiding place for dirt and diseases. After the floor is poured, a layer of plastic protects the concrete from drying out.

The filling- and emptying floor at the back of a shelf farm needs to be thicker, since it needs to carry trucks and machinry. A 15 to 20 cm thick, reinforced floor is generally used. Temperatures of a room that is cooked out differ very much with local outside winter temperatures. Therefore the joint between this floor with the rooms, especially if the filling- and emptying floor is outside, demands a non standard approach.

13.6.2 Steel structure

In mushroom growing, the steel structure has to withstand influences from disinfectant, hot and cold temperatures and moist air. Therefore the steel structure is made out of hot-dipped galvanised steel. Preferably mushroom houses have a double wall with cavities in between that completely separate one room from another. In practice, however, many rooms are built with a single wall, simply because this is cheaper. The steel structure is then integrated in the walls. In order to prevent heat- or coldbridges between rooms, the steel structure should be well covered.

13.6.3 Room cladding

Rooms are constructed out of a diversity of materials. The choice normally depends on price and availability. Most modern rooms are constructed out of stainless steel panels with polyurethane filling or gas concrete (also known as autoclaved aerated- or cellconcrete). These materials have proven to isolate well, which increases climate control possibilities, prevents condensation, smoothen's temperature fluctuations, saves energy and withstands cooking out the rooms. The pros and cons for both materials are described in chapter 13.4.3, Tunnel cladding. Under moderate outside climate conditions rooms are generally built from 8 or 10 cm thick stainless steel panels. Walls and ceiling are generally finished with polymer paint, so the room becomes damp-tight. To increase visible hygiene a white colour paint is normally used. This also permits better lighting. The products used must not only offer a high degree of damp proofing, but also water and heat resistance. The wall and roof surfaces must be as flat as possible to ensure that an even layer of paint is obtained.

13.6.4 Large and small doors

The door panels too, should be made of aluminium or galvanised steel. The doors should be sealed effectively with rubber strips. Door frames of aluminium or galvanised steel should be chosen. Cold bridges should be prevented as far as possible. The doors must be provided with appropriate hinges or carriers and locks that allow opening from both sides. The large doors at the back of the room are often sliding doors which may only be opened from outside. It is advisable to fit one of them with a personnel access or escape door. If the filling is done directly from outside, the doors can also hold overpressure grids with a filter cloth.
13.6.5 Shelf farms
In the past shelf farms were provided with fairly standardised shelving, making mechanisation easier; the sideboards being the rails on which most of the machines run. Nowadays shelves are built in various dimensions, usually out of aluminium. In some countries labour regulations limit the width of the shelves and the distance between the shelves at handpicking farms. Pickers need access and visibility towards the mushrooms, without having to squeeze themselves in all sorts of difficult angles to reach places. Also if traditional mushroom strains are grown, more height between the shelves is preferable in order to have a better buffer of air, and hence a better climate control.
On the other hand shelves in use at farms that are harvesting the mushrooms mechanically become wider with increasing machinery capacity. Side effects from the shelves are then minimised.
The area around the shelves must provide enough space for pickers, packaging, machinery and air movement. While setting up a farm enough space should be considered for pickers and especially also for the mushrooms to grow. The air/compost ratio in a room is very important when it comes to controlling the climate.
Shelves with a vertical central pole are also in use. This type of shelves can be integrated in the construction, so savings can be made on the steel construction.

13.6.6 Tray farms
Trays have never had the amount of standardisation compared to the shelves. Tray farms can often be found in existing buildings or caves. The tray size might therefore be limited to access doors. Normally they are custom-made and constructed out of wood. Some trays are constructed out of stainless steel with aluminium cladding and a plastic bottom. Due to the many movements of trays the construction is a lot heavier in comparison with shelves. Wooden trays are normally cheaper to buy, but also need a lot of maintenance. Tray farms that shift towards tunnels for spawn run, can normally reduce their maintenance costs tremendously, which justifies the investment in tunnels substantially.
13.6.7 Isolation of the outside building
The outside cladding of a mushroom farm should be based on outside climate conditions. Both walls and roofs are often constructed out of stainless steel panels nowadays. Of course also brick walls or other normal building materials are applied. Flat roofs are not advised, since they require more maintenance than roofs with a slope. In addition, it is very difficult, if not impossible, to keep a flat roof free from little puddles of rainwater. In the long run this always carries the danger of leakage, no matter how watertight the material is.

13.6.8 Ancillary rooms
Rooms such as the boiler room, cold store, canteen, machine store etc., should be situated as functionally as possible. The gas company or local legislation normally sets standards for the size and layout of the boiler room if natural gas is to be used. The storage of pesticides etc. is strictly controlled. The design and fitting of ancillary rooms, particularly the size and location of doors, must take account of the amount and type of traffic expected. Factors such as the movement of mushrooms on pallets, and the size of the machines are important. Here too, uneven floors or thresholds will cause problems for wheeled transport. Functional location of the ancillary rooms is extremely important. Regarding the canteen, toilets and entrances a hygienic audit is important for quality control.

13.6.9 Water supply
At a mushroom farm water consumption is relatively high compared to other agricultural activities.
For watering the mushrooms (semi) automatic systems are available. For watering the mushrooms, and also with cleaning being such an important task, there should be at least one tap in each room, one per two rooms on the working corridor, and also one tap per two rooms on the compost floor side. The taps should be fitted with quick-release couplings.
Water must also be available at the canteen facilities, toilets, showers etc. Boiler water and disinfectant supplies must be fitted with a backflow prevention valve.
If allowed, wastewater can be discharged via the usual siphon traps to the sewer. At most modern farms water management calls for feasibility studies to reuse the water for cleaning purposes or even spraying.

13.6.10 Lighting
Good lighting is essential, both to monitor the growth of the mushrooms, and to facilitate work within the rooms. Lights should be mounted both at the ceiling and the walls. Special fittings are available for mushroom houses.
In order to fill the rooms with as few flies as possible, most growers try to fill the rooms early in the morning. Therefore it is also necessary to have good visibility at the compost filling area.
Composting is a natural process that happens all the time in nature. Fresh or active organic structures are broken down and reformatted by micro-organisms, leaving a material that finally has such long and complex structures of organic matter that no further breakdown occurs. An intermediate stage - between too active and stabilised material - is what the compost producer needs to spawn its mycelium upon. In nature this process of breakdown happens under both aerobic and anaerobic circumstances and might take decades or even centuries. As written in the former chapter, in modern mushroom farming this process is speeded up and made more controllable by building aerated floors or bunkers and tunnels.

For various mushroom strains modern climate control also means creating a climate that simulates the most favourable conditions for the compost to break down further. Once the buttons are occurring, climate control is used for looking for the best climate for the fungi to grow. It means simulating autumn weather conditions: a humid, cool breeze of refreshing air, normally without direct sunlight.

Each process control system starts with searching for the actual status. This is then compared to what it should be, and if necessary, changes are made to reach the desired status. From there on it starts all over again.

Most modern mushroom farmers are facing the challenge of finding the right balance between quality and quantity on one side, against the costs on the other side. For that a good process control system is crucial.

### 14.1 Measurement

#### 14.1.1 Temperature measurement

Normally electronic temperature probes are in use for measuring air temperatures – both wet and dry bulb, and compost temperatures. Several probes are available, yet only probes and their wires specially manufactured for the mushroom industry, can resist the combination of ammonia, percolate water, high temperatures and high humidity.

Some compost producers and growers prefer temperature probes that can be calibrated. However, this means that often temperatures over time start to deviate from the reading. This is a huge risk, especially because temperature measurement can be regarded
as the most important parameter during composting and growing. Most reliable are probes that can not be calibrated and give either an accurate reading, or no reading at all.

For wet bulb temperature measurement a textile socket dipped in water is placed over a temperature probe. This has to be changed regularly. Most sockets need to be washed out before use. A regular check of the water level is necessary.

14.1.2 Ventilation measurement
Only measurement of both air volume and air pressure can tell the compost producer or grower something about the ventilation. Especially in phase I, many mistakes are made by trusting that when the fan is running at 100%, a certain amount of air volume is given to the compost. This might however not be the case. The fan can be running at 100%, yet blow air not in the desired direction, leaving a wide variety in air volume. Wet compost, too dense compost, or overfilling a bunker or tunnel can create the situation in which the fan runs, but little or no air is blown through the material. Therefore volume and pressure measurement are both to be considered, also when building a phase I composting installation. At most modern climate units for phase II/III and for growing, this combination of 2 parameters is measured.

14.1.3 Oxygen
In phase III and I, oxygen is normally measured. With oxygen measurement, a sample of air is taken and guided towards a sensor. In phase I this can be done with a lance that sticks in the heap of compost. Through the tip of the lance, air is sucked up. At the other end of the lance or outside the bunker, a sensor is installed. From this sensor a signal is sent towards an oxygen meter, situated outside the bunker. This meter compares outside fresh air with the measured value and determines the oxygen level.

The sensors used around the world are not specially developed for the compost industry. They can not stand heavy shocks, or a sudden change of air. The sensor works with a glowing spiral at 600-700 °C. Therefore their lifetime, if constructed in a lance that is in the compost, is only a couple of months.

For phase I tunnels that are fully enclosed and for phase III tunnels, samples of air for oxygen measurement are taken from the circulation ducts near the climate unit. Several suppliers provide both individual systems and central systems. In the individual system each tunnel has its sensor. The central system works with a multiplexing switchbox. From each tunnel a sample is pumped through tubes to the switchbox and measured.

14.1.4 Carbon dioxide
Carbon dioxide (CO₂) measurement during growth of mushrooms is essential for good buttoning. The measurement in itself works similarly to oxygen measurement of air, only the sensor is made for carbon dioxide rather than for oxygen.

Due to the importance of fine measurement, carbon dioxide measurement should be calibrated regularly.
14. MODERN CLIMATE CONTROL INSTALLATIONS

14.2 Control

After measurement and turning electrical signals into understandable data, these data are computed. In the following sub-paragraph the general controllers are described. Further down the specific required climate is described.

14.2.1 Computer system

At modern composting installations and mushroom farms the computer takes a central role in the control system. Most computer systems work as a central system for storage of measured data, processor of this data and sending signals to the control equipment. Several suppliers in the mushroom industry sell either standard or custom-made software along with other controllers. In general this software provides more options than software provided by a local climate company.

![Software system for control and for examining data.](image)

In search for most optimum results, against lowest costs, the control becomes more complex. Controlling on moist deficit, rather than relative humidity, or taking warmth, moist- and carbon dioxide as dimension for energy release and using that as control factor shows good results with better quality and lower costs. Besides a central computer all sorts of accessories emerge at mushroom farms, making the (remote) control easier, such as palmtops or internet connection with the control system via GSM phone.

14.2.2 Air valves

Air valves for fresh air intake and air circulation are crucial regulators. Normally valves are servo controlled from 0-100%. For a good air volume control and air mixing of different air streams, contra-rotating air valves give the best control possibilities as a result of least turbulence within the ducting.

14.2.3 Switchboxes

Most fans are equipped with an individual switchbox, providing the electronics that are necessary for the control of that particular climate unit. As a result of cost-saving also more tunnels or rooms can be controlled with one switchbox. Obviously there is a risk involved; in case of a failure two or more rooms or tunnels are without control. Particular for the mushroom industry is the climate in which these switchboxes have to operate. Warmth, moisture, frequent use of cleaning water, ammonia during composting and disinfectants can easily harm the fine electronics.
14.2.4 Frequency drive
Throughout the process, most fans are provided with frequency drives. This part of equipment adjusts the frequency to a lower frequency between 0 and 100% of 50 or 60 Hertz. This means that a fan or sometimes a pump can be controlled in that range, adjusting it to the desired frequency.

14.2.5 Water
For mushroom growth automatic watering systems are available. For fine-tuning the whole climate system, a good automatic or semi-automatic watering system is necessary.

14.3 Specific control requirements
14.3.1 Phase 1 measurement
Considering all the monitoring and measurement further down in the process of growing mushrooms, very little attention is normally given to measuring parameters in phase I composting. Apart from readings of compost and air temperatures much substrate is produced with little or no monitoring of any important parameter whatsoever. Especially with starting compost producers, or for those for whom biology is not their strongest point of knowledge, this makes it harder to find a good basis of composting, let alone fine-tuning the process.
A number or parameters are indicators of either survival circumstances for biological life, or from energy levels released by the compost. The most important ones are:
- Temperature of compost and air
- Humidity or moist content
- Oxygen level
Apart from the composting itself, the reactions of the system towards the preparation of the input mix are of great importance. The most important parameters in this field are air pressure and air volume.

14.3.2 Phase I climatisation
In practice it is shown that phase I composting requires between 5 and 15 m³ of air per ton per hour. The air is necessary to provide oxygen, and to take energy and water vapour out. This at a pressure between 3200 and 4800 Pascals, depending on how wet and how heavy the material is filled on the floor (100 Pascals equals 1 millibar or 10.2 mm water column). Should more pressure be necessary, then the compost is made too wet or too much compost is on the floor in general.
The use of air or oxygen differs with the temperature of the material. During the stage of heating up much air is required. In practice fans run 80-90% of the time. In the stage between 60-75 °C the fan runs around 50% of the time. In the peak heat stage, from 75 up to 84 °C, the fan is on only 10-20% of the time. Fermentation is then a chemical reaction rather than a biological one and does not require so much oxygen.

Fans for phase I that have air circulation possibilities should be constructed out of stainless steel, for a longer lifetime. If fans only blow fresh air, galvanised steel is sufficient.

14.3.3 Phase II/III measurement
Most phase II/III tunnels are controlled on air temperatures, compost temperatures, air volume and air pressure. For phase III oxygen measurement provides important information.

Carbon dioxide measurement during phase III — although built in at several tunnels — has never taken off. The differences in carbon dioxide between tunnel air and fresh air are too small. A little bit of fresh air intake has a large influence on the measurement.

14.3.4 Phase II/III climatisation
As mentioned in the previous chapter, phase II and III composting requires 150-200 m³/h per hour, per ton of fresh compost. Due to heavier filling of the tunnels, there is a tendency towards more backpressure. Normally this varies between 2500-3500 Pascal. However, fans should be capable of bringing more.

Cooling of phase III compost is only possible by evaporation of water out of the tunnel. For that purpose often a cooling coil is installed. Depending on the location of the tunnels, groundwater might be used for this purpose. However, most installations work with cooled water. The required cooling capacity differs around the world due to outside weather conditions. Fresh inlet air needs to be cooled back to 16 °C in order to be able to cool the compost.

If possible in the logistic plan, tunnels can be used for both phase II and III. By changing this, each tunnel is regularly filled with ammonia from the pasteurisation stage. This is a natural way of keeping tunnels clean. After phase III — if a tunnel is not directly used for phase II again — steam of 0,5 bar can be used, and the tunnel is cooked.
out at 70 °C for at least 8 hours. The steam can be blown in right after the inblowing duct.

14.3.5 Growing room measurement
In order to have an optimum airflow, air volume is measured. Most mushroom houses are equipped with 4-6 temperature probes, which are in the substrate, divided over different beds or trays. In the room dry and wet bulb temperature is measured, along with CO₂.

14.3.6 Growing room ventilation
In the growing room between 225 and 300 m³ of air is given per hour to a ton of spawn-runned compost. In practice this equals 22.5 to 25 m³/h per m² of growing surface. For manual harvesters, who would rather prefer some spreading in the growth of mushrooms, 22.5 m³/h is sufficient. For mechanical harvesters who want the majority of the mushrooms to be at the right size at one particular moment, rooms are designed with 25 m³/h of air per square metre of growing surface. Backpressures are in a range between 500 and 700 Pascal, depending on the resistance in the ducting and the dimensions of the room.

14.3.7 Growing room cooling and heating
For mushroom growing chilled water cooling, freon cooling and groundwater cooling are in use. The water is pumped through tubes along which the air flows. At that point exchange of heat occurs. The longer the tubes – circling in the coil – the more efficient such a coil can be used. In order to keep airflow over the beds or trays at a low velocity, less air volume can be used. Therefore cooling should be somewhat over-designed. Air coming through the cooling coil should be cooled back to 10-11 °C. In warm and especially humid climates this might be only possible in 2 cooling steps. Freon cooling works most directly, yet carries a risk of leaving bacteria spots as a result of overcooling. Also legislation towards the use of many freon sorts make this type of cooling less attractive.
Groundwater spillage creates problems in many parts of the world. Therefore this method of cooling is forbidden in some protected areas. Cooling water can be pumped back in the soil during summer and pumped up again during winter. In this situation, water is not spoiled, only warmed up and cooled down throughout the season. After de-humidifying air by the cooling coil, air is heated up to reach the desired temperature. Heating is done with coils through which hot water is pumped. Conical and symmetric ducting around the cooling and heating coil optimise their capacity. Carefully welded and shaped ducting in a unit therefore has benefits for climate control and energy consumption.

14.3.8 Growing room cooking out
As with tunnels, 0.5 up to 2.0 bar of steam is necessary to cook out the rooms. A temperature of 70 °C should be reached for at least 8 hours.

14.3.9 Humidification
In climates with cold outside weather conditions, the steam valve can do a part of the humidification of air. However with steam also a lot of energy is blown into the room. During warmer outside climate conditions, water humidification is required. A mist of small water particles is obtained by either a high-pressure compressor or by sonic sprinklers.

14.4 Energy-saving innovations
(Addition by P. Oei)

The latest developments in climate control pay more and more attention to energy saving. New climate control units and ventilation ducts are isolated to minimise energy loss during transport of the conditioned air.
to the growing rooms. Especially when cooling or peak heating, much energy is lost with traditional air ducts. Special PUR has to be used in order to withstand the high temperatures and ammonia. Other advantages of the isolated ducts include low air flow resistance, little accumulation of debris or bacteria in condensing water. These kinds of installations are of course more expensive than the traditional non isolated ones; the payback time depends on the local climate and energy prices and can be less than three years. Other means to save energy have been treated above, such as high pressure humidification systems and high efficiency steam or heating boilers.

A high pressure humidification system instead of steam saves much energy (courtesy Peeten).
15 Cultivation on fermented substrates in general

This third edition of Mushroom Cultivation contains much more information on growing *Agaricus* than the previous editions. The previous two chapters described modern farms (13) and climate installation (14). The following chapters provide more details on new developments like indoor composting (chapter 17) and the latest cultivation techniques (chapter 18) and Mechanisation (chapter 19). This chapter describes the cultivation of *Agaricus bisporus* in developing countries and briefly mentions some aspects of some other compost-thriving mushrooms, in the following sections:

- introduction on cultivating *Agaricus bisporus*,
- successive stages and requirements,
- organisation,
- substrate containers,
- substrate preparation,
- composting process,
- pasteurisation and conditioning,
- spawning and spawn run,
- casing soil,
- cropping cycle schedules,
- cultivation parameters for other mushrooms on compost.

15.1 Introduction on cultivating *Agaricus bisporus* in developing countries

A number of mushrooms can only be cultivated on fermented substrates. Among these are *Agaricus bisporus*, *A. bitorquis*, *A. arvensis*, *A. blazei*, *Coprinus comatus* and *Lepista nuda*. In nature these mushrooms grow on organic litter, which is left behind by the first stage decomposers. They are capable of breaking down ligno-cellulose complexes, which are difficult to degrade by other organisms.

The most cultivated mushroom in the world, *Agaricus bisporus*, is rather difficult to grow in tropical countries for a number of reasons:

- compost preparation is a sophisticated process, which requires expertise,
- the temperature for fruiting is rather low,
- the materials for the casing soil are in some places difficult to obtain. Another quite similar species has a higher temperature range for fruiting: *Agaricus bitorquis*. It takes longer to fruit, however, and the time between flushes is also longer. The substrate preparation is the same. Scientists in tropical countries should try to find other edible *Agaricus* species, which can be grown in a tropical climate without expensive cooling and with similar high yields. Two such strains, called W19 and W20, were presented at the Twelfth International Congress on the Science and Cultivation of Edible Fungi in
1989 in Braunschweig, Germany. The cultivation of the White button mushroom is well documented for circumstances in Europe, the USA and Australia. Its industrial cultivation requires huge investments and is thus unsuitable for rural development in the Third World. In Taiwan and mainland China more appropriate small-scale techniques have been developed. Because of higher wages, Taiwan can no longer compete on the world market, but it developed techniques that can easily be applied in other countries. Taiwanese techniques are nowadays being employed in Saudi Arabia, Paraguay, Malawi and Indonesia, to mention just a few places. In mainland China much research is being carried out on developing and adjusting techniques for small-scale farmers. The following procedures combine experiences from China, Taiwan and India.

15.2 Successive stages and requirements

The following stages can be recognised in *Agaricus* cultivation:
- Phase I: composting
- Phase II: pasteurisation and conditioning
- Phase III: spawning and spawn run
- Casing
- Pinning
- Fruiting and picking

A general requirement is some means of transport for the compost. Theoretically transport should be kept to a minimum to avoid labour and risks of contamination. On the other hand, the erection of rooms purposely built for only one stage of the process (e.g. pasteurisation) has proven to give the best results. The following table shows the other requirements to produce mushrooms on a fermented substrate.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I: composting</td>
<td>- concrete floor</td>
</tr>
<tr>
<td></td>
<td>- pile formers: wooden planks, one for each side of heap, 1.20 to 1.50 m high and 2.5 m long. Three- or four-sided bins can also be used</td>
</tr>
<tr>
<td></td>
<td>- pitch forks with a long handle to turn the compost heap</td>
</tr>
<tr>
<td></td>
<td>- a sprinkler or hose with spray nozzle</td>
</tr>
<tr>
<td></td>
<td>- long-stemmed thermometers</td>
</tr>
<tr>
<td></td>
<td>- optionally: a flat-bladed shovel</td>
</tr>
<tr>
<td>Phase II: pasteurisation</td>
<td>- steam boiler</td>
</tr>
<tr>
<td>(peak heating) and</td>
<td>- long-stemmed thermometers</td>
</tr>
<tr>
<td>subsequent conditioning</td>
<td>- tunnel or well-insulated growing house</td>
</tr>
<tr>
<td>Spawning</td>
<td>- spawn</td>
</tr>
<tr>
<td></td>
<td>- optionally spawning machine</td>
</tr>
<tr>
<td></td>
<td>- forks to mix the compost</td>
</tr>
<tr>
<td>Spawn run</td>
<td>- a well-insulated room with a temperature of 20-25 °C (the temperature in the compost should not be higher than 28 °C)</td>
</tr>
</tbody>
</table>
15. CULTIVATION ON FERMENTED SUBSTRATES IN GENERAL

<table>
<thead>
<tr>
<th>Stage</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spawn run (cont.)</td>
<td>• long-stemmed thermometers</td>
</tr>
<tr>
<td>Casing</td>
<td>• casing soil</td>
</tr>
<tr>
<td></td>
<td>• growing rooms with some means of climate control</td>
</tr>
<tr>
<td>Harvest</td>
<td>• growing rooms</td>
</tr>
<tr>
<td></td>
<td>• cold (2-4 °C) storage</td>
</tr>
</tbody>
</table>

15.2.1 Organisation

The different steps in *Agaricus* cultivation can be carried out by successive producers or all on one farm. In the first case, company X performs composting (Phase I), company Y buys the compost and performs pasteurisation and conditioning (Phase II), the final growers spawn the substrate, apply the casing soil and pick the mushrooms (Phase III). There are some advantages if inoculated or even fully grown substrate is delivered to the growers:

- the quality of the compost can be better controlled by specialised personnel,
- higher productivity, because equipment can be used more often than on one farm,
- the rate of crop failure will be lower at the level of the farmers,
- environmental impact can be reduced more easily compared to distributed diffuse compost production.

The disadvantages are:

- transport of heavy loads of compost is necessary,
- the influence of the individual farmers may be limited.

15.2.2 Substrate containers

The substrate can be handled in bulk throughout the whole process, or packed in containers after spawning. The following containers are in use:

- trays,
- plastic bags,
- rectangular blocks, lined with plastic.

Trays should be disinfected thoroughly after the growing process. Wooden trays are difficult to clean; viruses can survive the heat treatment in the wood. In the ideal case, the trays have smooth surfaces for easy cleaning. Wood, aluminium, galvanised steel, and recently polycarbonate are the most used materials for trays. Plastic bags have the advantage that they are used only once, thus reducing the risk of infections. Bags of up to 20 kg can be handled relatively comfortably. Often,
however, the bags are filled with more substrate, which makes filling and emptying the growing houses hard labour. The bags should not be closed entirely, to allow for some aeration. Rectangular blocks are quite similar to plastic bags. Their additional advantage is that they can be arranged in the shelves easier than bags. Production of blocks, however, requires a substantial investment in special equipment to press the substrate in the right size, and subsequently fold and seal plastic around it.

15.2.3 Substrate preparation
A substrate for *Agaricus* cannot be defined solely by the chemical compounds. The biological nature of the prepared substrate determines whether the substrate has be-

Chopping (left) and wetting (right) rice straw (courtesy TARI).

come selective during the preparation process. Important in this respect are the successive fermentation stages, the heat treatment, an even distribution of the individual ingredients, the water content (actually the water activity) and the C/N ratio. A large number of ingredients makes an even distribution in the substrate more difficult.

15.2.4 C/N ratio
The C/N ratio of *Agaricus* compost is very important: it should be 30:1 at the time of mixing the substrate, 20:1 at filling in a house or tunnel before the heat treatment, and 17:1 at spawning. A too high nitrogen content will result in a long period of ammonia release. Then the fermentation has to last longer, which will affect the quality of the compost. Both structure and nutritional value will become less suitable for *Agaricus*. If the C/N ratio is too high, carbon compounds will remain after the fermentation process. These may become food for competitor fungi. These competitors in their turn may attract mites.

The following tables provide several recipes which have been used successfully to grow *A. bisporus* in different parts of the world:
### Synthetic straw compost Ingredients

<table>
<thead>
<tr>
<th></th>
<th>kg Wet weight</th>
<th>kg Dry weight</th>
<th>N%</th>
<th>kg Total N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taiwan 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paddy straw</td>
<td>1000</td>
<td>850</td>
<td>0.62</td>
<td>5.3</td>
</tr>
<tr>
<td>urea</td>
<td>10</td>
<td>10</td>
<td>46</td>
<td>4.6</td>
</tr>
<tr>
<td>ammonium sulphate</td>
<td>20</td>
<td>20</td>
<td>21</td>
<td>4.2</td>
</tr>
<tr>
<td>calcium superphosphate</td>
<td>30</td>
<td>30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>potassium sulphate</td>
<td>8</td>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>calcium carbonate</td>
<td>25</td>
<td>25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>943</td>
<td>1.41</td>
<td></td>
<td>14.1</td>
</tr>
<tr>
<td><strong>Taiwan 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paddy straw</td>
<td>1000</td>
<td>850</td>
<td>0.62</td>
<td>5.3</td>
</tr>
<tr>
<td>urea</td>
<td>0.5</td>
<td>0.5</td>
<td>46</td>
<td>0.23</td>
</tr>
<tr>
<td>ammonium sulphate</td>
<td>20</td>
<td>20</td>
<td>21</td>
<td>4.2</td>
</tr>
<tr>
<td>calcium superphosphate</td>
<td>2</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>calcium carbonate</td>
<td>3</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>925</td>
<td>1.28</td>
<td></td>
<td>11.8</td>
</tr>
<tr>
<td><strong>South Korea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paddy straw</td>
<td>1000</td>
<td>850</td>
<td>0.62</td>
<td>5.3</td>
</tr>
<tr>
<td>chicken manure</td>
<td>100</td>
<td>63</td>
<td>4</td>
<td>2.52</td>
</tr>
<tr>
<td>urea</td>
<td>12-15</td>
<td>0.5</td>
<td>46</td>
<td>0.23</td>
</tr>
<tr>
<td>calcium superphosphate</td>
<td>2</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>calcium carbonate</td>
<td>3</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>925</td>
<td>1.28</td>
<td></td>
<td>11.8</td>
</tr>
<tr>
<td><strong>Fujian Institute of Light Industry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paddy straw</td>
<td>2496</td>
<td>2122</td>
<td>0.62</td>
<td>13.2</td>
</tr>
<tr>
<td>dry cattle manure</td>
<td>4160</td>
<td>3535</td>
<td>1.3</td>
<td>46</td>
</tr>
<tr>
<td>barley straw</td>
<td>1664</td>
<td>1414</td>
<td>0.64</td>
<td>9</td>
</tr>
<tr>
<td>peanut cake</td>
<td>333</td>
<td>333</td>
<td>5</td>
<td>16.6</td>
</tr>
<tr>
<td>ammonium water</td>
<td>33</td>
<td>6</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>wood-ash</td>
<td>70</td>
<td>70</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>calcium superphosphate</td>
<td>83</td>
<td>83</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>urea</td>
<td>42</td>
<td>42</td>
<td>46</td>
<td>19.3</td>
</tr>
<tr>
<td>lime nitrogen</td>
<td>83</td>
<td>83</td>
<td>21</td>
<td>17.4</td>
</tr>
<tr>
<td>gypsum</td>
<td>125</td>
<td>125</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>lime</td>
<td>83</td>
<td>83</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

### 15.3 Composting process

The objective of the fermentation process is to obtain a substrate suitable for *Agaricus*, but not for other fungi. Many experiments have been performed in order to develop techniques that yield a good substrate in a minimum of time. Unlike Oyster mushrooms, which can fruit well on substrates which have only received a hot water bath, *Agaricus* needs a thoroughly composted substrate. In nature, *Agaricus* grows on organic matter, which has already been partly decomposed. The organic material of this
specific layer of the soil can be substituted by a man-made compost. It has been shown that the quality of the compost for *Agaricus* is positively correlated with the amount of biomass of a thermo-tolerant fungus, *Sclerotium thermophilum*. This is one of the reasons why *Agaricus* compost preparation is time-consuming. All commercially viable techniques consist of a pre-wetting stage, a fermentation stage and a heat treatment. An additional treatment, which is called conditioning, can further increase the quality of the compost.

**15.3.1 Compost activity**

The temperature in the freshly mixed compost will rise rapidly due to microbial activity. If *Agaricus* were spawned at this stage, its mycelium would be killed by the heat.

Inside the compost heaps, easily available nutrients are degraded by other micro-organisms first; this will raise the temperature to 80 °C. The temperature of the compost (compared to the air temperature) is a measure of the compost activity. If many nutrients are available, then microbial activity will be high, too. The temperature inside the compost will be more difficult to control then. A conditioning period after the peak heating renders the right conditions for the available microflora to break down remaining traces of ammonia and easily degradable nutrients.

**15.3.2 Stacking and turning heaps**

When stacking the heaps it is important to get a high temperature inside the heaps with sufficient air flow to support microbial growth. The structure of the heap has to permit adequate aeration, but if aeration is too high, the generated heat will be lost in the air. The water content affects aeration. Too low a water content results in a rapid loss of temperature. Too high a water content will clog the air flow and create anaerobic circumstances. The water content may vary between 68% for rather dry compost to 76% for rather wet compost, with an optimum at 72%.

If the heaps are too small, the temperature will stay too low, resulting in only partial composting. The temperature would then rise too high for *Agaricus* at a later stage in the cultivation process.

It is very important to mix the substrate ingredients well to prevent uneven decomposition. Turning the
heaps is also practised to prevent long-term anaerobic conditions in the core of the compost. The compost pile will become more compact during composting. Microbes produce water and together with the compaction this will eventually stifle air flow. When the temperature in the core starts to drop, it is time to turn the dikes. Turning the heaps by hand is hard work, so special compost turners have been designed to turn the dikes efficiently. When machines from other countries are imported, it should be checked whether they have to be adapted to the structure of the locally available substrate materials.

15.3.3 Long composting versus short composting
Two ways of composting have been developed: long and short composting. Indoor composting (see chapter 17) can be considered as a next step, which further optimises substrate quality.

Long composting: This can be carried out completely outdoors. It requires more time and is used only if adequate equipment to perform short composting is not available. Sometimes not even a heat treatment is applied to the substrate, but this will result in many pests and diseases. If no heat treatment is applied to the substrate, then the outer shell of the piles has to be discarded after the last stage.

Short composting: This is the method that is used by most commercial growers in developing countries. It takes less time because optimal conditions during composting are created.

15.3.4 Time schedule for long composting

<table>
<thead>
<tr>
<th>Day</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>−10</td>
<td>Prepare synthetic compost ingredients: water the loose straw, add supplements which are high in carbohydrate content but low in nitrogen content. Animal manure also has to be added at this stage.</td>
</tr>
<tr>
<td>−5</td>
<td>Turn the heaps and add more water. Break up clumps of supplements.</td>
</tr>
<tr>
<td>−2</td>
<td>Wet thoroughly and mix; add all the remaining ingredients, except the gypsum.</td>
</tr>
<tr>
<td>0</td>
<td>Make a pile (or dike) 1.80 m wide and 1.20 m high. The core should be loose, but the sides should be tightly compressed. Use pile formers.</td>
</tr>
<tr>
<td>6</td>
<td>First turn. Turn the inside out and the outside in. Decomposition will make the heap smaller. Keep height and length constant, but reduce width.</td>
</tr>
<tr>
<td>10−12</td>
<td>Second turn. When microbial activity is reduced, the pile should be turned. This can be measured by monitoring temperature. When the temperature drops, it is time to turn. Add gypsum if it is listed as one of the substrate ingredients. Check</td>
</tr>
</tbody>
</table>
Day | Action
--- | ---
10–12 | Water content and add water if necessary. White-grey spots of Actinomycetes can be seen. Spread evenly through the compost.
13–15 | Third turn. Actinomycetes should be visible all over the compost. Their growth will decrease water content, so water has to be added. There is still a slight smell of ammonia. Make the pile 1 m high and 1.20 to 1.50 m wide.
15–17 | Fourth turn. The compost should be dark brown and spots of Actinomycetes can be seen all over the compost. Moisture content has to be 67 to 70% and pH 7 to 7.5. If this is not the case then turn again after another two days.

15.3.5 Time schedule for short composting

Day | Action
--- | ---
-10 | Spread the straw in a low pile of 60 to 80 cm high and wet thoroughly.
-7 | If animal manure is used, it should be mixed with the straw at this moment. Moisten the mixture very well, reuse water running off.
-3 | Remix and add water if necessary. If chopped straw is used, mix it well with the manure and wet well.
0 | Make piles 1.50 m high and 1.80 m wide. Add as much water as possible without run-off. Use wooden shelves to ensure vertical sides. Compress the top. The heap should be rather compact.
4 | First turn. Add gypsum and turn according to picture. Maintain the pile height but reduce width.
7 | Second turn.
10 | Third turn. Mix well, add water and reduce width to 1.50 m. Fill the tunnel or the beds if the compost is ready or continue composting by two day interval turns until ready.

The biochemical reactions in the middle of the pile are fast as the local temperature is rather high. In short composting the dike are higher, resulting in a larger area with a high temperature. Start on day -3 if chopped straw is used.
The compost prepared by the short composting method is ready for filling when:

Temperature zones for long (left) and short (right) composting.
15. CULTIVATION ON FERMENTED SUBSTRATES IN GENERAL

1. The straw is still firm but can be sheared with some resistance.
2. The colour has changed to a uniform deep brown.
3. The squeeze test reveals only a few drops of liquid.
4. The pH is 8.0 to 8.5.
5. Uniformly distributed white flecks of Actinomycetes can be seen.
6. The C/N ratio has been reduced to 20. It may still have a strong smell of ammonia.

15.3.6 Transport for pasteurisation process
After composting, the compost has to be put on the shelves or in a tunnel for pasteurisation. Some growers use a wagon on a rail to transport the compost from the composting area into the mushroom house. Others use conveyor belts. If the compost is pasteurised in the growing rooms, a long fork is necessary to move the compost from the wagon onto the shelves. The amount of compost per m² varies: if composting and heat treatment can be performed under relatively controllable circumstances, the layer may contain 140 kg/m², but more normal filling rates are 100 or even 50 or 60 kg/m² under poorly controlled circumstances in mainland China. When composting is performed poorly with inadequate techniques, the temperature would rise too high if the compost is put on the shelves in too thick layers.

15.3.7 Pasteurisation
In some developing countries Agaricus is grown without a heat treatment (peak heating), but then yields are low and unpredictable and many pests and diseases hinder the growth of the mushrooms. The functions of the heat treatment (pasteurisation and conditioning) are:
- To make the substrate more selective for Agaricus (by eliminating ammonia (NH₃) and creating a suitable climate for specific micro-organisms).
- To eliminate eggs and larvae of harmful insects, as well as nematodes.
Steam is blown into the tunnel or mushroom house to reach the desired compost temperature of 60 °C. Many farmers add wet rice, wheat bran or soy bean meal one day before filling or even during filling. The micro-organisms in the heap will use these nutrients very quickly, thus raising the temperature of the compost. This will speed up and lengthen the pasteurisation process, thus saving on energy costs during peak heating. Supplements can also increase the yield as they provide nutrients for the mushroom mycelium.

The higher temperature in the compost will also enhance ventilation in the mushroom house. The greater the difference between compost and air temperatures, the better the aeration in the compost. A ‘dead’ (low microbial activity) compost is more difficult to condition. A sufficient air supply is necessary to favour the growth of Actinomycetes and Scytalidium. These are biological markers for selectivity towards Agaricus. Agaricus can actually feed on the biomass of Scytalidium thermophilum.

Anaerobic conditions should be avoided, since these will result in the growth of unwanted fungi like Chaetomium, an olive green mould. This marks a less suitable compost. Lack of oxygen can easily be detected by lighting a match in the growing room or tunnel. If the flame is maintained, the oxygen level is high enough. An excess of fresh air will only cause problems if it leads to cooling the compost below the desired tem-
temperature. Fresh air is usually introduced when the compost temperature rises too high.

15.3.8 Case study: saving on energy by half bulk pasteurisation on shelves in China
White button mushroom yields in China were low before 1978: only 3.6 kg per m². The substrate was composted outdoors, filled on the shelves and subsequently spawned. Because no heat treatment was applied, many pests and diseases occurred and the quality of the compost was very variable. When peak heating (pasteurisation) was introduced, it proved to be difficult to perform on the vast countryside where the farmers had little resources. Because of the poor insulation and the low efficiency of the heating system, it took too much fuel. The Fujian Research Institute of Light Industry therefore developed the following technique:
1. Phase I composting outdoors is shortened to 10 days (rendering a more active compost with higher heat-generating properties)
2. The compost is stacked in half-bulk as shown in the figure (because of the

Peak heating is performed in the middle of the conditioning period (after Z.S. Wang and H.C. Wang, *Mushroom biology and mushroom products*).

The compost is filled on only three of the five available shelves during conditioning and peak heating (after Z.S. Wang and H.C. Wang, *Mushroom biology and mushroom products*).
poor heat-conductivity of the substrate, the temperature inside will be higher than in thin layers.

3. Peak heating takes places in between the fermentation/conditioning phase. The peak heating temperature could be reached by adding only small amounts of energy. The two fermentation/conditioning phases would enhance the microbiological properties of the substrate. The method proved easy to learn and has gained popularity among the farmers. The yields increased by 15-30% to 4-5 kg/m². Still this is rather low compared to Dutch standards, with yields of 30 kg/m². A further development would be to improve climate control, but that requires initial investment. Farmers often grow only one cycle of mushrooms and then use the farmland for other crops. They are thus reluctant to invest in equipment they use only part of the year.

15.4 Spawning and spawn run

Grain spawn is most commonly used in Agaricus cultivation. It is mixed through the compost with a small fork. Approximately 1.2 to 2 litres of spawn are used per m² when 100 to 140 kg/m² compost are filled. On thin layers less spawn is used. Now the mushroom house should be kept closed to promote mycelial growth. Only if the temperature rises too high (above 27 °C) is some fresh air allowed in for cooling. The temperature of the compost should be stable; if the compost cools down below 20 °C, the yields will suffer. After the mycelium has grown through the compost, a casing soil must be applied.

15.5 Casing soil (for developing countries)

Also refer to the new chapter 16 on casing soils. Experience showed that fully grown Agaricus compost hardly gives rise to any fruit body formation if there is no casing soil (a top layer with a different composition than the compost) applied to the full-grown compost. This casing soil fulfills the following functions:

1. to supply water for the growth of both mycelium and fruit bodies,
2. buffering climatic conditions in the growing room,
3. protect the compost layer from drying out,
4. provide an environment suitable for the stimulation of fruit bodies and their development.
15. CULTIVATION ON FERMENTED SUBSTRATES IN GENERAL

ad 1. The water holding capacity is especially important. Peat is the most abundantly used raw natural material for casing soil, because of its high water holding capacity. Many parts of the world have to do without peat, however. For these regions other formulations were tested (see recipes below).

ad 2. Humidity in the growing room will be influenced by evaporation of water from the casing soil. A specific microclimate is formed in the first few mm above the casing soil, with humidities ranging from almost 100% in the casing soil to the relative humidity in the ambient air. If the air is relatively dry, the mushrooms will 'root' deeper.

ad 3. Moisture is extremely important because the nutrients from the mycelium in the compost are moved to the fruit bodies by water. The water evaporates and it has been estimated that for each kilo of white buttons 3-4 kilo’s of water have evaporated. The compost can contain only limited amounts of water, whereas the casing soil has a high water capacity.

ad 4. Moist soil hosts large numbers of bacteria, which are indispensable for primordia-initiation. It has been suggested that the bacteria decrease the concentration of certain volatile metabolites of the mycelium.

**Casing soil formulas**

- farmyard manure
- loamy soil

or:
- farmyard manure
- fermented tree bark

or:
- coarse peat
- limestone flour
- limestone grit

1 part
1 part
1 part
4 parts
1 part
0.5 part

When farmyard manure is used, it is necessary to sterilise the casing soil. The manure contains numerous possibly contaminating moulds, insect larvae etc. Sterilisation can be performed with steam or with chemicals like formalin or chloropirerin. Try to avoid this kind of product; formalin for example may cause cancer.

The casing soil is laid on top of the full-grown compost in a layer of 1-5 cm. It depends on the compactness and water holding capacity of the casing soil and the temperature in the growing room how much soil has to be applied. When button mushrooms are grown at low temperatures in caves, a thin layer can be applied. If high yields are expected, e.g. 15-25 kg/m², the thickness should be 5 cm of a high water holding capacity soil. In Taiwan with its rather high temperatures, a thick soil would increase the activity in the compost (because it acts as a layer of insulation) thus delaying fruiting. Also, as no peat is available in Taiwan, the soil is rather compact. Only 2.5 to 3 cm casing soil is applied. In countries with a more favourable outside temperature and a casing soil with a higher porosity, a layer of 5 to 6 cm thickness is used. Two to six days after casing, fungicides are often applied, for example Benlate or TBZ. However, if hygiene can be maintained at a high level, it is often possible not to use any fungicides.
Before primordia initiation the *Agaricus* mycelium has to colonise the casing soil. This is promoted by maintaining a high CO₂ level in the growing room by minimising fresh air input.

### 15.5.1 Climate control during cropping

The following table shows which conditions have to be met during the successive phases after spawning for *Agaricus bisporus* and *Agaricus bitorquis*. A more detailed cultivation parameter guide can be found in the chapter Cultivation techniques.

<table>
<thead>
<tr>
<th></th>
<th><em>A. bitorquis</em></th>
<th><em>A. bisporus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature for fruiting</td>
<td>20-25 °C</td>
<td>10-20 °C</td>
</tr>
<tr>
<td>Temperature for spawn run (incl. case-run)</td>
<td>25-30 °C</td>
<td>20-28 °C</td>
</tr>
<tr>
<td>CO₂ concentration during pinning/cropping</td>
<td>&lt;2000 ppm</td>
<td>&lt;1000 ppm</td>
</tr>
<tr>
<td>Relative humidity for pinning</td>
<td>95-100%</td>
<td>95-100%</td>
</tr>
<tr>
<td>Relative humidity for cropping</td>
<td>85-92%</td>
<td>85-92%</td>
</tr>
</tbody>
</table>

When the mycelium can be seen running over half the surface of the casing soil, fresh air should be introduced in the growing room to stop mycelial growth and promote pinhead formation. Some growers wait until the mycelium has grown over 90% of the surface and then apply a very thin layer of fresh casing soil. Ideally the temperature should drop now. This can also be performed by spraying fine droplets of water in the ventilation tubes. Another way to control air and compost temperature to some extent is by ventilating at night when the temperature is lower. Humidity is controlled by spraying water on the walls and floor and by opening and closing the doors of the mushroom house. The doors must have a screen to keep insects away. The casing soil must remain wet all the time.

Keep in mind that for each kilogram of mushrooms produced, 3 kilograms of water have evaporated!

Harvest the mushrooms by gently turning them out of the casing soil. Only a little soil should stick to the stalk, otherwise the soil is too dry or the technique too crude.

**Yields:** From 3 kg/m² to 30 kg/m² can be harvested, depending on the quality of the compost, the strains involved, and how well the climate can be regulated.

![White button mushrooms ready to harvest (courtesy Cpoint).](image-url)
15. CULTIVATION ON FERMENTED SUBSTRATES IN GENERAL

15.5.2 Pests and diseases
Quite a large number of pests can be encountered when cultivating White button mushrooms. Especially if the heat treatment has been performed poorly, all kinds of pests can multiply after the compost mass has cooled down. A list of common pests and diseases and how to control them can be found in chapter 26 on Pests and diseases. A common disease is caused by a bacterium called Pseudomonas tolaasii. Under high humidity it can develop very quickly and form brown stains on the mushrooms. The remedy is to keep the mushrooms dry. If they remain wet for two to three hours after watering, an infection may have occurred. Temperatures above 20 °C and an air humidity above 85% promote bacterial blotch, especially with little air movement. Additional fresh air and air movement can ensure that the mushrooms will dry within two to three hours. Some growers use chlorine to control bacterial blotch. They spray concentrations of 125 ml chlorinated water (10%) per 100 litres of water per 100 m² even at the first flush. If an infection occurs, they will use 1000 ml chlorinated water per 100 litres of water for 100 m². If more water is applied, the chlorine should be added to the last 100 litres.

15.5.3 Cropping cycle schedules
Continuous production is only possible when rooms are regularly filled with new substrate. This will also provide more continuous labour as compared to seasonal production of mushrooms.

Two examples of cropping cycle schedules are given from mushroom farms with good climate control. In case less sophisticated mushroom growing rooms are built, the growing schedule cannot be as tight as in the examples. A 12-week schedule would be safer in the first example, to account for longer periods between flushes due to a fluctuating climate in the growing rooms.

**Example:** one particular White button mushroom farm had a 10-week growing cycle period. 10 growing rooms are used, each cell is one week ahead of the following one. Every week one room is emptied and filled again.

Activity or process per week

<table>
<thead>
<tr>
<th><strong>Activity</strong></th>
<th><strong>Week</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>filling and spawning</td>
<td>1</td>
</tr>
<tr>
<td>spawn run</td>
<td>1-2</td>
</tr>
<tr>
<td>casing and cacking *</td>
<td>3</td>
</tr>
<tr>
<td>first flush</td>
<td>5</td>
</tr>
<tr>
<td>second flush</td>
<td>6</td>
</tr>
<tr>
<td>third flush</td>
<td>7</td>
</tr>
<tr>
<td>fourth flush</td>
<td>8</td>
</tr>
<tr>
<td>fifth flush</td>
<td>9</td>
</tr>
<tr>
<td>emptying</td>
<td>10</td>
</tr>
</tbody>
</table>

* cacking: a technique to mix some full-grown compost through the casing soil, to speed up casing soil colonisation
15.6 Cultivation parameters for other mushrooms on compost

The following mushrooms can be cultivated in the same way as Agaricus bisporus. There are of course some differences in growth parameters, like light requirements and optimal temperatures. There are less data available on these species as they are much less studied than A. bisporus. The yield is usually lower than for the White button mushroom, because optimal conditions have not yet been established. Only forty years ago the production of Agaricus per m² was similar to that of the Wood blewit (Lepista nuda) nowadays. Researchers and entrepreneurs can improve the current techniques significantly to produce a wider range of mushrooms.

15.6.1 Lepista nuda (Wood blewit)

The substrate may consist of standard Agaricus compost, mixed with 10% pasteurised wheat straw. Alternatively, leaf mulch with sawdust can be used but spawn run time is much longer then. Some strains keep on growing through the casing soil and completely cover the casing soil. This makes moistening difficult. In this case, a new layer of casing soil can be put on top of the previous layer. Yields are approximately 6-7 kg/m².

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Optimal duration</th>
<th>Temperature</th>
<th>CO₂ concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>spawn run</td>
<td>90-95%</td>
<td>20-25 °C</td>
<td>2 to 3 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>on compost</td>
<td></td>
</tr>
<tr>
<td>pinhead</td>
<td>95%</td>
<td>12-18 °C</td>
<td>1 week</td>
</tr>
<tr>
<td>cropping</td>
<td>85-90%</td>
<td>12-18 °C</td>
<td>15 weeks</td>
</tr>
</tbody>
</table>

Note: it is not absolutely sure whether light is necessary or not

Casing soil overgrown with mycelium and harvested fruit bodies (right) of Lepista nuda (courtesy Cpoint).

15.6.2 Coprinus comatus (Shaggy mane)

Young fruit bodies of the Shaggy mane taste very well. The Shaggy mane can easily be cultivated, and offers relatively high yields but fruit bodies are difficult to keep. Within
four days after harvesting young specimens, some of them will start to liquefy and form the ink-like fluid for which the genus of Inkcaps (*Coprinus* sp.) is well known. One procedure would be to can them immediately after harvesting. The compost is easier to prepare than standard *Agaricus* compost. *Coprinus* growth is not hindered by ammonia, the compost is thus ready for *Coprinus* in a shorter period.

```
<table>
<thead>
<tr>
<th></th>
<th>Relative humidity</th>
<th>Optimal air temperature</th>
<th>Optimal substrate temperature</th>
<th>Duration</th>
<th>CO₂ concentration</th>
<th>Light requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>spawn run</td>
<td>90% and more</td>
<td>should maintain optimal</td>
<td>20-28 °C</td>
<td>2 to 3 weeks</td>
<td>5000-10000 ppm</td>
<td>none</td>
</tr>
<tr>
<td>spawn run</td>
<td>90-100%</td>
<td>should maintain optimal</td>
<td>20-28 °C</td>
<td>2 weeks</td>
<td>5000-10000 ppm</td>
<td>none</td>
</tr>
<tr>
<td>pinhead</td>
<td>90-100%</td>
<td>16-18 °C</td>
<td>18-21 °C</td>
<td>1 week</td>
<td>&lt; 1000 ppm</td>
<td>none</td>
</tr>
<tr>
<td>cropping</td>
<td>85-90%</td>
<td>16-18 °C</td>
<td>18-21 °C</td>
<td>15 weeks</td>
<td>&lt; 1000 ppm</td>
<td>none</td>
</tr>
</tbody>
</table>
```

### 15.6.3 Cultivation parameters for *Agaricus blazei*

The production of *Agaricus blazei* has spread from Taiwan and China around 1990 to many other places; the primary market used to be Japan. Marketing was spurred by the medicinal properties of the species; it has excellent gourmet potential because of the typical almond-like fragrance and taste of the fruit bodies. The substrate is similar to that of *Agaricus bisporus*. It needs a casing soil with an active bacterial flora.

```
<table>
<thead>
<tr>
<th></th>
<th>Relative humidity</th>
<th>Optimal substrate temperature</th>
<th>Duration</th>
<th>CO₂ concentration</th>
<th>Light requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>spawn run</td>
<td>90% - 100%</td>
<td>21 - 27 °C</td>
<td>4 - 6 weeks</td>
<td>&gt; 5000 ppm</td>
<td>none</td>
</tr>
<tr>
<td>pinhead after casing</td>
<td>80 - 90%</td>
<td>21 - 24 °C</td>
<td>1 - 2 days</td>
<td>400 - 800 ppm</td>
<td>Little, 100 - 200 foot-candles</td>
</tr>
<tr>
<td>cropping</td>
<td>75 - 85%</td>
<td>24 - 27 °C</td>
<td>15 weeks</td>
<td>&lt; 1000 ppm</td>
<td>Little, 100 - 200 foot-candles</td>
</tr>
</tbody>
</table>
```
Top: *Agaricus blazei* for the fresh market. Bottom: The same on a drying rack in China.
Well-incubated compost is covered with a layer of casing soil, 14-15 days following incubation. This casing soil layer is necessary to induce pinheading, with the end result being enough mushrooms. Casing soil provides water to enable the growth and development of mycelium and the fruit bodies, plus the layer of casing soil protects the compost.

16.1 Characteristic features of casing soil

In order for casing soil to successfully perform the functions described above, there are certain important features to be considered:
1. Water holding capacity
2. Acidity
3. Structure
4. Nutritional level

16.1.1 Water holding capacity
After casing, water is extracted from the casing soil through evaporation. Casing soil must therefore have a high water holding capacity, in other words it must be able to absorb and retain moisture.
The following features influence water holding capacity (or water supplying capacity):
- The moisture content of the casing soil
- The concentration of decomposed inorganic matter
- The capillary working.

The moisture content of the casing soil. The higher the moisture content, the more water is available for the mushrooms. Mushrooms use this water for growth and partly for the supply of nutrition via evaporation. At the moment of casing, the moisture content of the casing soil is between 68 and 76%. Growers can order casing soil with varying moisture contents. Each supplier has a different name for types of casing soils with different moisture contents.
The concentration of decomposed inorganic matter. The higher the content of decomposed inorganic matter (salinity) the more difficult it is to absorb or extract water from the casing soil. Compost, casing soil, mycelium and the mushroom all have different levels of salinity, which influence moisture absorption.
The EC (electricity conductivity) of the casing soil, with added salt, is between 0,5 and 1 mS/cm.
The capillary working. The capillary structure of the casing soil also partly determines how easily water is obtained, in other words how much suction is needed to
extract water from the casing soil. Large capillaries (macro-pores) supply water more freely than small capillaries (micro-pores). Casing soil must have a certain capillary working in order to absorb sufficient water; it must therefore have enough water absorption capacity. However, the casing soil must not release the moisture too easily either, but retain it—the water holding capacity should also be high enough. Little suction power is required to extract water from saturated casing soil. The water is taken from the larger capillaries and is relatively freely available. The more water is extracted, the more suction is required because the bond between water and the micro-pores is stronger.

16.1.2 Acidity
The optimal pH (acidity) for mycelium growth is ±7. This is the neutral pH-value and is comparable with clean tap water. The pH of casing soil is between 6.8 and 7.5 (Visscher). Levels above or below the optimal pH value will cause mycelium growth to stagnate.

The pH must not drop below 6.8, as this will greatly increase the risk of Trichoderma (competitor/green mould).

The acidity of casing soil is indicated by the pH (water). Distilled water is added to
casing soil at a ratio of 1:1 proportion. The pH can then be measured after approximately fifteen minutes. At casing pH levels of between 7 and 7.5 are often measured. When casing soil is sprinkled the pH usually rises caused by the spent lime (CaCO₃) in the casing soil dissolving in water. Reaction comparison:

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{OH}^- \]

\[ \text{HCO}_3^- + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 + \text{OH}^- \]

There are more OH⁻-ions in the solution which causes the pH to rise. During incubation in the casing soil, the mycelium forms oxalic acid (H₂C₂O₄). This acid is neutralised by a reaction with the OH⁻-ions. Casing soil contains an excess of CaCO₃ which means pH will stabilise to around 7.0 after blow down.

16.1.3 Structure

**Surface structure.** Mushrooms are formed in the surface of the casing soil. The surface structure and the corresponding microclimate are determining factors here. There must be sufficient gas exchange and no patches of surface sealing in the casing soil. **Micro-pores.** The structure in the casing soil is also very important. This determines the amount of mycelium in the casing soil. The heavier and greasier the casing soil, the fewer mycelium it will contain.

If the casing soil is too heavy it risks becoming anaerobic. Anaerobic means there is no oxygen available, which makes mycelium growth impossible.

16.1.4 Other features

Mycelium grows in the compost and in the casing soil but gains its nutrition only from the compost. Casing soil does not supply nutrition. The raw material used to prepare casing soil must be nutrient-poor. Nutrient-rich casing soil increases the risk of competitor moulds.

Casing soil must have a certain level of bacterial activity, as this is essential for good fruit body formation. This bacterium, *Pseudomonas putida*, occurs naturally in peat. This bacterium probably ensures that waste gases, such as ethylene, are extracted or converted.

16.2 Casing soil production

Good casing soil must meet certain requirements. The water holding capacity, which encourages optimal mushroom growth and development, plays an essential role. The casing soil must be nutrient-poor, have a certain level of acidity and an open surface structure. In The Netherlands, a mixture of peat and spent lime has been found to be the best.
16.2.1 Peat
Peat is an organic material formed by peat moss dying off under anaerobic conditions (in this case under water). The peat for casing soil mainly originates in Germany. In these moorlands, situated at higher altitudes, peat formation started approximately 6000 years BC. In the following 8000 years a peat layer of approximately 2.5 metres thick was created. The moors where peat is used for casing soil have an upper layer of rough peat that gradually changes to black peat.
The rough layer, the youngest peat, is mainly formed by sphagnum acid bog. Black peat is older and composed of a blend of sphagnum acid bog and cotton grass. There is a difference between upland and lowland peat. Casing soil is prepared only with upland moor peat and not with lowland moor peat, the reasons being:
- Consistent quality (homogeneous due to limited variation in vegetation).
- Sterile (pH: 3.5 more acidity than low peat).
- Lowland moor peat is often used as farm soil.
The peat must be dug from virgin plots, where no farming has ever taken place. Impure peat causes problems because it can contain diseases (e.g. nematodes) or weed seeds. The two major peat cutters are Nevea and Klassmann. The supply of rough peat is enough for another 20 years; there is enough black peat for 30-40 years. After that, alternatives must be found, or sourced from other countries such as Finland, Ireland or Russia. The best peat bogs for excavation of black peat are situated at 50 to 55 degrees latitude.
Much research has already been carried out into peat substitutes. In the past decades trials have been run with: clay, loam, coconut soil, champost (spent substrate), municipal waste, paper pulp, mineral wool flakes, elutrilite, vermiculite and various other products.
Peat was totally or partially replaced by these products. The results show lower mushroom production and/or the substitutes were more costly than peat.
The conclusion is that peat remains the best product as it gives the highest performance at the lowest cost. Other disadvantages of alternatives are: reduced selectivity, and the required bacteria do not occur naturally in these materials.
The peat is excavated using the vertical method. The entire peat layer (except the upper peat litter layer) is extracted at the same time.
The peat mixture used for casing soil consists of a large proportion of black peat (80%), the remainder is rough or brown peat (20%). Each peat type has its own characteristics. The degree of weathering greatly determines the water absorption capacity. Peat that is more weathered has a lower water absorption capacity. Rough peat can absorb a huge volume of water in a very short period. The water absorption capacity is >800 grams water per 100 gram dry matter. The water absorption capacity of black peat is 400-800 grams water per 100 gram dry matter.
The water holding capacity of casing soil is mainly determined by its capillary working. Casing soil has a multitude of air-filled spaces where water can be stored. Between the casing soil particles are larger areas (macro-pores) and smaller areas (micro-pores). In the micro-pores the water is bound strongly by the capillary effect. Black peat is more decomposed and therefore has more micro-pores than macro-pores. Black peat has a strong capillary working and as a result a high water holding capacity. Rough peat
has more macro-pores, which gives it a high water absorption capacity, but the absorbed water is bound less strongly.

A high level of black peat in the casing soil gives a heavier, greasier casing soil, which is often quite sticky. Casing soil with a high level of rough peat is light and spongy and generally quite sensitive to surface sealing.

16.2.2 *Spent lime*

For optimal mycelium growth, a pH (acidity) slightly above 7 is essential. A mixture of pure peat does not reach this level, so the addition of substances that increase the pH is necessary.

When casing soil is prepared, spent lime, \( \text{CaCO}_3 \) is added. Spent lime is an extremely fine deposit of calcium carbonate mixed with certain organic matter. In farming spent lime is used as a calcium fertiliser. Spent lime has an adhesive quality and helps coarsen the structure of the casing soil mixture.

Spent lime or Sugar Beet Lime occurs as a by-product of the sugar refining industry during the syrup purification phase. To obtain the purest possible sugar from the solution, calcium hydroxide is added during processing, followed by carbon dioxide gas added to the syrup. This causes various reactions whereby the calcium hydroxide is converted into calcium carbonate. At the same time deposits occur of mainly protein, indissoluble salts and colouring agents. The deposit is filtered and the hot sugar extracted. The spent lime is marketed as a by-product.

Sugar is refined from the syrup by crystallisation. There is no direct relationship between the amount of sugar beet used in the process and the amount of resulting spent lime. A global indication is around 50 kg of spent lime per ton of sugar beet.

There are various types of spent lime available:

**Liquid spent lime.** Liquid spent lime is the fresh spent lime obtained following filtering.

**Normal spent lime.** Normal spent lime is obtained by spraying liquid spent lime into tanks so the product can weather and dry out. This has the following advantages for mushroom growing:

the organic matter disappears during the yeasting process, which reduces the risk of competitor moulds during cultivation. The spent lime has a drier structure and can be spread more easily.

Research shows that spent lime, but also carbo calcium, must be stored for at least 12 months for optimal results.

**Filtered spent lime.** Spent lime that has been filtered under high pressure. This process gives better results in sugar refining.

**Carbo calcium.** Carbo calcium occurs during syrup purification when membrane filter rollers are used. Carbo calcium therefore has a higher dry matter content and is easier to process.

16.2.3 *Other additives*

**Stone grindings.** A substitute for spent lime is stone grindings, which change the structure. As the adhesive effect of the spent lime is removed, the casing soil structure will be finer.
Salt. Another additive used since recently is salt. It can be added in various quantities. The normal EC (electricity conductivity) of casing soil is 0.5 to 1 mS/cm. If a saline substance is added, the EC increases from 2 to 3 mS/cm.

Coconut. Coconut material from the outer layer of the nut is also sometimes used as an additive. The fibres help keep the pores in the casing soil open.

Marlstone. Marlstone (chalky tuff stone from the Upper Cretaceous) from South Limburg is also currently being used as an additive instead of spent lime. Marlstone gives a less greasy casing soil, with a reduced risk of surface sealing. Some growers leave the stems on the mushrooms then and sell the mushrooms as ‘cave mushrooms’.

Clay. Clay was formerly the major ingredient of casing soil. The disadvantages were that the casing soil quickly compacted and had a low water holding capacity.

16.3 Calculation example

The following example is a calculation for an average load of casing soil (based on 1 m³ peat):

1 m³ peat + ¼ m³ spent lime + 200 litres water.

Information: Volume loss during preparation: 15%

Specific gravity (kg/m³):

<table>
<thead>
<tr>
<th>Material</th>
<th>Specific Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>600</td>
</tr>
<tr>
<td>Spent lime</td>
<td>1500</td>
</tr>
<tr>
<td>Water</td>
<td>1000</td>
</tr>
</tbody>
</table>

Moisture content (%):

<table>
<thead>
<tr>
<th>Material</th>
<th>Moisture Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>75</td>
</tr>
<tr>
<td>Spent lime</td>
<td>40</td>
</tr>
</tbody>
</table>

Calculate the specific gravity and the moisture content of this load of casing soil.

The water added is absorbed by the casing soil and stored in the pores and capillaries. This means the calculated volume is not fully used. In addition, mixing and spraying also causes volume loss. The total volume loss is 15%. The calculated volume is: 1 m³ + 0.25 m³ + 0.2 m³ = 1.45 m³. The final total volume is 1.45 m³ - 15% = 1.23 m³.

Total weight is:

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 m³ peat</td>
<td>600 kg</td>
</tr>
<tr>
<td>¼ m³ spent lime</td>
<td>375 kg</td>
</tr>
<tr>
<td>200 l. water</td>
<td>200 kg</td>
</tr>
<tr>
<td></td>
<td>1175 kg</td>
</tr>
</tbody>
</table>

Specific gravity is:

\[\frac{1175 \text{ kg}}{1.23 \text{ m}^3} = 953 \text{ kg/m}^3\]

Total volume of water in the mixture is:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>450 l</td>
</tr>
<tr>
<td>Spent lime</td>
<td>150 l</td>
</tr>
<tr>
<td>Water</td>
<td>200 l</td>
</tr>
<tr>
<td></td>
<td>800 l</td>
</tr>
</tbody>
</table>

The moisture content = \[\frac{800}{1175} \times 100\% = 68\%\].
17 The indoor composting process for Agaricus cultivation

By Marc Maas, Cpoint

Introduction

This chapter describes modern composting procedures. Creating selectivity and homogeneity are the two main aims of composting. When composting outdoors, the direct environment suffers from the sulphur odour and ammonia. In the USA, some compost yards have been closed down by authorities because of odour problems. It has been shown that it is possible to produce a high quality compost with much pollution in tunnels.

17.1. Selective compost

Selective compost is a nutrient medium that has been especially prepared for mushroom mycelium and is less or completely unsuitable for competitor moulds. This is an essential quality as it is impossible to ensure a completely sterile environment in a mushroom farm – traces of competitor moulds are always present.

Research shows that the thermophilic mould, Scytalidium thermophilum, is the determining factor for the selectivity of compost.

The nitrogen contained by the raw materials is mainly present in a form that cannot be absorbed by mushrooms. The composting process therefore converts this nitrogen into a form that can be absorbed by mushrooms. During composting, amino acids and proteins are formed, and ammonia released. Nitrogen forms in the so-called lignin-humus complex. This complex is created by the breakdown of easily decomposable carbohydrates, which in turn builds up a supply of lignin and proteins.

Ammonia plays a special part in the composting process. At the start of the composting process a certain level of ammonia nitrogen is required for optimal fermentation. Ammonia encourages decomposition of the straw.

At the end of phase II, the compost should be selective for the mesophilic mushroom mycelium. Selectivity depends on certain factors:

- the nitrogen-rich lignin-humus complex has become a hostile environment for competitor moulds, but a suitable environment for mushroom mycelium. The easily decomposable components have disappeared by this stage. Lignin, cellulose and pentose, all carbohydrates that are difficult to decompose, are still present. As competitor moulds are denied the chance to absorb the difficult-to-decompose carbohydrates, the mushroom mycelium has an advantageous starting position on the selective compost. (Additives, however, are easily decomposable and influence selectivity.)
- there are no remaining mesophilic micro-organisms to compete with the equally mesophilic mushroom mycelium.
• the temperature drop to 25 °C means that thermophilic micro-organisms are unable to develop further. The presence of dead *S. thermophilum* in the compost encourages mushroom mycelium development.
• there is no more ammonia (NH₃) in the compost (the typical ammonia smell must have disappeared (max. 5 ppm) and is no longer detectable by humans). Nevertheless, this does not mean that the compost is odourless.

17.1.1 Homogeneous compost
In addition to selectivity, the homogeneity of the compost is highly important. Homogeneity determines the progress of development during cultivation. Homogeneous compost is compost with an even structure and moisture content. Anaerobic patches and undecomposed straw do not make good homogeneous compost.
Central composting in large volumes makes it easier to produce homogeneous compost.
Composting is homogenisation. The quality of raw materials available often differs widely. Try to eradicate this to prevent too much variation during the cultivation phase. Stable homogeneous compost produces a far superior product than a nutrient that fluctuates in quality. Specialised machinery achieves good homogenisation. Outside The Netherlands, where compost is mainly produced in small quantities, it is often a problem to create homogeneous compost with a consistent quality.
It is clear that a complex process such as composting is relatively difficult to approach and characterise. However, a number of traits have been defined over the years that can be used to assess the quality of homogeneous compost.
The main and most frequently used features are:
• structure
• C-content
• NH₄⁺-content
• pH
• moisture content
• N-content
• C/N-ratio
With the exception of «structure» all these traits can be expressed as a measurable number.
17.2 Raw materials

To create a good nutrient medium for mushrooms we need to know on which nutrient medium fungi naturally occur in the wild. The natural process of decomposition can then be simulated.

17.2.1 Basic ingredients and their function

The following five ingredients are required to produce compost:

a. Water
b. ‘Straw’ (difficult decomposable carbohydrates)
c. Easily decomposable carbohydrates
d. Nitrogen (proteins)
e. Gypsum.

In principle, compost can be composed from a wide range of raw materials, as long as they belong to the groups mentioned above and are applied in the correct proportion.

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**Development of moisture content during the composting process.**

**Development of pH during different composting phases.**

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**a. Water.** Water is an essential element required by the mushroom, as mushrooms consist of 90-93% water. Cave mushrooms, cultivated at lower temperatures with a marlstone casing soil have a very low water content of about 84 to 86%.

Water is also used as transport medium to provide the necessary nutrients to the mushroom. In general, each kilogram of mushrooms requires 2 litres of water; 1 l as a nutri-
ent material, 11 evaporates during cultivation. On average 70-90% of all the mushroom’s water requirements is extracted directly or indirectly from the compost (via the casing soil). The final amounts of water are extracted directly from the casing soil by the mushroom. Later flushes in particular extract a higher percentage from the casing soil. This last fact obviously depends on the amount of water sprinkled onto the casing soil. The above graph shows the decrease in water content during the subsequent phases of composting.

b. ‘Straw’. Wheat or rye straw or hard grasses etc. supply compost with difficult-to-decompose carbohydrates (nutrients) and give the nutrient medium the correct structure. Preserving the structure after wetting is extremely important. The length and hardness of the straw plays an important role. Finally, straw types must have high moisture-holding properties.

Suitable kinds of straw

- wheat straw
- rye straw
- rice straw
- corn leaves
- bagasse (pressed sugar cane)
- triticales (hybrid: rye wheat)
- barley
- oats
- grasses

| Mainly used in The Netherlands, retains structure well after wetting |
| These are not suitable for composting on their own but can be combined with other, harder straw types |
| Dutch hay is not suitable, however some foreign grasses are suitable, such as elephant grass |

Wheat straw accounts for 90% of all straw used in composting in Europe. 2/3 originates in France and Germany. These large bales of pressed straw have a long structure that is eminently suitable for preserving the final structure. Straw-rich horse manure can be used instead of straw, it is also much cheaper. Compost made entirely of horse manure has a much shorter structure. In some western countries, straw is sold to horse owners first; they then have to pay again for the manure disposal. Horse manure is therefore a cheap source for the mushroom industry and an environmentally safe way to deal with manure.

c. Easily decomposable carbohydrates. Easily decomposable carbohydrates should
be added to activate the fermenting process. As mesophilic micro-organisms can easily decompose these carbohydrates the entire decomposition process and inclusion of N is started under thermophilic circumstances. Broiler manure contains huge amounts of easily decomposable carbohydrates and has a nitrogen content of 3.5-4%. (Other decomposable sources are liquid chicken manure and ground feathers.)

d. Nitrogen. Nitrogen, in the form of proteins, is an important nutrient for mushrooms. The relationship between the amount of carbohydrates and proteins, the C/N-ratio, can be expressed as a parameter.

e. Gypsum. Gypsum is added to compost for three reasons:
- To improve the structure
- Reduces pH during composting (see figure)
- Forms a pH buffer during cultivation. Gypsum also acts as a buffer in phase 4: it neutralises oxalic acid, which is formed by mycelium growth.

17.3 Indoor composting

Odour nuisance and ammonia emission from substrates for mushroom cultivation, are hot items. In certain countries, ammonia emission has a lower priority. Two factors are: the government’s decision to drastically restrict the levels of ammonia emissions to limit environmental pollution and complaints under the odour nuisance acts from people living in the vicinity of compost processing facilities.

17.3.1 Research into ‘indoor compost’
The burden on the environment is caused by odour and ammonia emission when compost is processed outdoors. This process should therefore move indoors, so that the air can be purified in a closed environment.
Research into indoor composting in The Netherlands started in 1985. The first studies showed that with well-mixed and wetted raw materials it was possible to omit the pile phase and only carry out phase II (in other words, pasteurisation for 8 hours at approx. 56 °C, followed by a conditioning period of 5-6 days). This method of production yielded an equally high level of production as when the traditional method was applied.

It quickly became evident that preparation of the huge quantities of compost produced in Dutch companies couldn’t take place inside enclosed halls. The levels of evaporation, ammonia and carbon dioxide would cause immense difficulties. Adequate extraction would also be a great problem. Furthermore, enormous volumes of fresh air are also required to guarantee an optimal composting process. Tunnels were chosen as a good alternative. These are enclosed spaces with a slatted floor, through which fresh air is supplied. The experiences with phase II compost in tunnel facilities means that the extracted air could be properly treated to reduce the ammonia and odour to acceptable levels.

To maintain and even improve the productivity and quality of the compost, pile conditions are simulated in a tunnel. The conditions prevailing in a pile rely equally on the temperature and the supply of oxygen. Odours emitted by a pile during composting are caused by relatively oxygen-deprived conditions.

Odour and ammonia emission not only occurs during the pile phase, but also when the raw materials are mixed and during the ‘flat heap’ phase. Blending compost must therefore take place indoors and the flat heap phase must be reduced or omitted altogether. This means that the raw materials must be mixed and wetted as quickly and efficiently as possible. The compost is then placed in the tunnel. The compost’s own activity causes the temperature to slowly rise. The temperature can increase to 70-80 °C.

If the compost’s own activity gradually raises the temperature and a small volume of fresh air is introduced at the same time (enough oxygen, ± 6% measured above the compost) the bulk of compost in the tunnel will reach 80 °C. At the base of the tunnel the temperature will probably not exceed 45-50 °C. When the compost is taken out of the tunnel after a few days (±3-6) to continue the normal conditioning process, it is well mixed to ensure that enough useful micro-organisms are distributed through the entire mixture. Considerable levels of odour and ammonia are released during the composting process. NH₃ free compost, is not necessarily also odour-free.

Indoor composting employs various systems that will be described in detail later. The ingredients used roughly correspond to those described for traditional methods. Sampling and analysis methods are also the same (see the previous chapter for blend formulas). The systems can be divided into the so-called warm and cold method. The construction-technical demands differ according to the method used.

17.3.2 The ‘warm’ indoor composting method

With this method all the raw materials required for compost are supplied as fresh as possible. Normally, only a small supply is kept in stock – enough for 1 or 2 days. The straw is debaled and placed loose on the transport belt. A layer of well-mixed horse manure is laid on top of the straw. The percentage of horse manure is about 50 to 60%. 
If the straw is too hard it can be pre-wetted at a higher temperature. During this process, straw is placed in a phase II tunnel and heated at 60 °C for 2 to 3 days. After the straw and manure mixture has been placed on the transport belt, the entire mixture is immersed in a bath of liquid broiler manure and gypsum (percolate). The immersion process takes place in a matter of minutes. After immersion the mixture reaches a long transport belt where percolate sprinkling is repeated several times, as the first wetting was intensive but brief. Following the spray belt, the wet mixture (moisture content up to 80%) proceeds to the phase I tunnels (‘fresh’ compost). These tunnels have a traditional slatted floor with a net open surface area of 30%. This relatively large percentage means that lots of air is blown through the compost at low pressure, so that the entire layer of compost is brought to the same temperature. Immediately following ‘levelling’ (filling), the air inlets are nearly fully closed and with a minimum oxygen requirement (6 to 10%) the temperature of the entire layer of compost rises.

By exceeding the optimal temperature of 48 to 50 degrees a large part of the microorganisms in the compost is destroyed by the high temperatures. At 80 degrees, however, a caramelisation process occurs that attacks the waxy layer around the straw particles and produces soft, dark compost within a relatively short time. With this system, the process of controlled phase I at extremely high temperatures takes 3 to 5 days. With a 3-day duration, 2 complete cycles can be run per week.

After 3 days the compost is ready for phase II. The compost removed from the phase I tunnel is mixed with 1-2% phase II compost before being transported to phase II. This means that ‘sterile’ compost is spawned with the micro-organisms destroyed by the high temperature. This is not always necessary as the temperature at the sides of the tunnel does not rise above 50 °C.

It is advisable to allow the compost a ‘recovery’ night in an intermediate storage area before filling the phase II tunnels. This gives the micro-organisms a chance to develop if they are not spawned. In general, this is not essential, the micro-organisms recover naturally.

17.3.3 The ‘cold’ indoor composting method

With the cold indoor composting method used in The Netherlands, the raw materials are mixed in the same way as with traditional composting.

Straw is mixed with horse manure, preferably using up to 80% horse manure. The straw is pre-treated for 1 day by gently wetting with ‘clean’ water.
The blend of straw and horse manure is then mixed with a blend of dry broiler manure (wood chippings) and gypsum (CaSO₄).
This blend is then wetted as much as possible with percolate on a long transport belt, before being placed in a so-called pre-composting tunnel. These are tunnels with a spigot floor where air is blown through the compost under high pressure. In fact, this is the same as the old pile system, but now as a controlled process – anaerobic patches are avoided and the entire mass has a homogeneous temperature.
This pre-composting takes 3 to 5 days depending on the structure of the raw materials. After pre-composting the entire mixture is taken to the indoor composting tunnels. These tunnels also have spigot floors and use the same system of air blown through the compost under high pressure.
Unlike the method with a slatted floor, this method allows various temperature zones to be created in the compost. In this system the lower layer (0,40 m) is kept colder than the upper layer. The upper layer is 3.6 metres. The temperature in the lower layer is kept at 48 to 50 °C, this being the ideal range for thermophilic micro-organisms (i.e. *Scytalidium thermophilum*), while the upper layer can reach 80 °C. The temperature progress is gradual, but above 75 °C in the upper layer. This decomposes the compost in the upper layer well and creates enough spawning material in the same tunnel to bring the micro-life to an acceptable level again in phase II.
Composting in an indoor tunnel lasts 5 days. The tunnel is then emptied and the heap left to rest for a night. During this period the micro-life can recover as the highest temperatures in the largest layer of the compost dropped while the tunnel was being emptied. This is followed by a normal phase II.

### 17.4 Odour prevention

During the composting process considerable levels of odour and ammonia are released. Even if the compost contains no NH₄, it doesn’t mean it is odourless. The air contains many other elements that have an unpleasant smell.

**Ammonia removal.** Ammonia dissolves easily in water. This principle is used to remove ammonia from the air. The airflow is drawn through a so-called air washer, where the air comes into intensive contact with water. Tremendous volumes of water are needed to remove the high amounts of NH₃ from the air. If too little or circulated water is used, the water quickly becomes saturated, which prevents any further beneficial washing effect. As it is forbidden to discharge large volumes of water containing ammonia, circulation has to be used. The answer is to ensure that ammonia dissolved in
the water is chemically removed. This is done by adding acid to the cleansing water. Sulphuric acid or nitrous acid can be used. Ammonia is then converted to ammonium sulphate or ammonium nitrate.

The air washer can be designed so that a solution of 10-30% of the salt formed is present in the washer. The advantage is that relatively little water needs to be drained and the ammonium nitrate is available in a fairly concentrated form. The composter can use this form of ammonia as a source of nitrogen. The ammonia salt formed in the washer can be disposed of by adding it to the poultry manure combined with the compost at the beginning of the process. Tunnel companies that do not actually compost must dispose of the ammonium salt by bringing it to a composting facility, for example. An air washer has a resistance of between 300 and 500 Pa (30-50 mm water column).

**Odour removal.** An air washer can remove ammonia but can do little about other odour problems. The circulating water is quickly saturated by odours, then fails to absorb any more. An effective odour-reducing system is the so-called biofilter. A biofilter consists of a housing or cover containing a biomass. The biomass contains a filter medium such as a mixture of turf and heather, champost mixed with bark or bark with humus.

The polluted air is filtered through the biomass. The biomass absorbs the pollution in the air, which is then used as a nutrient medium for the micro-organisms. The extraneous substances from these micro-organisms are usually environmentally friendly products such as carbon dioxide, water and heat. Organisms live and multiply in the biomass. They die and this dead material is in turn used as a nutrient medium for other living organisms.

The biomass must have water-holding properties, in order to provide the damp surroundings required for micro-organic activity. Any dust particles in the airflow are detrimental for biomass functioning, as they block the filter.

The working life of a biomass can vary from 3 months to 5 years, depending on two important factors. The load on the biomass is influential (ammonia breakdown is time-dependent). Micro-organisms need a certain amount of time to do their work. If the amount of ammonia to be removed is more than can be handled in a certain time frame, the unprocessed ammonia will dissolve in the water in the biomass. This causes the micro-organisms in the biomass to die off. In practice, pre-conditioning is often used which washes the ammonia from the air.

The second factor is acidity. The micro-organisms will also die if their living conditions become too acidic. To keep a biofilter, primarily intended to prevent odour nuisance, in a good working condition for as long as possible, it is advisable to remove ammonia and other acid level-raising substances from the air prior to entering the biofilter. An air washer can be used.

Other influencing factors are:
- temperature in the biobed not above 35-40 °C and not below 15 °C.
- little or no NH₃, less than 30 ppm/m³ air
- air must be moist to prevent biobed dehydrating RH >95%
- airflow 150-200 m³ air/m²/hour
- moisture content of the biofilter 50-70%.

As well as using bio-filtration, air can also be cleaned chemically. The major volatile
sulphur compounds can be removed with a multi-stage air washer using hypochlorite (bleaching liquor). This treatment eliminates virtually all odours. Polluted air can also be cleaned via a combustion process, but this is an extremely expensive method.

17.5 Phase II and III

17.5.1 Introduction
During phase I composting, large temperature differences arise between the middle and edges of the compost pile. This means that not all the compost has the ideal conditions. Unwanted organisms such as mites and eelworms can develop in the outer compost layer, as the temperature there is low. For optimal quality compost, the entire pile must be brought to and kept under the ideal conditions for thermophilic micro-flora activity. This process, which can take place in either a room or a tunnel is called conditioning. Phase II composting is the continuation of the composting process under conditioned circumstances. Conditioning in mass means that air flows through the compost, in comparison to conditioning in rooms, where air mainly flows over and along the compost.

Exchange between compost and air takes place by conduction and diffusion. The big advantage of conditioning in mass is that the intensive contact between air and compost means the fermentation process can be better controlled. As with the filling of rooms, it is equally important to fill tunnels evenly. If the compost is too dry it must be thoroughly mixed during or after moistening. In general, a moisture content 1 to 2% higher than with rooms should be used. Moisture contents of up to 75% (depending on the structure) are no exception. In tunnels, the compost is laid in overlapping layers, which guarantees even air movement through the compost. 800-1100 kg compost is filled per m². The compost layer is ± 1.80-2.00 m thick. Most tunnels position the door on one side and the climate control system on the other (closed) side. The tunnel is therefore filled and emptied in the same space, which naturally has consequences for hygiene. Some tunnels have doors on both sides. This gives the advantage that fresh compost never comes into the same space as conditioned compost. The disadvantage is that the climate control equipment is placed on the roof – with all the consequences for repairs, inspections and measurements.
Compost temperatures are measured at the point where the highest temperature is expected, i.e. ± 30-50 cm beneath the surface of the compost layers. Four or five compost sensors are generally sufficient. The temperature of the supply air is measured under the slatted floor. The return air is measured above the compost.

Air movement and the air inlet temperature regulate the compost temperature and oxygen content. The air inlet temperature is in turn regulated by the amount of fresh air and possibly, open water cooling. Condensation of the circulation air may cause the compost to dehydrate. The air movement will certainly cause more condensation if the circulation air cools to below the dew point, then heats up again in the compost but absorbs a lot of moisture.

17.5.2 Pasteurisation
The purpose of pasteurisation is to destroy all damaging organisms that have survived phase I. According to the literature, they will be destroyed if the temperature is kept at 55 °C for 5 hours. Pasteurisation is determined by how high the air and compost temperature is and the length of time this temperature is maintained. In general, a pasteurisation time of 6-8 hours and a temperature of 56 °C should be sufficient. The air temperature is kept at 56-57 °C. This depends, however, on the reliability of the measurements. A higher air temperature will destroy too many useful micro-organisms. Compost temperature during pasteurisation will exceed 56 °C. If the air temperature is kept at 56 °C, the damage to useful micro-organisms will be limited (no yield loss). The aim is to keep as many of these useful micro-organisms alive as possible.

17.5.3 Conditioning
The purpose of conditioning is to increase the selectivity of the compost. The optimal temperature range for thermophilic micro-organisms must be maintained. This means a temperature course of 45-50 °C.

Air temperatures lower than 40 °C are not beneficial, as certain mesophilic micro-organisms will then have the chance to develop. Temperatures above 50 °C encourage the breakdown of proteins and amino acids to NH₃, a process that extends the conditioning process. High temperatures greatly slow down the integration process of nitrogen and carbohydrates. Luckily certain thermophilic micro-organisms are quite happy within a broad temperature range, so control does not have to be too precise.

During conditioning all ammonia should disappear from the compost; a part is integrated, the other part disappears as NH₃ in the air. All the remaining easily decomposable carbohydrates should also be broken down.

a. Levelling. After filling phase 1 compost, levelling is carried out for a certain period to lessen the internal differences in the compost temperature. The duration of levelling depends on the temperature differences, average compost temperature and compost activity. Levelling can vary from ± 1 to 15 hours. The amount of circulation air is 150-200 m³/hour/ton compost. The amount of ventilation air required also depends on compost temperature and activity.

The average compost temperature is brought to ± 45 °C. The corresponding air temperature depends on compost activity. When the differences in compost temperatures are less than 3-5 °C, warming up can start.
b. Warming up. The compost’s own activity is usually so high that it can be warmed up without steam. In general, aim for a temperature increase in the compost of 1.2 °C/hour. The oxygen content must not rise above 10%. There are reports from the field that the development of oxygen and carbon dioxide gas content does not follow ‘normal’ conditions. In certain conditions, the sum of O₂ and CO₂ is less than 21%. If the differences in compost temperature stay below 5 °C, circulation can be reduced. Too much circulation creates unwanted dehydration, with negative effects on compost activity. The amount of ventilation air required depends on how quickly warming up takes place and on the oxygen content. Pasteurisation starts when the air and compost temperature have reached 56 °C.

c. Pasteurisation. To ensure that all damaging micro-organisms have been destroyed, pasteurisation should last 8 to 10 hours. The air temperature remains at 56 °C (possibly 57 °C) whereby the average compost temperature can rise to 59-60 °C. It is important that the compost temperature does not rise above 60 °C. A certain minimum fresh air inlet position is often fixed during pasteurisation to avoid a lack of oxygen.

d. Cooling down. Following pasteurisation, the compost is cooled down by 3 °C per hour to 48-50 °C. The air temperature is controlled depending on this drop in compost temperature.

e. Conditioning. During conditioning the compost temperature is kept at 48 °C. The air temperature is lower than the compost temperature. During this phase minimum circulation applies too.

Approximately 3 days after pasteurisation, the compost temperature rises. The pressure in the compost also increases. Micro-life activity is exceptionally high at that moment. This is usually a sign that the compost is practically ammonia-free. To prevent compost temperatures from rising too high (> 50-51 °C) circulation can be increased. In this situation, the air temperature can be lowered to 43 °C.

A lower air temperature may have consequences for the selectivity of the compost. A higher compost temperature can be detrimental to the yield. In this case the tunnel company must decide which is the most important prevailing factor.

When the compost is ammonia-free, spawning can start. The ammonia content is generally measured using Dräger gas indicator tubes. Immediately following pasteurisation an ammonia concentration is usually measured of 600-800 ppm (parts of NH₃ per million parts of air, 0.06-0.08%). These concentrations decrease daily by a half to two thirds. Spawning can start when the concentration is lower than 5 ppm. In general, conditioning in tunnels can be carried out within a week.

If spawning has to be delayed it is better to keep the compost at conditioning temperature. This situation should however be avoided due to dry matter loss. Before spawning compost should be cooled to 25 °C.

Weight loss during conditioning in a tunnel is on average 25-30%.

17.6 The role of micro-organisms in composting

Composting is a process which uses micro-organisms to create a selective nutrient medium for the Agaricus mushroom mycelium. The term micro-organisms covers all
organisms, which are invisible or practically invisible to the naked eye. The study of micro-organisms requires a microscope (with electron microscope, if necessary). The composting procedure involves:

- Bacteria
- Actinomycetes
- Fungi.

17.6.1 Bacteria
Bacteria are microscopically small single cell organisms. Bacteria are found virtually everywhere: in the air, in water, in the ground, on and in plants, animals and human beings. Although some bacteria are pathogenic (causing disease) the majority play an extremely important role in helping to maintain the balance of life on earth. They differ from plant cells by having a much thinner cell wall. Unlike plants, most bacteria rely on organic nutrition sources.

Their most important activity is the decomposition of dead organic material into more simple, inorganic structures (mineralisation); this inorganic matter can in turn be absorbed by green plants.

When the word bacteria is mentioned, the usual association is with pathogenic bacteria that cause certain diseases such as tuberculosis, tetanus, typhus, etc. However, the number of pathogenic bacteria is very small in comparison to the total number of types. Many bacteria have a positive effect, such as in intestinal flora or in the casing soil used in mushroom cultivation.

Multiplication. This process is vegetative multiplication, and occurs via cell division or binary fission.

Multiplication can be rapid, if the prevailing circumstances are favourable. The time required for multiplication is referred to as the replication time.

The replication time of bacteria depends on the following:

- the type of bacteria
- the conditions (sufficient nutrients, suitable temperature, etc.).

Under favourable circumstances, the replication time is between approximately 20 to 60 minutes.

The table below gives an impression of the speed of replication: the times given apply to favourable circumstances and for a single bacterium with a replication time of 30 minutes.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Bacteria Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2 bacteria</td>
</tr>
<tr>
<td>1</td>
<td>4 bacteria</td>
</tr>
<tr>
<td>1.5</td>
<td>8 bacteria</td>
</tr>
<tr>
<td>3</td>
<td>64 bacteria</td>
</tr>
<tr>
<td>9</td>
<td>262,164 bacteria</td>
</tr>
<tr>
<td>10</td>
<td>1,048,576 bacteria</td>
</tr>
</tbody>
</table>

In a relatively short space of time, huge numbers of bacteria can be created. If there is more than a single bacterium at the beginning of replication, for example, 10,000 bacteria, the size of the increase is even more obvious.
An increase in the number of bacteria (the growth) is limited by the following factors:

- the nutrient supply is exhausted, further growth becomes impossible
- bacteria form metabolic products which have an inhibiting effect on growth in high concentrations
- the temperature becomes too high.

17.6.2 *Actinomycetes*

Actinomycetes cells form long, thread-like branched filaments which are extremely thin: 1 micron diameter (with fungi up to 5 micron). They are single cell organisms, with no visible core. For this reason they are classified under bacteria. Currently, the opinion tends towards placing them in a separate group – i.e. between bacteria and fungi. The name Rayfungi is confusing. Actinomycetes are a completely different group. Actinomycetes multiply as the cells separate from each other, these cells then develop into threads. Sometimes, cells at the end of the threads are cut off (spores), these are 1-2 micron in size and their only purpose is multiplication. In the same way as with bacteria, endospores can also be created, for survival. Actinomycetes form large, often powdery colonies usually white coloured. With Oyster mushrooms and Shiitake substrates, blue and green colonies also occur. Most of these actinomycetes are typical soil residents and have an earthy smell.

17.6.3 *Fungi*

Fungi are organisms that lack chlorophyll, no photosynthesis can take place. Contrary to bacteria and actinomycetes, fungi are multiple cell organisms. In the vegetative state fungi consist of long, filament-like branched threads. These long threads are known as hypha – a collection of these threads is referred to as mycelium. The hypha may be branched or unbranched. The entire mycelium can vary from just a few microns in size to a diameter of several metres or more. Chitin and glycogen are the main components of the cell walls of the hypha with macro-fungi. Micro-fungi mainly contain chitin, glycogen and cellulose.

17.7 *Conditions for micro-organism viability*

Micro-organism behaviour is heavily influenced by the surrounding circumstances. With behaviour we mean principally:

a. the rate of reproduction (growth)
b. the conversion process involved.

The combined surroundings and circumstances are referred to as the environment. To enable growth and other activities, the environment must satisfy certain parameters known as the vital conditions.

Vital conditions are the parameters which must be satisfied by the environment containing the micro-organisms, to allow micro-organism growth and conversion.

The most important vital conditions for bacteria, just as for every living organism, are:

1. the presence of (sufficient) nutrient medium
2. the presence of moisture
3. favourable temperature
4. oxygen levels (some micro-organisms survive under low oxygen conditions, most need higher concentrations)
5. favourable pH.

17.7.1 Nutrients
The majority of bacteria are heterotrophic, which means they are dependent on living or dead animals and plants. Their nutrient medium must contain carbohydrates, protein and inorganic materials.
Certain bacteria can absorb both organic N and inorganic N. These are referred to as nitrogen-binding bacteria (see also Rhizobium in paragraph 5.1).
The bacteria produce certain substances, called enzymes, which chemically break down nutrients to a liquid form that can be absorbed via osmosis through the cell wall. Vitamins are also essential in the nutrient medium, although certain bacteria can build vitamins themselves. A bacterium can absorb many times its own weight in food each hour.

17.7.2 Water
Many bacteria live in humid environments e.g. in ditch water, saliva, milk, the intestines, etc. Water can also be viewed as a nutrient medium as most bacteria, just like all living organisms, consist mainly of water. Nutrients and decomposition substances are also broken down in water, to allow them to pass through the cell wall. Water is therefore an essential part of every organism. This is one of the reasons that compost should have a high moisture content i.e. approx. 74%, at filling.

17.7.3 Temperature
Temperature has a huge influence on growth. Some micro-organisms grow best at high temperatures, others at lower temperatures. Each type has a certain optimal temperature for multiplication, whereby lower or higher temperatures inhibit growth.
Micro-organisms can be divided into three groups depending on their sensitivity to temperature:
a. psychrophilic micro-organisms (psychros = cold)
b. mesophilic micro-organisms (meso = middle)
c. thermophilic micro-organisms (thermos = heat).
The following global temperature ranges apply:

<table>
<thead>
<tr>
<th></th>
<th>minimum</th>
<th>optimum</th>
<th>maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. psychrophilic group (low temp.)</td>
<td>0 °C</td>
<td>15 – 20 °C</td>
<td>30 °C</td>
</tr>
<tr>
<td>2. mesophilic group (medium temp.)</td>
<td>5-25 °C</td>
<td>20 – 40 °C</td>
<td>43 °C</td>
</tr>
<tr>
<td>3. thermophilic group (high temp.)</td>
<td>25-45 °C</td>
<td>45 – 55 °C</td>
<td>60-90 °C</td>
</tr>
</tbody>
</table>

The thermophilic group is highly important for the mushroom grower during composting and cook out. Psychrophilic and mesophilic bacteria dominate the compost heap at the beginning. As the temperature rises, the thermophilic organisms start
to predominate; in the highest temperature range, thermophilic bacteria will be practically the only bacteria still active. When the temperature drops, the actinomycetes will take over the work from the bacteria and in turn the thermophilic fungi i.e. *Scytalidium* spp., will take over from the actinomycetes.

All these changes mean that various organisms will experience the optimal or nearly optimal conditions for viability applying to their kind, this means that domination of the compost by certain microorganisms is a changing process. The following can be found simultaneously and successively in compost: thermophilic bacteria, thermophilic actinomycetes, and thermophilic fungi. Regarding nitrogen, one group can more or less serve as a source of food for another group. Finally, after cooling down, the completely (dead) thermophilic flora will act as a source of nitrogen for the mushroom.

17.7.4 Oxygen
Bacteria need oxygen to breathe. Bacteria can be divided into two groups: aerobic and anaerobic bacteria. Aerobic bacteria can only survive if there is free air oxygen. They are the so-called free-air-oxygen users. They form the largest group, and are the most important for mushroom growing.
During the fermentation process, aerobic bacteria, including the fermentation bacteria, play an important role.

Nitrifying bacteria are aerobic, as nitrification is in fact an oxidation process. Anaerobic bacteria find it difficult to survive in the presence of free air oxygen; they extract oxygen from compounds. Anaerobic bacteria are also found in the compost heap during manure fermentation. They cause so-called ‘acid’ manure, which has the same odour as pig manure. Methane bacteria are also classed as anaerobic bacteria. In a flat heap their activity can produce up to 0.7 m$^3$ methane per ton daily.

17.7.5 pH

For optimal development each type of bacteria requires a certain degree of acidity. In general, bacteria need higher acidity than edible mushroom-producing fungi. Most bacteria like a pH of around 7.0. There are also various bacteria which can reduce acidity in substrate and it is known that milk acid bacteria, for example, develop optimally at a pH of 4.2.

17.8 The influence of other organisms

The most important criteria for viability of micro-organisms, and in particular bacteria, have just been discussed. Micro-organism growth can also be inhibited or stimulated by adding inhibitors and stimulants. Other neighbouring lower or higher organisms can also encourage or limit the growth of micro-organisms. The following two situations arise: symbiosis and antagonism.

**Symbiosis:** Mutually beneficial effect from co-habitation.

The classic example is the bacteria in the tubers on the roots of so-called papilionaceous plants (lupines, peas etc.): bacteria obtain carbohydrates from the plant, the bacteria supply N-containing substances to the plant. These members of the *Rhizobium* family fix nitrogen from the air.

**Antagonism:** Micro-organisms having a negative effect on the growth of other micro-organisms (and on the growth of their own type).

The major cause is competition for food, but the fact should not be overlooked that micro-organism growth is also limited by numerous extraneous substances. A situation where micro-organisms have a mutually negative effect is called antagonism.
18 Agaricus cultivation: from spawn run to harvest

By Marc Maas, Cpoint

This chapter has the following structure: the different strains are discussed first, then different aspects of supplements are treated. Then phase IV (the spawn run) is discussed, and the cultivation techniques during the subsequent flushes. Quality and harvesting are given special attention in the last paragraphs.

18.1 Button mushroom strains and their characteristics

Most of the fungi cultivated in The Netherlands belong to the A. bisporus species. There are four distinct sub-groups within this strain: brown strains, white strains, cream strains and hybrid strains. These four groups and their characteristics are described below.

18.1.1 Brown strains (Chestnut mushrooms)
Chestnut mushrooms are currently cultivated on a number of farms, often in combination with the common white mushroom. Chestnut mushrooms can be seen as an extension of the product range, alongside the white mushroom. The cap colour of chestnut mushrooms ranges from very light to dark brown; the stalk is usually white. The small fruit bodies begin life white, their colour changes during development. The cap is often scaly. Compost temperature is a very important factor with chestnut mushrooms, during cultivation it must be 17-18 °C. This causes slower growth, which means better quality. Currently chestnut mushroom yields are 10 to 20 percent lower than hybrid strains.

18.1.2 White strains and cream strains
Before the advent of the hybrid strains, growers producing mushrooms for fresh consumption often used white strains. White strain mushrooms have a white, smooth cap. Compared to the hybrid strains, the weight per piece is relatively low, which gives these strains a lower picking rate. Dutch growers started to use the name cream strains as the ‘intermediate strains’ have characteristics that lie between those of the brown and white strains. Cream strains
have a white (scaly) cap, but the piece weight is considerably higher than the white strains. Cream strains were mainly cultivated for mechanical harvesting. They were less suitable for manual harvesting as the scales on the cap discolour quickly. Another big disadvantage of the cream strains is that the gills of the open mushrooms quickly discolour to brown. This brown discolouration spreads to the fruit body and causes a grey colour after canning or processing. Neither strain is cultivated in The Netherlands any longer, but their characteristics can be found in the hybrid strains.

18.1.3 Hybrid strains
Due to complaints about the quality of the cream strains from the canning and processing industries and growers complaining about the piece weight of the white strains, the Experimental Station started to cross white strains and cream strains in 1976. Around 1980, hybrid strains (crossed) became a reality in the shape of Horst® U₁ and Horst® U₇. These strains have a high yield and are suitable for both mechanical and manual harvesting.

18.1.4 Small hybrids (U₁-types)
These strains have inherited the smooth white cap and pinkish gills from white strains and the short, thick stalk from cream strains. These strains also have spontaneous fruit body formation. In general, the small hybrids have more in common with a white strain than with a cream strain.

18.1.5 Big hybrids (U₇-types)
These strains have a white cap with a tendency to scale. The mushrooms remain closed for a long period and can therefore reach a higher piece weight. Big hybrids are more sensitive to the compost quality and conditions than small hybrids. In general, the big hybrids are slow to pinhead and have a high yield in the first two flushes. These strains are suitable for both the fresh and processing markets.

18.1.6 Intermediate hybrids
Since approximately 1984, strains appeared on the market with characteristics between those of the big and small hybrid strains. The average piece weight is slightly lower in comparison to e.g. big hybrids. Fruit body formation with these strains is generally quite spontaneous. Companies that harvest mechanically chiefly use these strains.

Overview *A. bisporus* Hybrid strains

<table>
<thead>
<tr>
<th>Spawn supplier</th>
<th>Big hybrid</th>
<th>Intermediate hybrid</th>
<th>Small hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le Lion</td>
<td>X1 X4 X8</td>
<td>X20 X25</td>
<td>X13</td>
</tr>
<tr>
<td>Eurosemy</td>
<td>285</td>
<td>280 288</td>
<td></td>
</tr>
<tr>
<td>Sylvan spawn</td>
<td>U1 205 608 S100</td>
<td>512 609 A12 A15 S130 S140</td>
<td>U3</td>
</tr>
<tr>
<td>Amycel</td>
<td>U1 2800 2000</td>
<td>112 2100 222 229</td>
<td>U3 104</td>
</tr>
</tbody>
</table>
18.1.7 Strain choice
The characteristics described for each strain can be greatly influenced by all kinds of environmental conditions. Possible influences are: raw material, climate, water, cooling down method, etc. The method of harvesting—manually or mechanically—is an important consideration when choosing the strain. For example, with mechanical harvesting a uniform flush is extremely important, while the opposite applies with manual harvesting.

The choice of a particular strain is closely linked to the potential or limitations of each farm. For continuity, it is preferable to keep with a certain strain once a choice has been made. In practice, at least one complete growing cycle must be completed to gain enough experience with a certain strain.

18.2 Mycelium growth in mushroom cultivation
When phase II composting has been successfully completed, it is important to ensure mycelium growth as quickly as possible in the selective compost. Rapidly growing mycelium can repress the growth of competitor moulds or even make their further growth impossible.

18.2.1 Spawning
Spawning is the process of mixing spawn through compost. It is essential that spawn is spread through the compost as homogeneously and hygienically as possible. Infections in the compost during filling can have disastrous consequences. The presence of only five spores of an aggressive strain of Trichoderma viride per 25 kg of compost can spoil the entire crop, which happened repeatedly in Ireland.

Compost is usually spawned on tunnel companies, when the tunnels are emptied. With tunnels, spawning can take place by spreading spawn over the compost before the tunnel is emptied, or compost can be spawned by spreading spawn on the conveyor belt.

Spawnable compost filled in the growing room at the growing facility is spawned during filling as the spawn is mixed with the compost at that moment.

18.2.2 Amount and type of spawn
The amount of spawn used depends on the compost incubation time, the yield and the risk of competitor moulds. Button mushroom growers always use grain spawn, 5 litres of spawn per ton of fresh (phase I) compost appears to be the optimal amount. This
corresponds to 7-8 litres of highly vigorous spawn per ton spawnable (phase II) compost.

**18.2.3 Climate**

Mushroom mycelium develops best at 25 °C. Compost can be brought to and maintained at the correct temperature using heating, cooling, circulation and ventilation. With spawn run in mass, correct temperature control is the most important factor. This is maintained through circulation and ventilation. Various factors influence compost temperature. To prevent dehydration, a high RH (Relative Humidity) is required (95% or higher) during spawn run. If the incubation period takes place in the growing room instead of tunnels, it is advisable to cover the compost with paper. By regularly wetting the paper and the floor and walls, the RH stays at the correct level.

During spawn run the CO₂ concentration runs parallel to the compost temperature. The presence of a certain level of CO₂ stimulates the spawn run. No more fresh air than absolutely necessary should be allowed in the growing room to maintain the temperature. This automatically results in a favourable CO₂ content (>3000 ppm).

Compost is at its most active just prior to and after casing. The CO₂ concentration during the vegetative phase depends on e.g. the type of substrate, filling weight, the construction of the room or tunnel, the process development.

Spawn run in tunnels takes just as long as in a growing room, after 14 days the compost is usually well incubated.

**18.3 Supplements**

Supplementing is the addition of high-protein nutrients to compost. The major aim of supplements is to increase mushroom productivity. Supplementing compost by adding
certain substances that are directly absorbed by the mushroom has been accepted practice since 1962. The real breakthrough of applying supplements only came in the early 1980s when formalin-treated supplements were developed.

18.3.1 Materials
Supplements can be divided into 2 groups:
- vegetable: based on soy bean meal
- animal: based on animal proteins (keratin)
The protein-like substances ensure increased productivity. Foreign literature often refers to the stimulating effect of oils and fats on the yield and quality (Schisler, 1983). In The Netherlands this effect has not been proved however.

18.3.2 Vegetable supplements
The main ingredient of vegetable supplements is usually soy bean meal. The protein content of the products used is between 48%-60%, depending on the supplement.
Soy bean meal must first be treated either with heat or formalin, before it can be used as a supplement. This treatment has a double effect:
1. delayed release or slow release of nutrients
2. disinfects the product
Formalin treatment gives the best results, particularly for the delayed release effect. A formalin treatment joins the protein chains in a certain way that makes them less easily

Influence on yield of supplements: yield in kg per m² per week after supplementing with soy bean meal, treated with 0, 0.2, 0.6, 1.2% formaldehyde. Left: supplementing at spawning, right, supplementing at casing. The figures above the columns indicate the temperature rise in °C in the supplemented compost, compared to the control, during the first five days (From Mushroom Cultivation 1988, used with permission PPO).
decomposable, i.e. released with a delay. This formalin treatment originated in the cattle feed industry. When preparing cattle feed, treatment with formalin resulted in a delayed release of proteins (including those from soy beans) to the animals. The animal accesses these proteins in the stomach and intestines and not in the rumen.

In mushroom cultivation this formalin treatment creates a kind of protection for the product which makes decomposition more difficult. The temperature peak after supplementing incubated compost is considerably reduced as well. Unfortunately the treatment is not yet so sophisticated that the delayed release-effect continues in later flushes. The increased productivity is mainly seen in the 1st and 2nd flush.

Research (Gerrits, *Mushroom Cultivation* 1984, nr. 8), shows that Millichamp treated with a 0.3% formalin solution gives the best results. For supplementing at spawning, Millichamp treated with a 0.6% formalin solution gives the best results. The extra yield is however only half the extra yield obtained if supplements are added at casing. There are currently various supplements on the market that can be added at spawning or before casing. Champfood, Millichamp and Substradd are examples of different supplements mainly composed of vegetable proteins.

18.3.3 Animal supplements
Keratin is the main ingredient of animal protein-based supplements. Keratin is mainly found in animal skins. Ground feathers are usually used in animal supplements. Feather meal contains approximately 80% crude protein. Champlus is an example of a supplement mainly composed of feather meal. Champlus contains ±76% crude protein and has been heat-treated. This heat treatment is necessary for disinfection, but also because animal proteins are not easily absorbed by mushroom mycelium. Using a so-called hydrolysis-treatment the protein is easier to absorb. Research carried out by Overstijns (Der Champignon, March 1988) shows that hydrolysed protein, such as Champlus, can increase productivity to a comparable extent as Millichamp.

18.3.4 Effects of supplements
Supplement material is not selective for mushroom mycelium. The risk of weed moulds increases after supplementing. It has been shown that supplementing with untreated soy bean meal increases the number of weed moulds in the compost. Supplementing with formalin treated meal resulted in no gain in the number of moulds when supplemented and non supplemented compost was compared. Heat treatment of supplements is less successful than formalin treatment.

The aim of supplementing is to increase mushroom productivity. However, the extent of this increase can vary greatly. The compost quality has a huge influence on the actual effect of supplementing. It has been shown that the ammonia content of compost influences the effect of supplements.

In 1987 Gerrits (*Champignoncultuur* nr. 8) found that mushrooms extracted more N (namely in the form of amino acids) from the compost after supplementing. The N content of the mushrooms was also greater. The influence of supplements on the amount of minerals absorbed was however very slight.
Research also showed the following:
- the amount of water in the compost has little influence on the effect of the supplement
- the richness of the compost (can be deduced from the N content) is the greatest influencing factor on the varying effects of supplements.
However, the increased productivity gained from supplementing cannot only be explained by the increased N-content of the compost.

18.3.5 Quantity of supplement
Research demonstrates that the optimal quantity of added protein is 600 gram/m². This corresponds to 1 kg of supplement/m² or 11 kg supplement/ton incubated compost.
In practice supplement quantities varying from 0.8 to 2 kg/m² are usually used. The consequences of using large quantities of supplement on cultivation must always be considered.

18.3.6 Moment of supplementing
Supplement can be added to compost at two different moments: at spawning or just before casing.
Supplementing at the moment of spawning has the advantage that the two actions can be performed together. The disadvantage however, is that non-selective material is added to the selective compost. This increases the risk of weed moulds. The extra yield is at most 50% of the extra yield that supplementing at casing provides. In theory, this means an increased productivity of max. 11%. If supplementing is done at spawning, a specially prepared supplement must be used.
Supplementing at casing gives the highest increased productivity and the least risk. The extra yield can be up to 22%.
The other advantages of supplementing at casing compared to supplementing at spawning are:
- it is a good moment to assess the condition of the compost (extent of incubation);
- the mushroom mycelium represses growth of other micro-organisms (antagonism).
Always bear in mind that mediocre or badly incubated compost will never be improved by supplements, but will only deteriorate in quality.

18.3.7 Cultivation techniques
In most cases supplementing occurs after incubation of the compost. If incubated compost is filled, the supplement can be added to the compost when the tunnels are emptied or the growing rooms filled. Supplements can also be added when the incubated compost is already in the beds. If spawned compost is used, supplementing takes place on the beds, when the compost is fully incubated. Whichever moment is chosen, the supplement must be thoroughly and evenly spread throughout the entire compost layer. If the supplement is not mixed properly, it is possible that patches of the compost burn with all the negative consequences; green mould, mites etc.

To reduce the risk of competitor moulds, the level of hygiene on a mushroom farm is extremely important. After supplementing or filling with supplemented compost, the compost temperature rises. It is important to start cooling in time. Supplementing maintains the level of activity better, which means the first flush develops more spontaneously. The extra yield is concentrated in the 1st and 2nd flush. In The Netherlands it is usual practice to supplement compost.

18.4 Incubation in the casing soil (phase IV)
18.4.1 Simultaneous filling and casing (SFC)
On modern farms, the cultivation begins with filling the incubated compost and casing with casing soil. The type of mushroom that will be harvested has already been chosen. The amount of compost per m² in kilos is determined by adjusting the height of the compost-dosing chain, this is between 80 and 100 kg/m². The longer the cultivation schedule, the more compost is required. The filling weight depends on the schedule, strain, whether plastic is used or not under the compost. When the dosing chain has applied the compost, it is compacted under pressure rollers. If the compost has a short, wet structure the pressure rollers are set as high as possible, and if the compost has a long, dry structure, the rollers are set as low as possible.

The casing soil is placed in a container above the compost. This container uses the same kind of dosing chain as the compost. The chain height depends on the quantity of casing soil required on the compost. This layer is usually between 4.5 and 6 cm thick. The height of the dosing chain for the casing soil and the casing soil layer on the compost depend on the casing soil structure. The casing soil structure also depends on how the
casing soil is processed by the cac-axle chain and the levelling roller on the SFC machine.
The height of the cac-axle chain can be set in various ways. The axle chain should just touch the compost or rotate through the very top of the compost layer. This allows casing to take place. The casing soil structure also depends on the number of teeth on the cac-axle chain and the rotational speed.
The last treatment is often levelling the casing soil to create an even surface. This breaks down large lumps and equalises holes and cracks in the soil. The casing soil surface is levelled to allow even absorption of water during sprinkling.

18.4.2 Disinfection
With casing there is always a risk that spores or parasitic moulds enter the growing room with the casing soil. To destroy as many of these spores as possible, a formalin solution can be used to disinfect the casing soil. After casing, the casing soil is sprinkled with a 2% formalin solution. This means sprinkling 2 litres of formalin (40%) in 100 litres of water per 100 m² growing surface. A 2%-solution should be sufficient to perform correctly under normal conditions, without damaging the microbiological life in the casing soil. The formalin solution is sprinkled over the casing soil but any contact between the formalin and the mycelium in the compost must be avoided at all cost. Sprinkling at a level of 1 l/m² should not be an issue. To allow the formalin to be well absorbed by the casing soil layer, all air movement in the growing room is switched off for several hours following sprinkling.
If casing soil has to be pasteurised to destroy pathogens, this can be done in a special disinfection container where the casing soil is steamed. The casing soil is kept at a temperature of 60-65 °C for 5 to 6 hours. Pasteurisation for too long or at too high a temperature must be avoided as this causes sterile casing soil. Pasteurisation is an expensive method of disinfecting casing soil and leads to deterioration of the structure. However, it prevents the use of formalin with its carcinogenic side effects. If the hygiene standard at both casing soil producer and mushroom grower are very high, no disinfection is necessary.

18.5 Cac-ing: Compost Added at Casing
C.A.C. is the English abbreviation for ‘Compost Added at Casing’ i.e. adding incubated compost to the casing soil. Cathal MacCanna first used this technique in Ireland in 1969. Since then, companies growing in trays have mainly applied the cac-ing method. With cac-ing, incubated compost is mixed through the casing soil as a casing or cac-ing spawn (C1: Cac-ing Inoculum) is used.
The advantage of the cac-ing method, is that the time between casing and blow down can be shortened. Time is gained because the casing soil is incubated far more quickly by the mycelium. Without cac-ing the mycelium must incubate the casing soil from the top layer of the compost. With cac-ing, incubation takes place from the compost particles in the casing soil. The casing soil is quickly and evenly covered by mycelium, ruffling is no longer absolutely necessary. In practice the term cac-ing is also used when 2 days are won during mycelium growth. This, however, is rather a form of flush control, where ruffling is also used. This process means it is possible to bring forward the first harvesting day by 2 to 7 days.
Cac-ing also allows the first harvesting day following casing to be brought forward by
19 to 20 days after casing. The time gained depends on the amount of incubated compost added. The more compost is mixed through the casing soil, the quicker the casing soil will become incubated. The maximum time of bringing forward is 6 to 7 days. By varying the amount of incubated compost, the moment of the first flush can be determined. The amount of incubated compost used for cac-ing varies from 300 to 500 gram/m² growing surface. Higher doses of compost can inhibit fruit body formation. If cac-ing is used, ruffling is no longer necessary. Cac-ing ensures the casing soil is evenly incubated enough for manual picking. If the surface of the casing soil has been compacted by sprinkling, ruffling may still be necessary. On tray farms, mechanical ruffling is virtually impossible. These cultivation systems have long benefited from the advantages of cac-ing, as it is the only method of encouraging uniformity besides manual ruffling. In bed cultivation, ruffling is a standard procedure and cac-ing for uniformity less important. The incubated compost mixed through the casing soil must be well-incubated (minimum 14/15 days) and completely disease-free. Infections in the raw material can easily spread through the entire growing room. The compost used for cac-ing must obviously be incubated with the same strain as the compost in the beds.

18.5.1 Methods
The compost added to the casing soil must comply with a few practical conditions. The structure of the compost must not be too long, as this makes it difficult to spread through the casing soil. In the past, compost was shredded until the straw particles were approximately 2 cm long. After shredding the incubated compost was mixed mechanically through the casing soil, then covered. In contrast to the original CAC system, the incubated compost is only spread over the compost after casing, then mixed through the casing soil using the ruffling machinery. An alternative method is to ruffle the casing soil, including the upper layer of compost, after casing. The advantage of this method of mixing compost and casing soil is that there is a minimal chance of spreading infection.

18.6 Sprinkling
Sprinkling the casing soil during mycelium incubation is an important and complicated aspect of cultivation. Irrigation partly determines the growth pattern of mycelium in the casing soil and has an indirect influence on the quality and total yield. The volume of water given between casing and ruffling varies between 5 and 30 l/m² (average 10-20 l/m²). The sprinkling pattern is determined by preliminary and future cultivation conditions such as:
- Moisture content and water absorption properties of the casing soil;
- Compost quality;
- Incubation of mycelium;
- Amount and type of cac-ing material;
- Compost activity;
- Number of days between filling and ruffling;
- Casing thickness.
Six to ten l/m² water can be sprinkled on well-incubated compost in the first two days. The majority of this water flows straight to the compost, which absorbs the water well, as the mycelium is not fully recovered. This can be a risky process, as the compost
must be of a very high quality. If this is not the case, the compost may possibly not recover, with all the resulting problems. Sprinkling stops after the first two days. Sprinkling begins again when the mycelium starts growing after approximately 1 to 2 days. A second alternative is to sprinkle very lightly during the first few days, so that the water does not reach the compost. Full sprinkling only begins when the mycelium starts developing.

If sprinkling is started too early, the mycelium does not have the chance to fully recover and water will leak through to the compost. As the compost can no longer absorb water, the upper compost layer will start rotting, and the link between the compost and casing soil mycelium will be disturbed. Disturbing this contact layer forms a barrier which obstructs the flow of nutrients from compost to casing soil. This considerably reduces the final yield.

Sprinkling too late causes a dry, crumbling contact layer between casing soil and compost or causes too much mycelium which will lead to problems with the second flush. With rapid mycelium growth in the casing soil, sprinkling to provide moisture can be started earlier.

Cultivation with a weak mycelium growth is far more sensitive. The mycelium forms thin, weak threads in the casing soil. After sprinkling the water leaks freely through to the compost. If the compost absorbs too much water, the mycelium will die and the compost will start rotting.

18.6.1 Ruffling

Another technique during cultivation is ruffling. The aim of ruffling is to mix mycelium through the casing soil, to encourage uniform development throughout the entire layer of casing soil. At blow down, the mycelium on all the beds must have the same rate of development to encourage uniform mushroom growth. The aim of ruffling is:

- To encourage a uniform first break
- To stimulate mycelium to vegetative recovery
- To remove any obstructive layers between the casing soil and compost. A rotten upper layer in the compost in particular and/or a dry incubated casing soil can obstruct transport of water and nutrients. This layer will be removed if ruffling is deep enough
- To encourage exchange between casing soil/compost and room climate.

After ruffling the casing soil has a loose and open structure. Before ruffling, sprinkling compresses the casing soil. In the vegetative stage this is not a problem, as the increased CO₂ concentration in the casing soil will stimulate mycelium growth. After ruffling, the casing soil structure is more open which allows a better CO₂ exchange. This is excellent for good generative development.

A ruffling machine consists of one or more revolving rods with short pins or teeth and often a pressure roller. The ruffling intensity depends on a combination of a number of technical variables:

- machine speed
- rod revolutions per minute
- number of pins and their configuration over the rod
- pin/tooth shape.
The moment of ruffling is determined by the amount of mycelium in the casing soil and the cultivation schedule. Changing the factors mentioned above allow the structure of the casing soil to be altered during ruffling. Deeper ruffling can increase the amount of mycelium in the casing soil.

Levelling is done using a pressure roller or rod with pins. When a pressure roller is used, the casing soil must not be compressed and/or spread too much, as the essential exchanges will be obstructed and anaerobic patches can occur in the casing soil.

Levelling with a rod is done by setting the pins so they only touch the upper centimetres of the casing soil. In patches where there is too much casing soil, the excess is moved to fill holes and cracks in other places.

The method of levelling determines the structure of the upper layer of the casing soil. The surface structure required depends on the harvesting method (e.g. cutting or picking) and the sophistication of the climate control. Equally important here is uniformity of raw material and consistent application of the methods used.

18.6.2 Climate during mycelium growth in the casing soil

After casing, an ideal compost temperature of between 25 and 27 °C is aimed for. With a green mould infection it is better to stay in the lower part of this range as green moulds grow faster at higher temperatures. The first 4 days after casing, cooling is possible with outside air. Afterwards it is better to use internal cooling to prevent pre-pinners because of low CO₂ levels.

Air movement in the room depends on the internal differences in compost temperatures. If the temperatures are practically equal, minimum air movement is sufficient. If the temperature differences are greater, increased air movement should be applied. With active compost, after phase III compost has been filled, the growing room should be intensively cooled combined with plenty of air movement. This extracts large amounts of water from the room, ranging from 6-8 litres/m²/per day. Under these conditions, the casing soil can dehydrate. Plastic, moisture content of casing soil, filling weight (per bag) and the amount of supplements all influence dehydration.

A high CO₂ content encourages mycelium growth in the casing soil. Minimising fresh air/leakage automatically results in the required high CO₂ content.

18.7 Recovery

During ruffling the mycelium threads are broken up. During recovery the individual threads form branches and the mycelium fragments regroup until the compost is entirely incubated with mycelium. With caicing, mycelium has the opportunity to grow above the casing soil.

After the recovery period, the ideal situation is a network of fine and coarse mycelium threads in and on the surface of the casing soil.

There must be sufficient contact between the mature mycelium in the compost and the younger mycelium in the casing soil. This allows an uninterrupted process of water and nutrient absorption and transfer from the compost to the surface of the casing soil.
18.7.1 Climate factors during recovery

A number of climate factors are crucial for good vegetative growth:

- temperature
- CO₂ content
- relative humidity

**Temperature.** Mycelium recovery, particularly on the beds, is important. An air temperature of 25 °C is ideal for good mycelium growth. However, the compost temperature may rise too much then; if the compost becomes warmer than 27 °C, an air temperature of >21 °C is often used.

**CO₂ content.** With a CO₂ content in the air exceeding 0.4% (4000 ppm), the mycelium will remain in a vegetative state. In compost the CO₂ content can increase to 6 to 7%. A high CO₂ content has a stimulating effect on mycelium growth. Above 7% the stimulating effect diminishes and even turns into a negative effect.

**Relative humidity.** RH of 95 to 100% during recovery is ideal for vegetative growth.

18.8 Cultivation techniques during recovery

The purpose of the recovery period is to incubate the casing soil layer and surface without encouraging premature fruit body formation. As the preceding paragraphs have described, minimal extraction of heat, CO₂ and humidity is necessary. This means no ventilation.

The biological activity of the developing mycelium will cause the temperature, CO₂ content and RH to rise. Climate control should only be used if the maximum values are exceeded.

Circulation is kept to a minimum and is only used to prevent great differences in compost and growing room temperature or to activate internal cooling. Under ideal circumstances it is possible not to circulate at all during this period. If cooling does become necessary, internal cooling should be used to keep the CO₂ content sufficiently high.

To avoid the problems stated above, the compost temperature is often cooled down to 24 °C just prior to ruffling. During the recovery phase the compost temperature can rise to ± 27 °C without cooling becoming necessary. The CO₂ content in the air is between 0.3% and 1% (i.e. 3000 and 10.000 ppm) in a well-sealed growing room and with actively growing mycelium.

Directly after ruffling sprinkling can be activated (11/m²) for a uniform start to recovery growth and to moisten the casing soil layer. An added benefit is stronger mycelium. With little mycelium it is better not to water after ruffling, as this can delay recovery. During recovery no water should be given. The total recovery period normally takes 1 to 3 days.

A too long recovery period may be caused by one of the following:

- too little mycelium for quick recovery in the casing soil; too little caking material (CI)
- too long incubation period; this can mean that the mycelium is actually too ‘ripe’ to recover quickly enough
- interrupted exchange layer between compost/casing soil; too much water or too shallow ruffling
- too low air temperature due to active compost
- too high fan position for circulation, differences in compost temperature too large
- the entire spawn run is weaker than normal (too much mycelium at the bottom of the casing layer).
- too compact/heavy casing soil layer.

18.9 Cooling down

The purpose of cooling down is to stop the vegetative mycelium growth and encourage mycelium to commence fruit body formation, generative growth.

18.9.1 Climate factors

Three factors mainly influence the stop of spawn run:
- temperature;
- CO$_2$;
- relative humidity (RH)

**Temperature.** One of the most important factors is creating a so-called temperature shock: the air and compost temperature are lowered so quickly that the mycelium shows a shock reaction. This shock reaction stops vegetative growth. The mycelium bundles and forms thicker threads.

**CO$_2$.** During cooling down the CO$_2$ content must be lowered to less than 2000 ppm (0.2%). This will stop spawn run through the casing soil.

**Relative humidity.** Reducing the temperature and CO$_2$ is generally enough to stop the spawn run. As an additional stimulant, the RH can also be lowered, which will increase evaporation. However, too much evaporation will cause the mycelium to dehydrate and die off. This mycelium will then tend to recover at a later stage (e.g. when circulation is decreased). In order to actually achieve changed climate factors as above, heat and CO$_2$ extraction will increase. The degree of extraction is regulated by increasing the volume of ventilation air. Total air movement in the growing room must be increased.

18.9.2 Cultivation techniques during cooling down

**Moment of cooling down.** The moment of cooling down greatly depends on the specific requirements of the grower.

If cooling down is slow, the mycelium will continue vegetative growth on the surface of the casing soil for several days. Cooling down often takes place at an early stage. Growers who harvest mechanically place strict demands on crop uniformity. The mycelium must be evenly spread throughout the entire casing layer and fully recovered, before cooling down is started.

**Sprinkling.** Sprinkling simultaneously with cooling down can have three functions and/or results:
- mycelium that has incubated too high in the casing soil will be pushed down, this will benefit general flush uniformity
- deeper fruit body formation
sprinkling will inhibit the spawn run, the transition to fruit body formation will be easier.

In general, to use sprinkling to push mycelium down is an incidental emergency measure. With manual harvesting sprinkling down is used less than with mechanical harvesting.

**Temperature patterns.** The speed with which the air and compost temperature are lowered, influences fruit body formation. Rapidly reducing the compost temperature will generally result in more spontaneous fruit body formation. Cooling down to a lower compost temperature (deeper intensive cooling) will also give more fruit bodies. A long, gradual cooling down period causes the mycelium to start the generative phase at different times, meaning irregular fruit body formation. It is important that there is enough activity in the compost to keep this process going.

In the illustration below two ways of reducing air and compost temperature during cooling down are shown.

Two ways of reducing air and compost temperature during cooling down.

**Line A: slow, gradual cooling down.** Mycelium growth is not stopped simultaneously everywhere but continues growing into the casing soil for another few days. The fruit bodies already formed show various stages of development. Harvesting can be spread over a period of days.

This method of cooling down stimulates fruit body formation less and is usually used with spontaneously fruiting strains. The amount of mycelium must be taken into account.

**Line B: quick, intense cooling down.** Mycelium growth stops simultaneously. All the fruit bodies show the same stage of development. Fruit body formation is stimulated more. This method of cooling down is usually used with slow growing strains and where mechanical harvesting is used. A problem that can occur with this method of cooling down, is too great a loss of compost activity. This can lead to problems later with fruit body formation and development.

If a slow growing strain is to be harvested manually, follow a line between A and B.
Outside air is often used for cooling down. This also reduces CO$_2$ and RH. Internal cooling can be used during cooling down, however only if the CO$_2$ is sufficiently low. During cooling there should be enough air movement to ensure sufficient CO$_2$ and evaporative moisture extraction. Too much circulation will cause unnecessary activity loss. If there is not enough ventilation (fresh air), too little CO$_2$ and evaporative moisture will be extracted.

The CO$_2$ content and RH in the air above the beds remains too high, which allows continued mycelium growth. Incubation can still take place in spite of the fact that CO$_2$ and RH in the growing room are low enough. In general an RH exceeding 93-95% and a CO$_2$ content of 1500-2500 ppm is maintained.

Incubation is often confused with stroma. The difference is that incubation usually occurs after, while stroma is visible long before cooling down, sometimes even on the compost. Stroma stops as soon as cooling starts and forms large clumps in the casing soil.

Incubation means that the mycelium fails to change from vegetative to generative growth, but remains as a downy growth on the beds.

This may have various causes:

- too high CO$_2$ content and RH: use more ventilation and circulation;
- too high compost and/or air temperature: use more intensive cooling;
- using plastic under the compost can encourage incubation: circulating and ventilating for a longer period is often the answer;
- supplementing, possibly combined with plastic, increases the chance of incubation;
- use of cac-ing material gives extra activity with increased risk of incubation;
- too wet, compacted casing soil stimulates incubation (gas exchange).

If, in spite of all measures taken, the mycelium continues to incubate, try giving $\pm$ 0.5 l water/m$^2$. This will usually stop the spawn run.

In summer and autumn there may not be enough evaporation during and after cooling down. It may be necessary to decrease RH via cooling and after heating (dehumidification).

In winter, the casing soil may dehydrate too quickly. To avoid this it is better to increase the RH by wetting the floors and walls or by using steam to humidify the air. To save energy in the winter a higher CO$_2$ is used in cultivation. This means that less cold dry outside air is introduced into the growing room, which in turn means less heating and humidifying. Dehydrated mycelium will no longer form fruit bodies, so the RH must be kept above 85%. Regulating the number of pins and their spread on the casing soil depends on a combination of factors. Circulation in particular determines the influence of temperature and humidity settings.

### 18.10 Fruit body formation

During cooling the spawn run stops and fruit body formation is encouraged. This pin-heading phase lasts 4 to 5 days and follows the cooling down period. During this phase the mycelium contracts and gradually starts to form fruit bodies.

The following factors influence fruit body formation:

- climate;
• strain traits;
• bacteria;
• compost and supplementing;
• casing soil;
• sprinkling.

18.10.1 Climate factors
During fruit body formation the major aim is an optimal combination of the various climate factors: temperature, CO₂ content and humidity.

Temperature. For bed systems the optimal temperatures for fruit body formation are:
• air temperature 18 °C;
• compost temperature 20 °C.
Slight differences are possible. It is extremely important that the air and compost temperature differential is maintained. This guarantees more active fruit body formation.

CO₂ content. The CO₂ content in the growing room air is determined by the CO₂ production of the growing mycelium and the amount of fresh air, which determines CO₂ exhaust.
Factors influencing CO₂ production are:
• growth rate mycelium;
• compost activity (type of compost, filling weight, plastic, amount of supplement);
• number of mushrooms on the beds;
• temperature.
The extraction rate of the CO₂ produced depends on:
• volume of fresh air;
• air movement over the beds;
• activity: a higher air and compost differential causes more natural air movement;
• extent of compaction, compost structure and casing soil;
• humidity content of casing soil and compost.
Fruit body formation takes place at a CO₂ content of 600-2000 ppm (0.06-0.20%). The CO₂ content is usually measured 15 cm above the middle beds. With a low CO₂ content, but further equal conditions, more fruit bodies will be formed than with a high CO₂ content.
If a high CO₂ content is maintained during fruit body formation, there will be fewer fruit bodies with thicker stalks. This indicates future development into heavy mushrooms.

Relative humidity. During fruit body formation, RH above 92% is required. This RH level means mycelium will continue to grow and contract slowly.
A low RH, combined with a high air speed, means a greater risk of mycelium dehydration. The result is that the fruit bodies will form deeper in the compost with more risk of mushrooms being soiled by casing soil. Another result of a low RH is more but smaller mushrooms.
With a too high RH, higher than e.g. 96% there is too little evaporation and the mycelium will continue to incubate. The state remains too vegetative. In places where the casing soil is covered with mycelium, no more fruit bodies will be formed.
The optimal climate for fruit body formation is of course determined by a combination of the factors mentioned above. The RH, temperature and CO₂ cannot always all be ideal, but aim to approximate this state as closely as possible.

18.10.2 Strain traits
A fast fruiting strain (intermediate hybrid) forms more fruit bodies than a slow fruiting strain (big hybrid). The fruit bodies of a fast fruiting strain usually also grow quicker. Strain traits partly determine fruit body formation, but cultivation conditions and mycelium activity play an equally important role.

18.10.3 Bacteria
In the past many studies were carried out into the influence of bacteria on fruit body formation. This research showed that a favourable microflora in the casing soil is essential for spawn run and fruit body formation. As mycelium incubates in the casing soil, selection takes place in favour of these bacteria that are essential for fruit body formation. One of these bacteria, Pseudomonas putida has been identified. If sterilised casing soil is used for cultivation under sterile conditions, little or no fruit bodies develop (assuming sterile irrigation water was used). However, if a bacteria-suspension is sprinkled over the sterile casing soil, the result is normal fruit body formation. A quantitative relationship between the number of bacteria and the number of fruit bodies has also been proven. A too heavily disinfected casing soil has an inhibiting effect on fruit body formation.

After casing, the development of bacteria is stimulated by various volatile metabolic products of the growing mycelium. Natural selection takes place, namely of Pseudomonas putida. A too high metabolic products content (acetone, acetaldehyde, ethylene, ethylaconal and ethyl acetate) represses fruit body formation. Pseudomonas putida absorbs the excess of these substances so that fruit body formation is not inhibited. This theory is backed up by research where active carbon is added to sterile casing soil. The active carbon also compounds the gaseous extraneous substances and a normal number of fruit bodies were formed on the sterile casing soil. On the other hand, it is known that very small concentrations of certain substances (e.g. ethylene) encourage fruit body formation.

18.10.4 Compost and supplementing
In general, the more compost (dry matter) filled per m², the easier the process of fruit body formation is. This is explained by the fact that the extra nutrients increase activity. With sufficient activity there is more spontaneous natural evaporation which leads to easier fruit body formation. Supplementing has a comparable effect.

18.10.5 Casing soil
The lumpier, heavier and wetter the casing soil is, the less mycelium grows in the casing soil and therefore less fruit bodies will be formed. Compacting casing soil also means less fruit body formation.

In both cases the extraction of metabolic products from the compost and casing soil will be inhibited, making fruit body formation more difficult. The amount of cac-ing
material (Cl=Cac-ing Inoculum) should be adapted to suit the quality of the casing soil: wetter casing soil requires more Cl.

18.11 Cultivation techniques

During fruit body formation the climate demands high attention. The climate factors temperature, CO₂ content and relative humidity, combined with a certain degree of air movement, have an enormous influence on fruit body formation. The grower can only influence the number of mushrooms and their spread, if growth is high enough on the beds. If fruit bodies are formed under the casing soil surface, the influence of the grower will be limited, this is a difficult aspect due to dehydration and limited climate installations.

**Temperature.** After cooling down the compost temperature can drop too low. To prevent this, the air temperature is increased and air movement reduced. For spontaneous fruit body formation a 1.5-2 °C differential between the air and compost temperature is required (e.g. 18 °C air temperature and 20 °C compost temperature). This difference is partly influenced by the method of cooling down. A slow and even cooling down process will usually result in the compost retaining more activity. This means there is a more obvious difference between air and compost temperature.

With quick and intense cooling down more heat (activity) will be extracted and the compost temperature will drop, resulting in a small difference between air and compost temperature. Plastic under the beds ensures higher activity.

**CO₂, RH and air movement.** When fresh air is introduced based on CO₂, a CO₂-value of 0.06 to 0.20% is often set. This value determines the minimum level of fresh air. The exact CO₂-value set depends firstly on the outside climate conditions. During the winter a higher CO₂-value is set than in the spring and autumn. Compost activity, the type of climate installation and the strain also play a part in selecting the required CO₂-value.

During fruit body formation only slight air movement is required. However, there must be enough ventilation to extract evaporation and CO₂.

If there is too little air movement, the CO₂ content and the humidity will become too high, particularly close to the fruit bodies. In addition, the temperature differences will become too great.

Too much air movement causes the casing soil to dehydrate and lessens compost activity. Increased air movement also causes increased extraction of evaporation and heat. Evaporation requires energy that is taken from the compost in the form of heat. The difference between compost and air temperature will become smaller.

Recently formed fruit bodies are highly sensitive and benefit from an even growing room climate. This explains why only small temperature differences are acceptable.

In order to extract enough CO₂ and evaporation from the growing room, use ca. 5 m³ fresh air per m² growing surface per hour.

To keep humidity above 90% the air can be misted or humidified with steam. Wetting the floor and walls regularly can also humidify and cool. A too low RH, combined with a too high air speed will result in activity loss and cause dehydration.

**Micro- and macroclimate.** For efficient climate control it is important to distinguish
between the macro- and microclimate. Microclimate is the climate between the lumps in the immediate vicinity of the pinhead. Macroclimate is the climate in the rest of the growing room.

The grower regulates the condition of the macroclimate, which is measured above the third bed or in the central gangway.

The microclimate is however more important because it is closer to the actual fruit. Between the lumps there is less air movement and therefore less exchange with the air above the casing soil. The fruit bodies between the lumps are protected against abrupt changes in the growing room climate.

As air movement lessens, the difference between the macro- and microclimate will increase. A lumpier casing soil structure can provide better protection for the fruit bodies. The changes in the macroclimate will therefore have less direct influence on the microclimate. The difference between the macro- and microclimate is therefore greater than with a finer casing soil surface (with even air movement). The influence of air movement and casing soil structure on the macro- and microclimate can be expressed as follows.

**Influence of air movement on the microclimate**

<table>
<thead>
<tr>
<th>High air movement</th>
<th>Low air movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH = 90 %</td>
<td>RH = 87 %</td>
</tr>
<tr>
<td>CO₂ = 0.10 %</td>
<td>CO₂ = 0.08 %</td>
</tr>
</tbody>
</table>

To maintain the required climate around the fruit bodies a higher RH and CO₂ with high air movement should be set than in situations with low air movement.

During cooling down and fruit body formation, the number of fruit bodies is determined and also how deeply they are formed. Where casing soil has a rougher surface structure, fruit bodies will form where the conditions for fruit body formation are optimal. At the start of fruit body formation, this is generally high on the casing soil and between the lumps, as the influence of the macroclimate is greatest at this point. Deeper between the lumps the CO₂ content and the humidity will remain higher for a longer time period, causing later formation of fruit bodies. In this way various stages of fruit body formation are achieved, which spreads the flush.

A smooth upper surface of casing soil with very fine lumps creates a less defined microclimate around the fruit bodies – the influence of the macroclimate is therefore more direct. Combined with quick and intense cooling down, the pinheads will be formed simultaneously and more uniformly, leading to the development of a consistent first flush.

**Sprinkling.** Newly formed pinheads are extremely sensitive to water. Pinheads of slow fruiting strains are the most susceptible in this respect, so irrigation should not take place during fruit body formation.
In certain exceptional cases, sprinkling may be necessary:

- after cooling down, mycelium still incubates (re-growing). Sprinkling should be used to stop the spawn run – 0.5 l water per m².
- if cooling down has caused the upper surface of the casing soil to become too dehydrated.
- if overpinning occurs, sprinkling (4 to 5 l/m² in a short period) can be used to reduce the number of fruit bodies, that would otherwise develop into the 1st flush. The effect depends on air movement and the set values.

18.12 Fruit body development

When sufficient pinheads have formed, these fruit bodies should develop into mushrooms. Evaporation is essential for growth. The following climate factors determine evaporation.

18.12.1 Climate factors

The growing fruit bodies will produce more humidity, CO₂, and heat. Enough of these metabolic products must be extracted. The extent of extraction depends on the amount of ventilation air. As the mushrooms grow ventilation must be increased. Air movement in the growing room will usually also increase. Evaporation is a combination of relative humidity, circulation, ventilation, air temperature and compost temperature. The RH is generally reduced to 88-90%. The required values for CO₂ and temperature at the beginning are equal to the values required during fruit body formation. The CO₂ content at the beginning of fruit body growth determines the shape of the fruit bodies. The increasing heat production will gradually raise the compost temperature.

18.12.2 Cultivation techniques

Climate. The actual amount of extracted evaporation, CO₂, and heat is determined by three factors: air speed over the beds, the amount of ventilation air and the outside climate conditions compared to the growing room climate.

In the winter too much humidity is often extracted, the RH drops and dehydration can occur. For this reason, ventilation during the winter is often based on a higher CO₂ content. This generally provides considerable energy savings.

In the summer and spring, extracting evaporation is often very difficult due to the high moisture content of the outside air, resulting in evaporation problems. The CO₂-rule is often ‘ignored’ in this case and ventilation is based on RH.

For each degree of temperature rise in the compost, ventilation should be increased by 20%. In this case moisture production and outside climate conditions are not taken into account. Evaporation problems may occur.

With computer regulated control the minimum and maximum CO₂ content limits are usually indicated.
The moment when fruit body growth begins depends on the fruit body formation period. The more spontaneous fruit body formation was, the earlier evaporation can be stimulated. If large numbers of fruit bodies have been formed, a higher RH and CO₂, combined with a lower air movement can limit the growth of a number of fruit bodies. If fruit body formation is less spontaneous and therefore takes a longer period, evaporation must be additionally stimulated during pinheading.

Under ideal conditions sufficient fruit bodies should have formed to develop further under gradual, unforced conditions. Mushroom qualities will consequently improve. The transition from pinheading to fruit body formation, approximately 7 days after the start of cooling down, is a critical stage of cultivation. The biggest problem at this stage is too little compost activity. This makes evaporation difficult, delays growth and will possibly even result in growth stopping altogether. If this situation lasts too long, the fruit bodies will die.

In this situation all cultivation techniques should be applied to stimulate evaporation, and growth, as quickly as possible. The growing room climate must be considerably altered. When faced with a choice of two evils, bad fruit body development or a fluctuating climate, the least bad should be chosen.

Emergency measures to stimulate growth and evaporation are:

- increased ventilation and air movement, possibly combined with dehumidification. The disadvantage is even greater loss of activity. This can also cause the casing soil and fruit bodies to dehydrate. To prevent dehydration, sprinkle at a rate of 0.5 l/m²;
- to raise the compost temperature, the air temperature is temporarily raised: the so-called heating up. The purpose is to create a greater difference between air and compost temperature.

The opposite situation can also occur: too many fruit bodies. The cultivation techniques applied here are all aimed at considerably reducing evaporation.

Various examples are:

- ‘CO₂-dope’ is a method used by certain growers at the end of fruit body formation. It can be a rigorous method to reduce the number of fruit bodies. During a certain time, ventilation stops and hardly any fresh air is introduced. The CO₂ content and the humidity, particularly immediately above the casing soil, can rise to extremely high levels. The longer this condition persists, the more fruit body development will be delayed. This method of selection has a number of disadvantages. If used for too long, too many fruit bodies will fail to develop and the yield in the 1st flush will be too low. The exact moment this method is used also has a tremendous influence on the outcome. In a young/early stage the effect can be great. A drawback is that it delays the first flush.
- Increasing the temperature to reduce the difference in compost and air temperature (0.5 to 1.0 °C).
Sprinkling can also be used as a way to reduce the number of mushrooms. Sprinkle at least 4 l/m², but not with dry ventilation. The water film around the mushrooms only stops evaporation if it is maintained for some time and ventilation will cause the mushrooms to dry too soon.

Experience shows that with active compost, fruit body growth should not be a problem. Even with a high RH and CO₂ and early sprinkling, enough fruit bodies develop. If there is little compost activity, the yield and quantity will still be disappointing in spite of all extra cultivation techniques used.

18.13 Cultivation techniques from pinheading up to and including first flush

Growth curve of mushrooms (S-curve). During fruit body development, the speed of growth of the mushrooms increases but after day 7 it slows down dramatically.

As the speed of growth increases, so does the production of heat, CO₂ and moisture. The excess must be extracted. The weight increase of the mushrooms is namely a result of water absorption. During this period of high water demand, the water supply to compost and casing soil must be increased.
18.13.1 Temperature and activity
During the development of mushrooms towards the first flush, the compost temperature will rise while the air temperature stays the same. The growing nutritional needs and increased metabolic activity produce more heat. Part of this heat will be extracted through evaporation, the residual heat causes the compost temperature to rise. The higher compost temperature encourages growth. For better quality mushrooms (firm structure) the temperature is often kept lower than would be expected until just before harvesting.

The mushrooms have, by evaporating, continually extracted heat from the compost. When the mushrooms are harvested, metabolic activity gradually decreases. The compost temperature during the first flush can rise to $\pm 24 \, ^\circ C$, a higher temperature is often detrimental to the second flush, especially when growing in bags. If mushrooms are harvested mechanically, the effect is greater. A period of increasing activity (rising compost temperature) is the best moment for irrigation.

18.13.2 CO$_2$, RH and air movement
In principle, the CO$_2$ content and RH are kept at the same values as during pinheading. To maintain these values the amount of ventilation should be gradually increased. Too little air movement and ventilation cause evaporation problems (glassy patches on mushrooms).

Too much air movement and/or ventilation causes unnecessary activity loss. Excessive air movement combined with a low RH will cause scaly mushrooms (winter problem). If the mushrooms are close together, extraction of CO$_2$ and evaporation will be inhibited in spite of a high air movement. This leads to long thin stalks and open mushrooms. The second flush will also be negatively influenced.

Too late harvesting of the first flush will have a negative result on the second flush. The paragraph ‘Intermediate flush’ (18.13.4) describes this influence on the fruit bodies of the following flush.

18.13.3 Sprinkling
When mushrooms are sprinkled a number of factors must be taken into account:

- mushrooms must be a minimum size before they can be sprinkled. For big hybrids a minimum of a pea-size applies, with intermediate hybrids sprinkling may be started a little earlier. Sprinkling too early will cause growth stagnation;
- excessive sprinkling can also cause growth stagnation. This certainly applies with disappointing compost activity;
- enough water should be sprinkled to ensure that the casing soil after the 1st flush is soft enough for the 2nd flush. To avoid sprinkling the growing fruit bodies of the second flush, start sprinkling when the mushrooms of the first flush are being harvested; Sprinkling the mushrooms will improve the quality of the intermediate flush and the moisture balance of the 2nd flush;
- to reduce the risk of bacterial blotches, the mushrooms should dry in 2-3 hours after sprinkling; if the sprinkling water has to be chlorinated for bacterial blotches, chlorinated water should only be used for the final sprinkling. Generally, where there is sufficient compost activity, sprinkling should not cause too many problems during
cultivation. Natural evaporation should dry the mushrooms quickly enough without using extra air movement and ventilation (drying program).

18.13.4 Intermediate flush
At the end of the first flush, the grower has two options:
- to fully pick the bags, trays or beds;
- to cultivate an intermediate flush.

The greatest benefit of removing all the first flush mushrooms is that the cultivation techniques required to obtain a good second flush can be carried out as successfully as possible. These activities include sprinkling, increasing/decreasing temperature, crop protection etc.

An intermediate flush consists of the fruit bodies formed last in the first flush. After the first flush has been harvested, these fruit bodies are left to grow for a further 3-4 days to form an intermediate flush.

An intermediate flush usually supplies extra yield that can be harvested with a very high picking rate. The quality of the mushrooms in an intermediate flush fluctuates. Another advantage of an intermediate flush is that it allows the spread and moment of harvest of the second flush to be influenced. A large intermediate flush that is harvested late results in a delayed and better spread second flush.

A second flush intended for mechanical harvesting should have little spread, which is why the intermediate flush must be picked while it is as small as possible. The intermediate flush is removed using an aluminium strip that knocks the large fruit bodies (approximately four days before harvest) from the bed. The fruit bodies can also be removed using stalk uprooters.

Mushrooms in an intermediate flush tend to have a tapered stalk and a dented cap. Sprinkling and climate control should be managed to achieve good development of the second flush. If too much attention is paid to the quality of the intermediate flush at this stage, the quality of the second flush will suffer.

18.14 Cultivation techniques during the second flush
18.14.1 Temperature and activity
At the end of the first flush the compost temperature rises. The corresponding air temperature depends on the cultivation method.

A constant air and compost temperature differential can be set which maintains activity better. The danger of a higher air (and compost) temperature during the growth phase is that the mushrooms will develop too quickly with all the consequences for possible loss of quality.

The air temperature is usually controlled to a constant value (17-18 °C) during and after the first flush. This control does however lead to increased loss of activity, particularly with high air movement. To prevent the compost temperature dropping too quickly, the air temperature may sometimes have to be raised temporarily at the end of the first flush.

Growth of the second flush starts immediately after the drop in compost temperature, when activity increases. As there is a large temperature difference between air and
compost temperature at the beginning of growth, the progress of the second flush is easier and more gradual than the first flush. A temperature peak, as with a first flush, is not usually noticed with a second flush. To avoid losing activity during a third flush, air movement and ventilation should be kept to a minimum during the growth phase of the second flush.

18.14.2 CO₂ and RH
The CO₂ and RH values maintained during a second flush are slightly lower than during the growth of a first flush. If, during the otherwise healthy, first flush too few fruit bodies developed fully, a massive explosion of fruit body growth can be expected in the second flush. To limit the growth of too many fruit bodies, a higher temperature, RH and/or CO₂ is maintained following the first flush. There must however be enough activity left after the 1st flush to allow temperature to be used for control.

18.14.3 Sprinkling
Wetting the casing soil for a second flush stops at the end of the first flush. Sprinkling is only restarted when the growing fruit bodies are large enough. The amount of water depends on the expected yield. Stopping sprinkling in time has a beneficial effect on the quality.
For a good third flush it is important that the casing soil is wet and soft enough. When 80% of the mushrooms have been harvested, sprinkling can start.

18.15 The third flush
When the current big hybrid-strains (namely the U1-like types) are cultivated, the third flush delivers highly fluctuating yields. To cultivate a good third flush, it is crucial to retain activity after a second flush and provide soft casing soil.
There are two ways to create a forced greater difference between air and compost temperature:
- temporarily raise the air temperature to exceed the compost temperature (22-23 °C). As soon as the compost temperature has reached ±21 °C, the air temperature is lowered again.
- an extra decrease in air temperature, if the compost can remain at the same level as before the drop in air temperature, this also creates a greater difference between air and compost temperature. This will however delay the third flush (see diagram).
A number of greatly differing techniques are also used by growers to achieve a good third flush, for example:
- excessive ventilation following the second flush, i.e. extracting large amounts of CO₂, evaporation and heat to give an extra stimulus to evaporation to create more fruit bodies. Pay attention to activity loss.
- limited ventilation after the second flush for a few days, namely less evaporation and CO₂ extraction. The result is downy mycelium growth between the fruit bodies. As soon as the fruit bodies appear, ensure that exchange can occur. CO₂ production is very low during this period, ensure constant and sufficient fresh air.
The moisture content of the casing soil is an essential factor. If it is too dry, the result is
often massive fruit body formation. The individual mushroom weight will be disappointing and the picking rate low.
Activity is not only the difference between air and compost temperature. Activity is mainly determined by the humidity difference between the air and the casing soil surface. Activity is the ability of the compost to provide heat, moisture and CO₂.

18.16 Cooking-out, emptying and disinfecting

The room and contents are cooked out and emptied at the end of the harvest. Prior to cooking-out, inlet and exhaust openings are sealed off and remote thermometers placed in the bed and the air space. The temperature is then taken at a number of points. Fans and lights with control boxes mounted outside the rooms may be left on during cooking-out. This reduces the risk of failure. Lighting units with integral control equipment must be switched off. In order to kill all the infections (also viruses) that affect mushroom cultivation, the compost temperature must be raised to 70 °C. This also applies to the least favourable (coldest) parts. This temperature must be maintained for 8 hours. Increase and decrease the temperature (10-15 hours) slowly to prevent damaging the building.

In cooking-out, preference is given to heat treatment before the room is emptied. This prevents the spread of nematodes, mites, flies and harmful fungi. It also prevents the spread of die-back virus by spores or by living mushroom mycelium as the room is emptied.

After cooling, the room is emptied. The beds are often emptied using built-in conveyor nets. Spent compost is then immediately loaded on a truck and removed from the farm. When the compost has been removed, the room and compost-floor are thoroughly sprayed clean. This also applies to conveying mats. Once the room is cleaned out, any necessary maintenance can be performed.

Often old filters are removed and replaced with new ones at this point. If wooden trays are still used, they must be disinfected once the wood has dried (see chapter 26. Pests and diseases).

18.17 Quality of button mushrooms

18.17.1 Introduction

What is quality? ‘Quality’ is the extent to which a product fulfills the expectations that consumers have of the product in question. Quality is therefore relative. The consumer confers the term quality on a product as it is the consumer who actually makes the purchase.

Quality can be determined by maintaining various criteria used to distinguish variations in the product. The following criteria have been developed for button mushrooms: colour, size, stage of development, shape, and soiling.

Consumers buy mushrooms guided by the visible aspects listed above. But their willingness for repeated buying of the product is also influenced by other, equally important factors. For instance: flavour, smell and consistency, shelf life, ease of use in recipes, nutritional value, and healthiness. It is clear that the better the product meets consumer expectations, the higher its marketing potential. Consumer behaviour therefore
determines the quality demands placed on a product. Growers, traders and processing industries must aim to meet these demands. Growers must try to translate consumer wishes into standards that can be used to measure the quality of their produce. These standards can be laid down in quality regulations (representing the minimum quality standards) which the mushrooms must comply with. Part of these quality regulations are grading norms based on the size and stage of development of the mushrooms. In these regulations mushrooms are divided into class I, II and III. Classification depends on the mushrooms’ stage of development.
The choice of a grower to cultivate a certain quality depends on the financial results that can be achieved with that quality. The grower can, for example, try to cultivate a constant quality 1-1-40, but if the harvesting costs are too high it is not worth trying to continue.
In addition, traders and the processing industry will assess quality based on the suitability of the product to be sold at the highest possible market price for the lowest possible purchasing price.

18.17.2 Factors that influence quality

**Strain choice.** The relationship between quality and mushroom strain is difficult to illustrate.
There are certain general strain traits relating to mushroom shape, colour and firmness. The currently most commonly cultivated types are the U1-types. The U1-types produce larger mushrooms with a slightly better colour and firmness than U3-types. U3-types produce smaller mushrooms with a smoother cap. The final quality is determined more by the cultivation techniques than the chosen strain.

**Cultivation techniques.** In order to harvest a good quality mushroom, undisturbed growth is essential. Undisturbed growth means that sufficient evaporation is always possible and that the mushrooms have the space to develop.
With manual harvesting in particular, it is important to prevent too many fruit bodies growing during cooling down, fruit body formation and fruit body growth as such.
The treatment and quality of the raw materials such as compost and casing soil are also very important, plus the correct growing room climate is crucial during the various stages of cultivation.

18.17.3 Sprinkling
The relationship between sprinkling and quality is one of the most frequently discussed aspects of cultivation. On the one hand we want large, heavy mushrooms with a high yield (within a certain time frame) and a high picking rate. This cannot be achieved without sprinkling. On the other hand, we want white, compact mushrooms without discolouration, watery patches or bacterial blotches.
To meet both requirements we can mention certain aspects that can be used as a good average guide towards obtaining both good quality and the required quantity of the produce.
By sprinkling mushrooms, evaporation stagnates as the water forms a sealing layer around the fruit bodies. Depending on the age of the mushroom and the speed of growth, the effect of sprinkling is more or less beneficial.
It can lead to rapid deterioration in quality and in the worst case, growth will stop. To limit this growth repressing effect, the mushrooms can be ventilated to dry for 2 to 3 hours after sprinkling. The mushrooms will often dry earlier. The subsequent growth is so strong that the extra water hardly has a damaging effect and even seems to encourage good growth. At this stage, sprinkling may be done with no fear of quality loss. The cultivation techniques mentioned all have a huge influence on the final mushroom quality. The more attention is paid to the quality of work in the preparation phase of cultivation, the greater the chance of harvesting good quality mushrooms.

### 18.18 Manual harvesting

Cultivation techniques are not the only factors influencing quality. The harvesting method also affects the final quality. Mushrooms lack an epidermis and are therefore a highly sensitive product. Needless to say, the number of dents and bruises must be minimised. To harvest good quality mushrooms, pay attention to the following points:

- Keep handling to a minimum during picking and sorting, e.g. by putting the mushrooms immediately in the final container in which they will be sold.
- Do not pick too many mushrooms in a single action. It is easier to cut the mushrooms straight if they can be held upright in the hand.
- Wipe your hands regularly to avoid soiling the mushrooms with casing soil. Clean the blade of the knife regularly to avoid soiling or stripes on the cut edges.
- Never begin picking a full bed from the centre, but work from the outer edge to the centre. This prevents bruising of and casing soil on mushrooms that still have to be picked.
- A good posture and an overview of the various grades in the picking rack are very important.
- A bad posture means you tire more quickly and suffer from lapses of concentration. A good posture means that bending or reaching are limited to a minimum. Important here is the height of the picking platform, height of the second bed and equipment which facilitates picking from the lower bed.
- The various grades must be easy to reach.
- The picking rack must be constructed to contain all the necessary packaging material and allow mushrooms to be easily placed in it.

A good posture can only be maintained if bending and reaching are kept to a minimum. Not an easy condition in rooms with shelves 3 metres high and 1.40 m wide! (courtesy Cpoint).
If a manually harvesting farm’s aim is to constantly harvest a certain quality percentage it is essential that the pickers receive adequate instructions. The employees should be aware of the various grades, but most importantly, they should be aware of the effect that the way they pick has on the quality of the produce. When new pickers are instructed the emphasis must be placed on the quality of the produce picked and not the quantity. Once the correct picking technique has been learned, proficiency and speed will naturally follow.

### 18.18.1 Picking rate improvement form

The following form can be used to assess the picking process.

<table>
<thead>
<tr>
<th>Equipment and organisation</th>
<th>Good</th>
<th>Improve</th>
</tr>
</thead>
<tbody>
<tr>
<td>The equipment (trolley, rack and knife) must be adequate for the job: for example, tell your supervisor if the trolley sticks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plan ahead: what do I need if I change tier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per tier/bed empty the stumps, remove full containers, place on the trolley in the gangway and place empty containers on your picking trolley</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Easy to stack packaging: type with type, boxes and crates stacked separately</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicate your needs in time: let people help you and don’t make others walk for nothing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t walk around the growing room with empty hands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimate if you can finish a growing room before the break, so you don’t have to return later for just five minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consult colleagues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keep the growing room and gangway tidy, first empty a pallet of packaging before starting on a new one</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Personnel and picking</th>
<th>Good</th>
<th>Improve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start work in the morning at the agreed time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive picking attitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A good picking technique gives 25% more speed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevent diseases so no time is wasted tackling dry/well bubble, for example</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t pick too many different grades, this increases manual work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not pick zigzag over the bed, but in diagonal 30cm sweeps in a forward direction</td>
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<td></td>
</tr>
<tr>
<td>Picking forward is more efficient and less tiring for the muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t look behind, you will always see mushrooms you’ve missed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t hesitate, if doubtful continue working</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If there are only a few mushrooms, only use a few pickers in the growing room</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Be confident enough to move forward (‘racing through the growing room’), if you can’t see anything, there’s nothing to pick</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t obstruct others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mushroom size, the bigger, the heavier. Don’t pick mushrooms too small. A mushroom can double its weight in a single day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t pick too many mushrooms in one hand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The spread of mushrooms</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Good posture</th>
<th>Good</th>
<th>Improve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stand in a relaxed posture, do not overbend your knees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pick with relaxed shoulders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keep your back straight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Try to look straight ahead as much as possible, don’t constantly bend your head</td>
<td></td>
<td></td>
</tr>
<tr>
<td>When picking in sweeps place the foot closest to the shelves slightly in front of the other foot with your toes facing the picking direction. If you have to stretch, bend your knees.</td>
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<td></td>
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</tbody>
</table>
18.18.2 The picking rate
The picking rate is an equally important aspect of manual harvesting, as picking costs form a significant part of the cost price of mushrooms. The rate per hour depends on many factors, such as:
- spread, size, weight of the mushrooms
- preparation
- organisation
- supply prognosis
- logistics
- registration
- instruction/supervision
- quantity of mushrooms
- harvesting method
- stalk length
- cost price
- proficiency of the picker
- humour, chatting
- throwing mushrooms
- overtime
- working conditions. The amount of light; space above the mushrooms (tray system or bed system); adjustability, picking height, etc.

The art is thinning out carefully and knowing when not to pick. Preparation is half the work of harvesting.

18.18.3 Supervising new personnel.
A new employee is often left to his/her own devices. What exactly does the introductory period involve? What should new pickers know and how should they perform? Observe new personnel closely during the initial 6-8 weeks. Write down what you expect the new picker to know. List the points and describe them for yourself. Also make a timeframe to indicate when a new picker should be proficient at a certain task. For example, thinning on Mondays, harvesting a third flush without damage, correctly harvesting three mushrooms per hand, posture, dry/wet bubble, hygiene, house rules etc. It is not only important to know what or how to do something but also when. Try to indicate just how detailed the knowledge should be. Also note the expected picking rate after a certain time, and what you consider the word 'quality' to mean.

The following aspects should also be included in the checklist for the introductory period of new pickers:
- picking method (thinning out; harvesting 1st, 2nd or 3rd flush, etc.)
- damage (nails, bruises, sandy stalks etc.)
- uniform filling of the boxes ('heads up')
- overweight (limit by using individual scales)
- colour (depending on customer or packaging)
- grade (amount)
selective harvesting (S-curve)
stalk length
hygiene/Eurep-GAP
pests and diseases
posture (during harvesting and weighing)
harvesting several times a day.

18.18.4 The S-curve or growth curve
Mushrooms consist of roughly 90-92% water. Mushrooms grown in caves at low temperatures contain ± 88% water, while mushrooms, grown for industrial use in The Netherlands, can contain up to ± 94% water in certain cultivation conditions. Most water is absorbed during cell division. In mushrooms, this is the period 4-7 days after fruit body formation. In the primordial the cells for the stalk and cap are already present from the beginning. Subsequent differentiation follows. Between the ring and the underside of the cap further cell division occurs. Weight increase takes place at the same time as plasma and elongation. From day 4 to day 7 the weight increases by 100% per day. If the growth course and weight increase is expressed in graph form, the S-curve appears. This information is essential for the picker. He/she can then determine the exact moment to harvest the mushroom. This costs time and energy but ensures higher production and performance.

From the moment that the mushroom cap opens, becomes flatter and loses its spores, the mushroom weight will no longer increase under normal conditions. It can in fact decrease due to breakdown of organic matter and spore loss (as is the case with Portabellla and Flats).

18.18.5 Forward harvesting
By harvesting in a forward direction, the picker has a better impression of what still has to be picked. Middle and fine grades can be picked in one hand. Pests and diseases are detected at an early stage. The back, neck and shoulder are less strained. With backward harvesting there is an increased risk that the picker harvests ‘behind’ the shoulder, this increases stress on the shoulder. Pickers are more confident with forward harvesting. When harvesting backwards they see what has already been picked and tend to take a few more ‘expensive’ mushrooms. Harvesting randomly over the beds is still a disadvantage with forward harvesting, but is easy to learn. With forward harvesting the picker can plan ahead and estimate the quantity of packaging required much better. With this method the picker must ensure that he/she does not overreach, otherwise the picking rate will drop and the shoulders will be strained.

18.18.6 Instructions
Good instructions are difficult to give. Ensure that an experienced person with good didactic skills supervises new personnel. The following points will help form a solid communicative base.
Instructor
1. Before starting to give detailed instructions, give a global indication of the entire process; explain what the end product looks like.
2. Explain why tasks must be performed in a certain way and sequence.
3. Find out what the other person already knows about the subject.
4. Ensure the people being instructed are familiar with the jargon and terminology used.
5. Supply information and instructions in a logical order.
6. Supply information in easily ‘digestible’ chunks and regularly check if you are being understood.
7. Pay attention to non-verbal signals given by the people being instructed; facial expression and body language often indicate if the instructions are being understood or not.

Pickers
1. Listen actively and ask if items are not clear.
2. Tell the instructor if you do not understand certain words or terms.
3. Ask the instructor to summarise the instructions, if necessary.
4. Do not assume that others will explain certain points again to you later, but ask the instructor straight away to repeat the instruction or explain in more easily understandable terms.
5. Do not assume that the instructor is a mind reader! If your body language doesn’t show that you are confused, angry or unhappy, the instructor may assume that you have fully understood the instructions.
6. Remember that communication is a two-way process and that both parties involved are responsible for good mutual understanding.

Ten rules for correct posture
1. Vary posture/movements as much as possible
2. Bend the knees slightly when standing. Never overstraighten the knees
3. Keep your head upright above the back and spine
4. Use your muscles correctly
5. Bend through the knees with all bending and lifting movements
6. Bend forward through the hips
7. Carry objects close to your body (never bend backwards when carrying and do not balance weights on your hips)
8. Use the edge of the bed or your legs to support your back
9. Never lift with straight arms (lever effect)
10. Do not stretch from the shoulders.

18.18.7 Picking equipment
The aim of picking equipment is to lower harvesting costs by increasing the picking rate. This could be achieved by allowing the picker to use both hands, automatic stump cutting, centralised transport of the mushrooms and grading by size (not quality!).
18.18.8 **Quality**
This must still be maintained even if picking devices are used. Pay attention to:
- Falling height
- Handling/touching rate increases
- ‘shaking’ during transport may cause bruising
- ‘heads up’
- Stalks at equal length
- Mushroom assessment. The picker only sees the top of the mushroom
- Grading. With picking devices it is usual to harvest several times a day. The picker
takes one grade each time. With equipment that grades on size there is less variation
in the containers and everything can be harvested in a single go.

18.18.9 **Managing changes in picking methods**
Changes can cause problems for the pickers. The way in which change is introduced is
very important. When purchasing equipment to help with harvesting, bear the follow-
ing in mind:
- with the device, can you now harvest from the lower bed?
- limited view of mushrooms (bed distance and bed width)
- limited reach
- straight in front of trays
- (change of packaging).
It is possible to raise the picking rate with the help of picking devices. Clearly, each
individual farm must consider the type of equipment best suited for their purposes.
Increasing the picking rate is only possible if the mushrooms are well developed on the
beds. Ideal are dry mushrooms high on the beds. Organisation in this case is more
important than ever, because there is less flexibility possible. Ensure all preparations
are made properly before starting work:
- Make worksheets for the pickers.
- Place all the equipment ready, so picking can be started immediately.
- Register picking data, so any problems can be traced and remedied.

18.19 **Mechanical harvesting**
This method of harvesting is totally different from manual harvesting. The greatest
emphasis here is on uniformity throughout the entire process. For this reason, sprink-
ling for example is done in the final few days before the harvest. Organisation is impor-
tant. Particularly in cutting companies there is a huge variety in the way in which the
mushrooms are processed. Work preparation is therefore highly important.
During harvesting, the following points are important:
- Moment of harvest. This depends on the quality required. The time of harvest is
  also closely tied to the second flush.
- Machinery setting and speed. This determines the yield, but also the number of
  stumps. Sorting on a belt is preferable to ‘shaking’ containers. Cutting affects the
  work still to be done after cutting.
• Edge cutting or picking. This picking device is currently used in slicing companies, mainly for sorting and pre-picking.
• Grading. Graders can be used on slicing companies. Especially companies specialised in the smaller grades can achieve a higher percentage of fine mushrooms using a grader.
• Filling containers/stacking/unloading.
• Evacuating: treating the mushrooms to replace air with water in special tanks. Certain companies do this themselves, but it is normally done at the canning facility. The advantage is that mushroom deterioration is kept to a minimum.
• Stump uprooting. The fruit bodies of the second flush are immediately under the first flush. This is why it is important to uproot stumps at the right height. If uprooting is too deep, the next flush could be damaged.
19 Mechanisation in Agaricus cultivation

By Ruud Thielen, Thilot, The Netherlands

This chapter gives an overview of the mechanisation in the production and handling of compost and the cultivation process of button mushrooms. The chapter follows the subsequent stages in substrate preparation:

- Phase I: green compost
- Phase II/III: pasteurisation, conditioning and spawn run
- Transportation of compost and casing soil
- Phase IV: the actual growing of the button mushrooms.

Several methods may be applied for compost making, handling and mushroom growing. This survey is by no means complete. It is based on general opinions of the author and his ideas and describes the state of the art in mechanisation. The equipment described is based mainly on Dutch circumstances.

19.1 Phase I compost preparation

In The Netherlands, phase I compost needed for growing mushrooms is generally prepared by larger companies. The relatively small to medium size of the average Dutch mushroom farm renders phase I compost not profitable, also because of environmental regulations in The Netherlands.

Current modern methods of compost preparation, for instance the bunker composting or the pre-wetting concept, make it possible that emissions of ammonia and bad smells are reduced considerably in combination with a suitable building.

Dutch legislation is aimed at limiting environmental pollution and is not permitting the establishment of any new conventional compost production companies in a traditional open-air method. Compost preparation is performed completely indoors, minimising odour pollution; only straw storage is admitted in the open air at CNC for example.

The situation is totally different in other countries, where growers – who generally have a larger growing area – do have to make their own compost i.e. phase I, II and phase III compost.

19.1.1 Straw processing

Producing phase I compost is very much handling of straw, being one of the main materials for compost production. Straw-rich horse manure is often another important basic ingredient, since it is often cheap and relatively widely available. For compost preparation, it is eminent to pre-treat the straw. The quantities of straw necessary for composting purposes are quite large, therefore several machines have been developed for handling and processing the straw. In The Netherlands, mainly square bales are
being used nowadays, which are somewhat easier to transport, stack and eventually handle (see also paragraph 17.2.1. under b).
Strings, wound around the bales to keep them in shape, first have to be removed either manually or by automated equipment. After removing the strings, the bales of straw are loosened up and pre-treated.
In addition (manure) liquids, horse manure, chicken manure and gypsum may be added. After absorbing sufficient water and manure liquid, as well as proper mixing with the other ingredients, the compost mixture may be formed into large piles or filled into composting bunkers for further fermentation.
Basically these 2 concepts can be determined for a further phase 1 compost production:
1. Pre-wetting/turning concept
2. Bunker concept.

19.1.2 Pre-wetting/turning concept
With the so-called pre-wetting system, the bales of straw are stacked in a pyramid shaped pile with a width up to 6 m, covering a certain length preferably on an aerated concrete floor. Strings are removed first. In this set-up, instead of moving the straw (mixture) around on the yard from one place to another, it stays more or less on one spot. Here, a pre-wetting/turning machine is being used for breaking up, wetting and eventually mixing the straw and its ingredients for composting purposes.
During this process, chicken manure, gypsum, horse manure, manure liquids are added to the pyramid piles. For this either a front-end loader may be used or specially developed spreading equipment.
In combination with a flow of air coming from beneath through the aerated floor, a very natural way of composting is created. It requires a minimum use of equipment and keeps maximal flexibility. There are no limitations like the need for spare empty bunkers for a turn-over nor a fixed time schedule.
Turning the compost mixture piles every couple of days and subsequently turning compost as with traditional compost-turning machines, proves to be an excellent solution for production of phase 1 compost in regard to quality and economics.
For smaller scale compost production, the pre-wetting concept for phase 1 compost production may be executed with the traditional somewhat smaller compost-turning machine. Again the bales of straw are put in a traditionally smaller row and the ingredients are added subsequently. But in this case only one or two bales are put side by side, covering the length of the compost wharf.
For a good composting process the stacks have to be turned a number of times by the compost turner to make the compost as homogeneous as possible and aerate the compost pile.

19.1.3 Bunker concept
The bunker concept generally requires more equipment for handling straw and straw mixtures.
In an automated straw processing line, the bales are placed on a chain conveyor for feeding the bale string-removing device. After removing the strings, the bales are torn apart and pre-treated by means of a pick-up drum and spinner installation. Addition-
ally, ingredients like e.g. horse and chicken manure and gypsum may be added as well as the necessary liquids. Several spinners and mixing devices take care of a proper handling and mixing of the ingredients. An attached elevating conveyor belt dumps the compost mixture onto a static larger pile.

In some instances it may be necessary to run the mixture through the processing line again to obtain a better mix, and to moisture it by a sprinkler/watering system. Eventually, the straw is taken from the pile into composting bunkers either by the front-end loader or better with the help of a bunker-filling machine or conveyor system. Obviously a better fill may be achieved by the latter. A drawback of this set-up is the amount of equipment and buildings needed.

Several Dutch companies follow the described methods, each with their own variations.

19.2 Required equipment for Phase I

19.2.1 Pre-wetting/turning machine

Pre-wetting/turning machines have been developed from the traditional compost turners. Their main task is to make the compost as homogeneous as possible and aerate the compost pile. They are highly productive and versatile, as their operating width is 6 metres. A pick-up drum in combination with a spinner takes the compost mixture and deposits it onto an auger/conveyor system for discharge.

The interior of the pre-wetting/turning machine is usually equipped with water supply tubes for watering purposes. Spreader discs attached to the conveyor, spread out the mixture/compost between the two sidewalls of a towed make-up box. These machines have capacities reaching up to about 400 tonnes of compost per hour; therefore diesel/generator drives are common.

19.2.2 Compost-turning machine

Compost-turning machines have served the
industry for several decades. Based on a pick-up drum and spinner system, they take care of mixing and aerating compost. Capacities range between 30 to 400 tonnes per hour. Traditionally their operating width is limited to approx. 2.5 m wide piles. Generator driven machines are preferred considering the power requirements for pick-up drum, spinner and wheel drives as opposed to dragging a heavy electrical cable.

19.2.3 String-removing device
Square strawbales are supplied to the string-removing section by means of a chain conveyor. A fork construction attached on a carriage, is pushed into the bale. The saw mounted in the top section of the machine, cuts all the strings of the bale. Next, the fork will make a rotation to roll up the strings and eventually it will be pulled backwards to remove the strings from the cutting section. Loose pieces of string wound on the fork, then come away at the bottom of the machine and are deposited. To end the sequence the next bale in line will push out the ‘cut’ bale. A length and height measuring device accounts for the proper handling of each bale as they may differ from each other.

19.2.4 Straw breaker
The straw breaker is a machine specially designed for tearing up square bales of straw. It is usually used in combination with a bale string-remover. The machine consists of a chain conveyor feeding the milling section of the straw breaker with straw bales. This milling section is equipped with a main pick-up drum and spinners for loosening and breaking up the bales of straw.

19.2.5 Bunker filling machine
Bunker filling machines are specially constructed for filling composting bunkers with a compost mixture. These machines are basically provided with a hopper equipped with a chain conveyor and an oscillating gun conveyor, all mounted on a driven undercarriage.
The hopper of the machine is filled by means of a front-end loader. The gun conveyor distributes the compost inside the bunker covering the full width and length for a proper composting process.

19.2.6 Bulk tunnel phase II/III compost preparation

Producing phase II and III compost in bulk, a development which has been taking place since the mid seventies, is usually the following step after phase I compost production in compost preparation for mushroom growing. In Holland nowadays, practically no farms use phase I compost for filling their growing rooms anymore.

The purpose of bulk pasteurisation and spawn-running in tunnels, is mainly to shorten growing cycles. Also, the tunnel is specifically designed and equipped for a better controlled environment for compost treatment. Thus the cost of mushroom growing is reduced through a more intensive utilisation of buildings and machines in combination with major quality improvements.

The dimensions of the tunnels are generally either 3 or 4 metres wide with a length of approx. 12 to 40 metres. Usually bulk handling of compost occurs in a number of tunnels simultaneously i.e. larger quantities of compost are handled. This makes great demands on the building structures and machines, which have to be capable of processing the compost in a short time. Filling of the tunnels with phase I or II compost and subsequently taking the substrate out, pasteurisation and spawn-run, can be mechanised to a great extent.

19.2.7 Filling bulk tunnels

Conveyor systems are generally considered best for filling tunnels. Modern conveyor lines provide a uniform fill and homogeneous distribution of compost inside the tunnel, important for the air flow through the layer of compost. A proper distribution of compost is obtained using an oscillating gun-conveyor attached to the front of the conveyor system. Generally two types of tunnel filling machines can be determined. One type of machine is the so-called filling cassette. The
other model is based on a system consisting of several separate conveyors placed behind each other in combination with a filling head. Basically the lay-out of the tunnel building and to a lesser extent the amount of compost to be handled, are of importance for this choice.

Filling bulk tunnels usually starts with filling phase I compost into the compost hopper which provides a uniform flow of compost to the tunnel filling machine. A front-end loader is normally used for this.

The distance between the compost hopper and the oscillating gun-conveyor operating inside the tunnel, is covered by a system of one or more conveyor belts. These supply conveyors may either be fixed inside the tunnel building or movable.

The number and size of the belts obviously depends on the number and length of the tunnels. During the filling sequence of the tunnel, the filling machine is driven backwards. Subsequently the same filling machine is used for filling the spawn-running tunnels with compost.

19.2.8 Emptying bulk tunnels

After pasteurisation and conditioning the compost, the bulk tunnels can be emptied in various ways. The most basic solution is using a front-end loader. However this method is not preferable considering the risk of damage to the tunnel walls. Clogging of the grid floor because of driving in and out of the tunnel is also a problem as this obstructs the air flow.

The best way to empty tunnels, is to apply a glide and pulling net. In this set-up two air-permeable nets are placed on the grid floor before the tunnel is filled with compost. The glide is fixed inside the tunnel and is used to reduce the friction between the concrete grid floor and the pulling net.

After pasteurisation or spawn-run, the pulling net is then connected to the winding shaft of the tunnel winch which pulls the compost out. The winch, equipped with one or more spinners, regulates the amount of compost coming out of the tunnel for further discharge onto the attached conveyor system.

Tunnel winches are available in a wide spectrum of designs, based on the width of the tunnels, the method of compost discharge i.e. by a chain conveyor design, or a combination of an auger with elevating conveyor or by a troughed conveyor belt.

19.2.9 Spawning & supplementation

Production of phase III compost requires spawn to be added after pasteurisation and conditioning. This may either be done manually by adding it on top of the compost inside the tunnel before emptying or one may use a spawn-applicator attached to the tunnel winch or conveyor system. As the compost is transported by several conveyors or other means of equipment, spawn is eventually mixed through the compost properly.
In a similar way, supplementation material can be added during emptying the tunnel with spawn-runned compost. In some instances, however, it may be preferable to do supplementation during the filling of the growing rooms with compost.

19.3 Required equipment for Phase II/III

19.3.1 Compost hopper
A compost hopper is based on a large dump box equipped with a driven chain conveyor inside. The box is being filled by means of a front-end loader. The dumping width is dependant on the required capacity and also the width of the front-end loader bucket. Usually a levelling drum is mounted which provides a uniform flow of compost to attached conveyors or filling machine.

19.3.2 Overhead conveyor with discharger
Many tunnel facilities use an overhead troughed conveyor belt system for transport of large amounts of phase I, II and/or phase III compost. Such a conveyor is usually installed parallel to the tunnels at a certain height inside the tunnel building. Loading the conveyor takes place using a compost hopper with an elevating conveyor. For discharging compost from the overhead conveyor onto for instance a tunnel filling cassette, a discharger is being used. The discharger can be repositioned across the overhead conveyor in order to bring it to a desired position in front of the tunnels.

19.3.3 Tunnel filling cassette
The tunnel filling cassette is a specific type of filling machine for filling phase I or II compost into pasteurisation and/or spawn-running tunnels. The design of the machine gives a compact construction combined with a high filling capacity and easy operation. The machine consists of several horizontal conveyors, positioned above each other for transport of compost to the gun-conveyor. A gun-conveyor takes care of distributing the compost evenly inside the tunnel with an oscillating movement. Conveyors and gun-conveyor are placed on an undercarriage for replacement of the complete unit in cross direction in front of the tunnels.
19.3.4 Tunnel winch

The tunnel winch is applied for emptying composting tunnels filled with phase II or III compost in combination with a glide- and pulling net.

The machine is installed parallel to the tunnels and movable sideways to cover all of the tunnels. A heavy duty net reel is installed for pulling the pulling net with the compost on top of it, out of the tunnel. One or more spinners take care of breaking up the compost and provide a uniform flow of compost to the attached conveyor for further discharge.

19.3.5 Net cleaning machine

It is preferable to clean the tunnel pullings nets after usage with a net cleaning machine.

The frame, provided with an electrically driven net reel for the net, is equipped with spraybars for cleaning purposes.

19.4 Transportation of compost and casing soil

As described above, in The Netherlands phase I compost is made by several larger companies. Apart from phase I compost production, in some instances these companies also produce phase II and III compost. In any case, compost has to be transported to either the bulk tunnel composting facility or the mushroom growing site.

19.4.1 Transportation of phase I compost

Since phase I compost still has to be pasteurised and conditioned when it arrives at the tunnel facility (or mushroom farm), no specific transportation requirements, i.e. lorries are needed.

Both tipping trailers and self-unloading lorries are applied for this job. The latter can be equipped with a pulling net or chain conveyor on the lorry floor or a walking-floor system may be applied. The compost is dumped on the concrete floor of the tunnel facility, near to the tunnels to be filled.

In other instances where phase I production is at the same site as where the tunnels or farm is located, front-end loaders may be used for transport of the compost between the two sites.

19.4.2 Transportation of phase II or phase III compost

Operations which have their own phase II/III tunnels on the farm, can use various methods for transportation of phase II or phase III compost from the tunnels to the growing rooms to be filled.

One of them would be the use of fixed or movable conveyors, if the distance between
the tunnels and the growing rooms allows this and the lay-out of the buildings is suitable.
Another way would be using lorries or trailers equipped with a conveyor chain, pulling net or walking floor system in combination with a discharge spinner installation. Even a simple tipping trailer may be applied. However, a suitable compost hopper is then necessary at the growing room site for feeding the filling equipment. Capacities, execution and individual design of the machines and equipment, depend largely on the lay-out and size of the tunnel operation. In all circumstances, great care must be taken to ensure hygienic conditions with regard to the equipment used and the environment. In The Netherlands, phase III compost suppliers generally opt for trucks and lorries for compost delivery, being the most suitable and flexible system for supplying the growing rooms.
These trucks take phase II or phase III compost from the tunnels to the mushroom growers and usually operate in combination with a head filling machines for filling growing shelves.

19.4.3 Transportation of casing soil
Casing soil is transported by (dump) trucks, specially designed containers and trailers or big bags. In some instances trucks may travel from one grower to another if its capacity is sufficient, whereas a transportation container may stay on site at the grower’s farm.
Lorries and containers are usually equipped with an unloading system like a chain conveyor, pulling net or a walking-floor system often in combination with an output levelling device. In many circumstances the transportation equipment feeds straight into the growing room filling system at the grower’s mushroom plant.
Fewer growers nowadays dump the casing soil on the floor in order to load a separate casing soil hopper for filling purposes. Mainly because of hygiene, the available labour as well as the extra cleaning effort after applying casing soil, account for this.

19.5 Required equipment for transportation of compost and casing soil

19.5.1 Compost trailer
Compost trailers for phase II and III compost, come in various designs and sizes depending on the travelling distance and truck lay-out. For emptying the trailer, they are either fitted out with a chain-conveyor with carriers on the floor of the trailer or a net is being used which is to be wound on a net reel attached to the frame. A third method for emptying is the so-called walking floor system.
All compost trailers are usually fitted out with a device for dosage with spinners for a regular discharge of compost to an external conveyor.
19.5.2 Casing soil trailer
Casing soil trailers are built up similarly to compost trailers in such a way that this kind of trailer is also equipped with a chain conveyor or pulling net inside for emptying and a device for dosage either towed by a tractor or truck. In The Netherlands these trailers normally feed directly into an external conveyor feeding the head filling machine with casing soil.

19.6 Filling and emptying growing rooms
Three different substrate containers for the compost can be distinguished: shelves, blocks or bags. It depends on the local infrastructure and cost of labour which method is the best in a specific situation.

19.6.1 Shelf system
The design of head filling equipment and implementation of the growing net, were a major step for the development of mechanisation for the Dutch shelf system. In Holland the head filling machine in combination with large entrance doors and metal shelves of the growing rooms, form the backbone for the mechanised filling effort. The growing net on which the compost is being filled, makes it also possible to empty the beds after mushroom growing. Initial developments for filling equipment were focussed on phase I compost. Nowadays, filling phase III compost simultaneously in combination
with casing soil, is applied on the majority of the Dutch mushroom farms. Basically a head filling machine brings the compost to the front of the growing bed at a certain width, layer height and density. A pulling winch to which the growing net is attached, pulls the compost (and casing soil) into the respective bed. Compost and casing soil are usually brought in bulk, by trucks and lorries. Dependent on the compost being filled, i.e. phase I, II or III, different types of head filling machines are being used. This in order to cope with the differences in compost structure and requirements such as e.g. adding casing soil to phase III compost. After completion of the growing cycle, the beds are emptied using the growing net and pulling winch again in combination with a wide belt conveyor attached to it. The pulling winch is used for taking out the compost by means of winding the net on the net reel. Compost and casing soil coming out of the bed, fall onto the attached wide belt emptying conveyor. This conveyor dumps the spent compost into a container or truck for further discharge.

19.7 Required equipment for the shelf system

19.7.1 Head filling machine
A head filling machine is generally used in The Netherlands for filling mushroom growing shelves with phase III compost and casing soil simultaneously. This is done by a combination of a pulling winch and a growing net. The filling machine is equipped with a hydraulic scissor lift mechanism which makes it possible to reach up to a 7th bed. Compost and casing soil are fed to the machine using two external supply conveyors. A gun-conveyor for compost is fitted for distributing the compost across the bed width. A levelling chain sets the required filling height for compost in combination with press-rollers.
Casing soil, first collected in the mounted casing soil unit, is spread out evenly and then levelled to the required layer and subsequently deposited on the pressed layer of compost.
Tools like a cacking, ruffling and levelling apparatus may be installed on the head
filling machine for an improved fill of compost and casing soil. In many circumstances, an automated filling system controls the supply of compost and casing soil as well as the flow of it through the head filling machine. Generally a drive system is fixed to the frame, enabling the machine to be driven across the filling floor in front of the growing rooms. Distinctive models are available one of which is being used by contractors. These machines are transportable by a truck for quick road transportation across great distances. Furthermore they are highly versatile with regard to the variation in filling speeds and settings like bed widths, filling density etc.

19.7.2 Pulling winch

![Pulling winches.](image)

The pulling winch is used to pull a growing net with phase I, II or III compost on top of it, into the beds in conjunction with a head filling machine. A casing trolley for mechanical casing with attached casing net and casing soil can also be used by such a machine. The speed of the pulling winch is set in accordance with the filling machine and its operation is usually controlled by the head filling machine.

Pulling winches come in different designs among which e.g. the multiple-arm and single-arm winches.

Multiple-arm winches consist of up to seven reels for the cables and/or nets at the same distance as the beds in the growing shelf.

Single-arm pulling winches consist of one reel only. The winch is generally provided with a lifting mechanism for lifting it to the required bed level. In some instances it is also possible to use it as a lift for smaller tools.

19.7.3 Emptying conveyor

The growing net, which was pulled into each bed with fresh, spawned or spawn-runned compost, has to be removed from the shelf again at the end of the mushroom growing cycle. A pulling winch is used for taking out the compost by means of winding the net on the net reel. Compost and casing soil coming out of the bed, fall onto the attached wide belt emptying conveyor. This conveyor dumps the spent compost into a container or truck for further discharge. In The Netherlands several contractors use sophisticated
19. MECHANISATION IN AGARICUS CULTIVATION

One advantage of this system is, that sophisticated equipment is not needed for transportation of the compost and filling the growing rooms, thus eliminating substantial investments for (smaller) growers.

For this purpose, however, the tunnel operation needs block pressing equipment.

Such a machine presses the phase II or III compost into blocks generally measuring 600×400×180 mm.

After pressing, these blocks are shrink-wrapped for transport and stacked on pallets. All that is needed is a lorry taking the blocks to the mushroom farm. The blocks are either placed manually inside the growing beds or with the use of a simple conveyor system, pulling winch and growing nets.

Inside the bed, the blocks form a layer of compost, very well suited for further mechanisation like applying casing soil, watering etc., although not as perfectly as when applying a head filling machine.

Putting the blocks on a growing net also makes it possible to empty the beds mechanically using a winch and conveyor set-up.

Generally this way of filling growing rooms is very well suited for areas where the infrastructure of the tunnels and growing rooms as well as the means of transport and handling of compost are less sophisticated.

19.8.1 Required equipment for compost blocks: block press

The block press produces compost blocks with a size of 600×400×180 mm, shrink-wrapped in plastic.
Phase II or III compost is fed to the machine either by the tunnel winch or by a hopper or compost truck. A chain conveyor transports the compost \textit{i.e.} compost blocks through the machine. The levelling chain is set for the desired compost layer height. In combination with fixed compression rollers and the movable press/cutting trolley, which is provided with the main press cylinders and knives, blocks are being formed into shape and subsequently cut. A shrink-wrapping device attached to the press, is used for wrapping the compost blocks for transport reasons.

19.9 Plastic bags

Filling compost into plastic bags is a predecessor of the block system. Considered less advanced, plastic bags are obviously more difficult to handle and transport because of their specific shape. Furthermore, placing plastic bags inside the growing rooms is practically limited to one or two layers high only. Little mechanisation means can be applied, making it quite a labour-intensive operation. Filling a growing room, casing and emptying it, must still be done manually to a great extent. On the other hand it is a very cost-effective method with regard to the investment of equipment involved.

19.9.1 Required equipment: bag filling machine

Bag filling machines are commonly used for filling phase II compost into plastic bags. The filling weight is approximately between 20 and 25 kg. Two models are available where the simple machine is provided with two filling stations. Machines with bigger capacities may have five stations or even more. The compost is supplied to the bag filling machine by means of a compost hopper or tunnel winch and conveyor belt. An adjustable compost weighing device is applied for setting the amount of compost fed to each bag. A spawn feeder is mounted for adding spawn to the phase II compost.

19.10 Mechanisation during growing

19.10.1 Casing machine

In The Netherlands, fewer growers use phase I and II compost as a growing medium. In those instances, after spawn-running, a casing machine is needed for applying casing soil to the compost inside the growing shelves. Basically the design of a casing machine resembles that of a compost filling machine for shelves. Casing soil is collected in a hopper and levelled off by a levelling chain or drum. The hopper can be filled either by hand, tractor or by a casing soil supply truck. The soil is transferred
onto a net and subsequently discharged on the compost in the shelf by means of a casing trolley running over the bed. This trolley is pulled into the shelf by a winch. Often a levelling machine is mounted on the casing machine for improvement of the structure of the layer of casing soil.

19.10.2 Ruffling/levelling machine
The ruffling/levelling machine for the casing soil usually consists of one drum with teeth for ruffling and one for levelling the casing soil in the mushroom growing beds. Both drums are adjustable in height. Often a compression roller is installed as well, for a smooth finish of the layer of casing soil after ruffling/levelling. The machine is travelling on top of the sideboards of the shelf. The driving and rotation speed of the drums is variable.

19.10.3 Machine lift
A machine lift comes in for lifting equipment like a ruffling/levelling machine or mechanical mushroom harvesting machine to the required bed level on a shelf farm. Generally a machine lift consists of a platform on castors and cable or gear system for raising the lifting platform. In some instances the lift can be attached to the two outer uprights of the shelf. Other machines are equipped with two or four uprights attached to a frame and provided with a cable lift for more heavy duty lifting jobs. Furthermore there are lifts which can not only be used for bringing machines up to a required bed level but can also operate as a pulling winch.

19.10.4 Net cleaning machine
Nets used in the growing rooms as well as casing nets can be cleaned with purpose-built net cleaning machines. These consist of a frame with a winding mechanism for the net and a number of spray bars and brushes.
The net, while electrically wound on the net reel, passes by these spray bars with their waterjets spraying against the net, combined with a brushing action.
Automatic watering system by a watering tree.

19.10.5 Watering

Water is needed for mushroom growing. This water is stored in the layer of casing soil. Obviously water has to be applied equally across the layer of casing soil. Nowadays watering trees or lorries are mostly used for watering the growing beds, either in four, five, six or more beds simultaneously from the aisles. Watering can be done from both sides of the growing bed as well as from one side only. Basically the watering tree is constructed from a foldable galvanised frame with several spray lances and swivel castors. The more advanced spraying lorry is hung to a rail fixed to the growing shelves. Suited specifically for one-sided spraying operation, the spray lances of the lorry also have a fixed position relative to the growing beds during water supply.

Further automation has led to an automated watering system. A water supply system that operates without anyone present leads to considerable time savings for the farmers. An additional advantage of an automatic drive is a constant velocity, which guaran-
tees that the water distribution in the length direction of the bed is constant. Automatic driving watering trees for centre and side aisles make use of a rail system attached to the shelves and walls and batteries for traction. The advanced automatic lorry system is based on a rail-and-cable system attached to the top of the shelves. The spraying lorry is hung on this rail locked to the cable cart. Each growing room is equipped with a motor drive unit for moving the cart with the attached spraying lorry. Generally automated watering devices are equipped with a control system which provides several possibilities like:

- Hand controlled as well as full automatic start through a built-in time clock.
- Adjustable number of watering turns each time.
- Possibility to program the base water supply schedule for the entire growing cycle.
- Registration and read-out of data of watering cycles.
- Adjustable drive speeds and flow-through quantities for the water, etc.
20 Cultivation on pasteurised substrates

The cultivation practices in this chapter differ from those in the preceding chapter in that the extensive composting procedure is not required. This chapter discusses the following topics:

- substrate containers for pasteurised substrates,
- pasteurisation by steam versus immersion in hot water,
- steam boilers, tunnels, growing Oyster mushrooms on pasteurised wheat straw, case study: pasteurised corn cob substrate, immersion in hot water:
  - case studies: cultivation of the Phoenix mushroom in China, cultivation of Oyster mushrooms on coffee pulp in Mexico,
  - feasibility aspects of Pleurotus cultivation on coffee pulp in Mexico,
  - cultivation of Stropharia rugoso-annulata,
- different Volvariella volvacea cultivation methods:
  - indoor cultivation,
  - traditional outdoor cultivation,
  - pests and diseases,
  - intercropping corn and Volvariella: a case study from mainland China.

20.1 Substrate containers for pasteurised substrates

Quite a few different types of containers can be used for pasteurised substrates. There is no need to use plastics which can stand temperatures of 100 °C and higher, like PP, as the maximum temperature involved is about 70 °C. Details of the cultivation technique are affected by the type of containers used and vice versa. If a concept is followed where substrate is moved from one room to the other, then easy transportability of the substrate containers is a very important requirement. Substrate containers have to meet the following requirements:

- they should provide sufficient airflow to avoid anaerobic conditions in the substrate,
- they have to be clean,
- their size should be such that spontaneous fermentation will not raise temperatures above the range of the desired mushroom (usually 35 °C). Compact bags should therefore not contain more than 20 kg of substrates; the layer of substrate in beds should not be thicker than 20 cm,
- they should be economical in use.

Plastic bags are much used for pasteurised substrates because they are cheap, available in many formats, and hygienic if used only once. The most commonly used material is polyethylene. Some manufacturers offer micro perforated bags; when using these
there is little risk of insufficient gas exchange between substrate and environment. If airtight bags are used, perforations will have to be made to avoid anaerobic conditions. A typical yeast-like odour emanates from compost or straw with clogged airflow (either because it was too wet or because the bags precluded sufficient air exchange). **Plastic trays** are less used because of the high initial investment; a further disadvantage is that they have to be cleaned thoroughly after removal of the spent substrate. A plastic foil in each tray can help in cleaning them. Completely closed trays may slow down the growth rate because of insufficient aeration; heat will also dissipate less easy. Trays with grates are safer in those respects. The advantage of trays is that they can easily be stacked.

**Wooden trays** will be colonised by the mycelium of Oyster mushrooms. The use of wooden trays is therefore limited to *Agaricus* cultivation. Usually a plastic sheet is laid in the trays to prevent any unwanted organism in the wood to contaminate the substrate. A disadvantage of wooden trays is that they are difficult to pasteurise. Viruses may survive the pasteurisation process. An advantage of trays is that they can easily be stacked.

**Baskets** of straw, bamboo or other locally available materials can be used only once, as the mycelium will degrade the basket material. Their use has been reported on the countryside in Africa, where plastic bags are difficult to obtain. Take care that the substrate doesn’t dry out, as these kinds of substrate containers usually have many openings. The relative humidity of both spawn run and cropping rooms should carefully be checked when using baskets.

**Blocks of substrate** are produced by a number of substrate manufacturers by pressing the substrate in a machine, wrapping a plastic sheet around it and heat sealing the sides of the blocks. The blocks shouldn’t be completely covered by the plastic to allow for gas exchange. Compost-like substrates are easier to pack in plastic than straw substrates because compost is less elastic. Straw should be pressed in a form and the plastic should be wrapped around it while it’s still in the form, because the straw is very elastic compared to compost. Plastic-wrapped substrate blocks are generally cheaper than substrate-filled bags, because the former process is easier to mechanise than filling bags. Also, the blocks can be filled more efficiently on shelves in growing houses because of their rectangular size. The major disadvantage is the high cost of the block-making machines.

**Beds** can contain large quantities of substrate at a time. A disadvantage is that infections are more difficult to isolate, as compared with all the other containers mentioned above. Their main advantage is that little work is needed in moving the substrates
around the mushroom farm. Special equipment (winch with dragnet) eases the filling procedure of beds, similar to filling the beds in *Agaricus* cultivation.

### 20.2 Pasteurisation by steam versus immersion in hot water

In the early years of *Pleurotus* cultivation the substrate was sterilised. Later it was found that a heat treatment at a lower temperature was also sufficient. Steam-pasteurisation and immersion in hot water share the low temperature in comparison with semi-sterilisation and sterilisation. Steam or water is used to keep the substrate at an adequate temperature for some time (usually about 60-70 °C). At this temperature most competitors of the cultivated mushroom are killed, but favourable micro-organisms will survive.

Immersion in hot water differs somewhat from pasteurisation by steam: besides killing most competitor fungi, the hot water will remove the easily soluble components from the substrate. Since these would also be the first to be attacked by contaminants, their removal renders a more selective substrate. It is recommended, however, to avoid an excessive number of pasteurisations (normally not more than 2-3) using the same water as it may lead to contamination problems. Pasteurisation by steam requires a tunnel of some sort. For immersion in hot water, only containers to hold the water and means to keep it hot are necessary.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>1. pasteurisation of pre-wetted straw by steam</td>
<td>large amounts of substrate can be produced, depending on the size of the tunnel; efficient method</td>
<td>relatively high investment, for both steam boiler and tunnel; keeping the right temperature for a specified period is more important than in method 2</td>
</tr>
<tr>
<td>2. immersion in hot water</td>
<td>simple method; very low investment level; solar energy can easily be used to heat the water</td>
<td>more difficult to treat large quantities; more labour force required; high hygienic standards; draining, cooling down of substrate and water can be difficult and time-consuming</td>
</tr>
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A third technique pasteurises dry substrate with steam in a large, tumbling container and subsequently cools it with cold water. The hot straw will easily absorb the water. This technique, however, demands rather high investments and the substrate is more easily contaminated compared to the other two techniques. It is therefore not discussed in detail in this manual.

### 20.3 Pasteurisation by steam

The necessary equipment depends on the choice whether to pasteurise in beds or in a separate tunnel. The advantage of a tunnel is that the process can be better controlled.
Pasteurisation in beds means less labour as the substrate doesn’t have to be transported from the tunnel to the beds. In both cases a steam boiler is necessary. If the capacity of an improvised steam boiler is not sufficient to pasteurise the complete growing room at once, then it may be possible to pasteurise only a number of shelves. The shelves have to be adapted then, to allow a plastic sheet to cover the construction.

20.3.1 Steam boilers
The capacity of the steam boiler should be high enough to allow for a rapid increase in substrate temperature. As a rule of thumb, each tonne of wet substrate needs about 1.1 kW capacity for pasteurisation; for sterilisation, additional capacity is necessary. If the steam boiler is also used to heat rooms or control humidity in the growing rooms, its capacity has to be increased even more.

20.3.2 Tunnels
Tunnels have initially been introduced in Agaricus cultivation because they allow for a more uniform heat treatment as compared to pasteurisation in beds. A detailed description of high tech tunnels can be found in chapter 13 of this book.
A simple tunnel can easily be built in developing countries. The aim of the tunnel is to heat pre-wetted substrate to a specified temperature. The size obviously depends on the volume of substrate that needs to be pasteurised per batch. A volume of 1 m³ can contain about 400-450 kilo of wet straw. The maximum height of substrate in a tunnel is about two metres. The total weight on the grate will then be less than 1000 kg per m². The construction should be strong enough to withstand the pressure. The fans should be able to blow air through the substrate.
The substrate should rest on a relatively open, mesh-like structure to allow for air and steam circulation. There should be provisions to blow in the steam from below this mesh. This mesh may consist of concrete, with as many holes as necessary to reach a 30% open structure. Alternatively, it may consist of a non-corroding metal (galvanised iron), aluminium or painted iron. A high capacity fan is needed for blowing air through thick piles of substrate. The tunnel cannot be filled to the rim with substrate. The top 50 cm of the tunnel should be left empty to allow for air circulation. A standard cargo container (40 or 20 foot long) can be adapted to serve as a tunnel.

20.4 Oyster mushrooms on pasteurised substrates
How Oyster mushrooms can be grown on two different substrates, pasteurised by steam, is discussed in the following paragraphs. Apart from the mentioned substrates (wheat straw and corncob), many other lignocellulose-rich materials can be used, such as Elephant grass, dried Water hyacinth, bean straw, etc. Some experiments will be necessary to find the suitable procedure for each substrate material.

20.4.1 Cultivation of Oyster mushrooms on a straw substrate
Substrate formulation: Wheat straw and water (sometimes a supplement of 5 to 10% (weight percentage of the dry straw) alfalfa hay and/or lucerne meal is added).
Substrate preparation: Wheat straw is shredded into pieces of 3 to 6 cm. It has to be
moistened until it has a moisture content of 75%. This may take up to one or two days, because the straw has an outer wax layer that makes it difficult to moisten. Barley straw can also be used, but care has to be taken because it easily absorbs too much water. Fill the substrate in the tunnel and pasteurise for six to ten hours at 60 °C. Some growers add a so-called conditioning period of one to two days at 48 °C. The micro-organisms which develop during this stage do not hinder the growth of Pleurotus but several of them are antagonistic towards green moulds and therefore offer more protection to contamination. Aeration during pasteurisation and conditioning is necessary.

**Spawning:** Take the substrate out of the tunnel when it has cooled down to room temperature. Spawn with 30 to 35 litres spawn per tonne wet substrate. Take care to distribute the spawn evenly. Fill substrate containers with 10 to 25 kg of the substrate.

**Spawn run:** The mycelium will colonise the straw substrate in two to three weeks at a temperature of 23 to 28 °C inside the bags; the air temperature may be considerably lower. Especially when using large bags, the temperature difference between the centre of the bags and the air can be as high as 10 °C. This means that the substrate would start fermenting if more than 25 kg is packed per bag. Especially if supplements have been added, the activity of the substrate can be high. It is safer to lower the ambient room temperature or to pack limited amounts of substrate in each bag.

**Fruit-body formation:** As soon as the mycelium has grown all over the surface of the substrate the fruit body formation can start. Most growers will keep the plastic around the substrate, to protect it from becoming too dry. They will cut holes in the bags from which the mushrooms can grow. Others use plastic that has been perforated with holes of 1 cm diameter, totalling 4% open surface. All Oyster mushrooms need light for the proper development of the cap. Some differences between species and strains with regard to the colour of the light and the required amount exist. The mushrooms themselves will show whether they are getting sufficient fresh air and light. If the caps remain very small in comparison to the stipe, this can be due to either lack of light or too high a CO₂ concentration. Usually there is enough light if it is possible to read a newspaper in the growing room.

The sensitivity for CO₂ also differs among strains and species: Pleurotus ostreatus is more sensitive than Pleurotus pulmonarius. Humidity is very important in fruit body formation; in the beginning the humidity should be 95%. Later, when the mushrooms are about 1 cm long, it has to be brought back to 85%. The nutrients are transported to the mushroom by a flow of water. If the water cannot evaporate, no nutrients can be transported to the mushroom. When the caps have almost completely spread out, the mushrooms

Oyster mushrooms on a pasteurised wheat straw substrate.
should be picked. Usually it takes five to seven days from tiny pinhead to the harvested Oyster mushroom.

20.4.2 Case study: pasteurised corncob substrate
In Hungary a method has been developed that gives high yields of *Pleurotus* on corncocks. Several hybrid strains of *Pleurotus*, like the famous HK 35, perform very well on this substrate.

**Substrate material:**
- Corncocks, shredded into pieces of 1 to 2 cm long,
- 0.5% feather meal,
- 1% CaCO₃, to stabilise the pH of the substrate.

Moisten the heap of corncocks for one to two days. Then add the other ingredients, mixing very well. Moisture content should be as high as 70%. Pasteurise the substrate for six to eight hours at 60 °C, then lower the steam input to reduce the substrate temperature to 48 °C. Condition the substrate for another 36 hours. After cooling, the substrate can be spawned with 3% spawn and is filled in light tight brown plastic bags. Usually 12 kg is filled per bag. The plastic is perforated with 1 cm holes every 100 cm². The mushrooms will later grow out of these holes, because primordia will form mainly here, at the only spots accessible by ambient light. Few primordia will develop under the plastic. During the spawn run the holes provide aeration. The first harvest of *Pleurotus pulmonarius* is possible after 17 days. Yields of up to 30% can be achieved within three months after spawning.

20.5 Immersion in hot water
This method is easier to perform, as no tunnel is necessary. The aim of the heat treatment is to kill competing micro-organisms and to easily remove soluble nutrients. Case studies from China and Mexico (developed by Dr. Martínez-Carrera) will be discussed. Materials and equipment required:
- substrate material (see formulas),
- substrate containers (e.g. plastic bags or trays),
- containers for hot water and means to keep the water hot (fuel, solar energy, steam, etc.),
- wire mesh to let the substrate drain.

The size of the water containers depends on the scale of the operation. A 240 litre container can hold about 90 kg of wet straw substrate. The same container can be used a number of times a day, because the actual immersion time is only about 30 minutes to one hour.

20.5.1 Case study: cultivation of the Phoenix mushroom in China
A white variety of *Pleurotus sajor-caju* is grown in China under the name of Phoenix mushroom. It has some favourable characteristics: fast growing mycelium, short harvesting period and its taste is considered better than that of other Oyster mushrooms.
Substrate formulas:
1. 99% cotton seed hulls, 1% CaCO₃,
2. 98% rice straw, 2% CaCO₃,
3. 74% cotton seed hulls, 24% rice straw, 2% CaCO₃,
4. 74% cotton seed hulls, 24% spent tea leaves, 2% CaCO₃.
The main substrate ingredients have to be kept in hot water (above 75 °C) for at least an hour. Afterwards, they should be drained thoroughly. Add the small quantities of buffer CaCO₃, after the immersion in water, otherwise they will be lost. The substrate should be spawned as soon as it has cooled below 30 °C. Relatively large amounts of spawn are used: 7 to 10%. If smaller percentages give similar results, then there is no need to stick to these figures. Different types of bags can be used to hold the substrate. Never fill more than 20 kg per bag; spontaneous fermentation would raise the temperature inside the bags to more than 30 °C, the upper limit for mycelial growth of most Pleurotus species.

One type of bag, used in China, consists of 20 cm diameter cylindrical plastic, filled up to 50 cm height, with a perforated pipe in the middle. The ends of the bag are tied to the pipe, and aeration proceeds through the pipe. The aeration pipe will also allow heat to dissipate, even if it is formed in the core of the substrate.

Spawn run: It will take the mycelium 20 days at 25 °C to colonise the substrate. The plastic and aeration channel can be removed entirely if a very humid environment can be created, for example in a shed. Alternatively, the plastic can remain on the substrate, in which case cuts have to be made in the plastic for the mushrooms to grow out.

Fruiting: Keep the humidity high (80-90%) by spraying water several times per day. When the small mushrooms emerge, their form will reveal whether they receive sufficient light and aeration. If the stipes are long and the caps small, the aeration and light requirements were not met. After five to seven days the mushrooms can be picked. It takes another five to nine days for the second flush. In total, three or four flushes can be harvested.

20.6 Cultivation of Oyster mushrooms on coffee pulp in Mexico

(Information and pictures kindly provided by D. Martinez-Carrera, Mexico).

Prof. Dr. Martinez-Carrera and his staff have performed many experiments with Oyster mushrooms. They found that fresh, dried, and ensiled coffee pulp can be used as a substrate for Oyster mushrooms. The yield on dried and ensiled coffee pulp is slightly lower. A number of commercially operating farms nowadays employ the techniques which have been developed at the research station.

20.6.1 Substrate preparation

Fresh coffee pulp: The fresh material is usually very wet when it has just been processed. When produced by wet processing the fresh coffee pulp is immediately subjected to microbial degradation, as yeast, fungal, and bacterial populations occur naturally. Natural fermentation develops rapidly following different pathways (e.g., acetic, lactic, anaerobic, aerobic), depending on physical, chemical, biological, and environmental factors. For these reasons, coffee pulp should be managed appropriately and
pretreated in order to be used as substrate for mushroom growing, not only for Oyster mushrooms (*Pleurotus*) but also for Shiitake (*Lentinula*), Wood ears (*Auricularia*), and reishi (*Ganoderma*).

In the case of oyster mushrooms, the coffee pulp has to drain for four to eight hours and then it has to be fermented. Make long pyramidal heaps of 1 to 1.2 m high. If needed, cover these with plastic to protect from the rain, or to prevent water and energy loss. The water content in the heaps should be 60 to 80%. In two days, the temperature inside the piles will have risen to 50-60 °C. Certain easily accessible components of the coffee pulp will be decomposed during this stage. After the second or third day, the heap should be turned to avoid anaerobic conditions. Anaerobic micro-organisms can produce toxic metabolites during their growth. Coffee pulp can also be mixed or supplemented with other agricultural by-products to favour aerobic fermentation, such as straw (barley, wheat), maize stubble, and sugar cane bagasse. After ten days of fermentation the substrate becomes less suitable for *Pleurotus* cultivation. It will turn dark and is easily compacted. The fermented coffee pulp is ready for immersion in hot water when:

- the pH is between 6.0 and 7.0,
- the colour of the compost, having better structure and consistency, has turned to brown (the fresh coffee pulp is cherry red).

**Dry coffee pulp:** Fresh coffee pulp is only available during the harvesting season. To spread mushroom production some fresh pulp has to be dried for later use. The pulp should be dried thoroughly for four to six days, for instance by spreading it in a layer on a concrete floor, on a wire mesh, or in a commercial drier. No moulds should be present and the pulp has to be air- and sun-dried. Then it can be stored for later use. As the chemical composition of the pulp will change slightly on drying (in a similar way as during fermentation) the substrate can later be used directly. It only needs to be rehydrated in water for two to three hours. Dry coffee pulp also offers transportation advantages, as it is less bulky and has a higher water retention capacity.

**Ensilaged coffee pulp:** Fresh coffee pulp can also be ensiled. Large amounts of pulp (ca. 5-10 tonnes) are compacted within a cubic concrete chamber, and wrapped with plastic sheets in order to promote a natural lactic fermentation under anaerobic conditions. If the pH decreases rapidly up to 3.6 and stable lactic conditions are maintained, the coffee pulp can be easily preserved for a year and used for mushroom cultivation even after 21 days after having been taken out of the silo. Before ensiled coffee pulp is used as substrate, it should be treated with lime in order to raise the pH up to 5.0-7.0 to favour mycelial growth.

Dried, fermented, or ensiled coffee pulp (either as a sole substrate, mixed or supplemented with other organic materials) should be pasteurised in the same way.

**Immersion in hot water:** The substrate is put in wire mesh cylinders in hot water. The water has to be kept at 70 °C for at least 15 minutes, but 30-60 minutes is safer. Some variation in time and temperature does not affect the growth of *Pleurotus* very much, but higher temperatures and a longer time would consume more energy. Immersion in water at lower temperatures and for shorter periods than 15 minutes would be insufficient to kill all contaminants. The same batch of water should not be used for more than two or three batches of substrate. Drain the heat treated substrate and let it cool in a
clean plastic sheet on a table or on the floor inside the farm.

**Sterilisation:** Fresh, dry, or ensiled coffee pulp, having a water content of 70-80%, can also be mixed with other organic materials and sterilised at 121 °C for 1-2 h using polypropylene plastic bags. After sterilisation, the substrate is used for mushroom cultivation.

### 20.6.2 Spawning and tending the mushrooms

**Spawning:** Appropriate mushroom strains should be selected according to local environmental conditions, considering that coffee plantations occur at a variety of altitudes (300-1,400 m). Strains of *Pleurotus ostreatus* and *P. sajor-caju* (but possibly this case concerned a mislabeled *P. pulmonarius*) have been used successfully. When the substrate temperature has dropped to about 29 °C, it is spawned, either by hand or mechanically, with 250 g of spawn per 10 kg of wet substrate, approximately 25 kg per tonne. Then the substrate is put in perforated plastic bags. The mycelium can breathe through the holes. The usual size of the polyethylene bags is 50 x 70 cm; 9 to 11 kg of substrate is filled per bag. Sterile coffee pulp used for the cultivation of Shiitake, Wood ears, and reishi should be spawned under aseptic conditions.

**Spawn run and fruiting:** The mycelium will colonise the substrate in two or three weeks, depending on the strain and temperature. A temperature of 25 to 30 °C inside the bags is recommended. The mycelium will start to form small fruit bodies in two to three weeks after spawning. Then the plastic is removed and the humidity is kept very high: 90 to 95%. Only if the relative humidity is rather low, some plastic is left on the bags to prevent the substrate from drying out. When the pinheads have grown to a size of 1 cm, the humidity is lowered somewhat to 85%. Average biological efficiency, defined as the yield of fresh fruit bodies as a percentage of the dry weight of substrate at spawning (Tschierpe & Hartmann, 1977), and recorded in edible mushrooms
using different types of coffee pulp, either as a sole substrate or mixed with other organic materials, is shown below. Mushroom yields vary according to biological factors, environmental conditions, as well as pests and diseases present during cultivation. The yield in commercial production is almost 20% of the wet substrate in fresh Oyster mushrooms. In subtropical regions, fresh mushrooms should be cooled down or processed further in order to avoid rapid deterioration before marketing.

Edible mushrooms which can be cultivated on coffee pulp (modified from Martinez-Carrera et al., 2000).

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>Dry weight (g)</th>
<th>Average yield (g)</th>
<th>Biological efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Auricularia fuscocinerea</em></td>
<td>Inga sawdust + coffee pulp³</td>
<td>159.3</td>
<td>59.2</td>
<td>37.1</td>
</tr>
<tr>
<td><em>Lentinula edodes</em></td>
<td>Corn cobs + coffee pulp + Leucaena³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pleurotus sp.</em> cfr. <em>Florida</em></td>
<td><em>Quercus</em> sawdust + wheat bran + coffee pulp⁴</td>
<td>232.7</td>
<td>50.9</td>
<td>21.8</td>
</tr>
<tr>
<td><em>P. ostreatus</em></td>
<td>Coffee pulp⁵</td>
<td>999</td>
<td>1,756.5</td>
<td>175.8</td>
</tr>
<tr>
<td></td>
<td>Coffee pulp + coconut fibre⁶</td>
<td>500</td>
<td>447</td>
<td>89.4</td>
</tr>
<tr>
<td></td>
<td>Dry coffee pulp (1-24 months)⁵</td>
<td>999</td>
<td>1,598</td>
<td>159.9</td>
</tr>
<tr>
<td></td>
<td>Fermented coffee pulp (5-10 days)⁴</td>
<td>976.5</td>
<td>1,227</td>
<td>125.6</td>
</tr>
<tr>
<td></td>
<td>Ensiled coffee pulp (6-12 months)⁵</td>
<td>884</td>
<td>654</td>
<td>73.9</td>
</tr>
<tr>
<td></td>
<td>Coffee pulp + sugarcane bagasse⁶</td>
<td>1,350</td>
<td>1,309</td>
<td>96.9</td>
</tr>
<tr>
<td></td>
<td>Coffee pulp + barley straw⁶</td>
<td>1,611</td>
<td>1,607</td>
<td>99.7</td>
</tr>
<tr>
<td><em>P. sajor-caju</em></td>
<td>Coffee pulp³</td>
<td>999</td>
<td>1,280</td>
<td>128.1</td>
</tr>
<tr>
<td><em>P. opuntiae</em></td>
<td>Coffee pulp³</td>
<td>999</td>
<td>1,437</td>
<td>143.8</td>
</tr>
<tr>
<td><em>P. salmoneostramineus</em></td>
<td>Coffee pulp³</td>
<td>999</td>
<td>1,549</td>
<td>155</td>
</tr>
</tbody>
</table>

¹ Yield of fruit bodies (fresh weight) as a percentage of the dry weight of substrate at spawning.
² Sterile substrate (121 °C for 1-2 h); proportion 1:1 on a dry weight basis.
³ Sterile substrate (121 °C for 1-2 h); proportion 94:3:3 on a dry weight basis.
⁴ Sterile substrate (121 °C for 1-2 h); proportion 1:1:1 on a dry weight basis.
⁵ Pasteurised.
⁶ Pasteurised; proportion 1:1 on a dry weight basis.
⁷ Pasteurised; proportion 2:1 on a dry weight basis.

### 20.7 Feasibility aspects of *Pleurotus* cultivation on coffee pulp in Mexico

The site for the Oyster mushroom farm has to be very near to a ‘beneficio’, a place for wet coffee pulp processing. For a farm with a daily output of 50 kg fresh mushrooms, the building has to have a surface area of at least 100 m² and it has to be divided into sections and growing rooms. A simple spawn multiplying ‘laboratory’ should be available in the vicinity or within the farm. An outdoor composting area of 120 m² with a concrete floor is necessary, too. It can also be used for drying the fresh coffee pulp.
20.7.1 Economics of a rural mushroom farm using coffee pulp in Mexico

Main production and operation costs from commercial rural production of edible mushrooms are salaries (48.3%), raw materials and energy (33.6%), travelling expenses (5.5%), maintenance (6.8%), and regional marketing (3.3%). A cost-benefit analysis of a mushroom farm operating commercially indicates that this biotechnological process is profitable, even under rural conditions (c/b ratio = 1.10). In comparison with other crops and agro-industries, mushroom cultivation is also an efficient process for using and converting energy or water into a human food. Overall data show that 28 litres of water are required for producing 1 kg of fresh Oyster mushrooms using rustic technologies, in a considerably shorter period of time (25-30 days after spawning). This is a smaller amount in comparison with estimations for other foods or forages, such as potatoes (500 L/kg), wheat and alfalfa (900 L/kg), sorghum (1,110 L/kg), corn (1,400 L/kg), rice (1,912 L/kg), soybeans (2,000 L/kg), broiler chicken (3,500 L/kg), and beef (100,000 L/kg). The production of 1 kg of beef requires 3,571 times more water than the amount needed to produce 1 kg of Oyster mushrooms. Several environmental, economic, and social indicators have also been identified to assess sustainability of rural mushroom cultivation.

Costs (US$) of production and operation in a rural commercial farm from Cuetzalan, Puebla, Mexico, which was operated by five workers and had an average mushroom production of 5,771 kg per year (Martinez-Carrera et al., 1998).

<table>
<thead>
<tr>
<th>Years</th>
<th>Salaries and energy</th>
<th>Raw materials expenses</th>
<th>Administration expenses</th>
<th>Travelling expenses</th>
<th>Maintenance expenses</th>
<th>Marketing expenses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992-1997</td>
<td>17,845.09</td>
<td>12,418.45</td>
<td>928.78</td>
<td>2,042.04</td>
<td>2,505.22</td>
<td>1,197.01</td>
</tr>
<tr>
<td>(%)</td>
<td>(48.3)</td>
<td>(33.6)</td>
<td>(2.5)</td>
<td>(5.5)</td>
<td>(6.8)</td>
<td>(3.3)</td>
</tr>
</tbody>
</table>

Financial analysis of the commercial mushroom production (5,771 kg per year) in a rural farm from Cuetzalan, Puebla, Mexico (Martinez-Carrera et al., 1998).

<table>
<thead>
<tr>
<th>Years</th>
<th>Production costs ($)</th>
<th>Gross incomes ($)</th>
<th>Fresh oyster mushrooms</th>
<th>Profits ($) Spawn</th>
<th>Total</th>
<th>Cost-benefit ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992-1997</td>
<td>36,936.59</td>
<td>40,576.57</td>
<td>2,536.60</td>
<td>1,030.36</td>
<td>3,639.96</td>
<td>1.10'</td>
</tr>
<tr>
<td>Average data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Estimated amount of water required for producing 1 kg of fresh Oyster mushrooms using rustic Technologies, in comparison with that for other food and forage crops (Martínez-Carrera et al., 1998).

<table>
<thead>
<tr>
<th>Product</th>
<th>Litres of water/kg</th>
<th>Protein content*</th>
<th>Litres of water per gram of protein(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster mushrooms (Pleurotus)</td>
<td>28</td>
<td>2.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Potatoes</td>
<td>500a</td>
<td>2.1</td>
<td>23.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>900b</td>
<td>14.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>900d</td>
<td>6.0</td>
<td>15</td>
</tr>
<tr>
<td>Sorghum</td>
<td>1,110b</td>
<td>11.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Corn</td>
<td>1,400b</td>
<td>3.5</td>
<td>40.0</td>
</tr>
<tr>
<td>Rice</td>
<td>1,912b</td>
<td>6.7</td>
<td>28.5</td>
</tr>
<tr>
<td>Soybeans</td>
<td>2,000b</td>
<td>34.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Broiler chicken</td>
<td>3,500b</td>
<td>23.8</td>
<td>14.7</td>
</tr>
<tr>
<td>Beef</td>
<td>100,000b</td>
<td>19.4</td>
<td>515.4</td>
</tr>
</tbody>
</table>

* Composition in 100 g, edible portion (fresh weight) [Watt & Merrill, 1975; Duke & Atchley, 1986; Chang & Miles, 1989].

b Data according to Pimentel et al. (1997).

The market potential of this technology is significant in Mexico:
- The technology is simple and has been tested on several commercial farms.
- The coffee pulp is available for free and in large quantities in coffee producing areas.
- Climatic conditions in the coffee producing areas are suitable for the production of Pleurotus.
- There is a growing market for the product at a national level.
- Cheap labour is always available in coffee producing regions.
- Financial support is available from banks and government programmes, because mushroom farms help to avoid the serious ecological problems caused by the coffee pulp.

However, further utilisation of coffee pulp is limited due to:
1. Seasonal availability during the year,
2. Active natural fermentation,
3. Impractical and uneconomic transportation over long distances,
4. Large-scale handling for substrate preparation is more demanding and laborious, in comparison with other agricultural by-products (e.g., straw and corn stubble).

This technology can be applied in any coffee producing country in the world with a
demand for Oyster mushrooms, although other lignocellulose-rich agricultural wastes can be easier to use.

20.8 Cultivation of Stropharia rugoso-annulata

A bulbous reddish brown cap with a thick, white stem: *Stropharia* resembles the famous *Boletus edulis* in its appearance, but it lacks the tubes of the *Boletus*, being a gilled mushroom. The species has been reported from the USA, the former Soviet Union, India and Europe. It typically grows on composted cow dung, woodchips, soils enriched with sawdust and where straw was covered by soil. Prof. Lelley has a nice story how farmers in Berlin ‘discovered’ this mushroom: it grew from the straw which was used to cover potatoes in the soil. These kinds of places are rare in a truly natural environment; under influence of mankind this species is becoming more and more common. Especially the increasing use of woodchips has contributed to its current abundance.

Although easy to grow, the species has not found much acceptance outside Central Europe. It is mainly cultivated by amateurs and farmers as a secondary source of income in Germany, Hungary, China, Poland and the Czech Republic. It can stand relatively high temperatures (some strains up to 32 °C) but the best temperature range for fruiting is 16–25 °C. Outside, it can thus be cultivated in subtropical areas in the colder period and in temperate regions in spring and fall. Cultivation in climate-controlled rooms seems uneconomic, as spawn run is relatively long and yields are somewhat unpredictable. This is the main reason why this mushroom is not commonly available at the market.

With its nice appearance and pleasant taste and texture, there is in my opinion certainly room to market this mushroom as a seasonal speciality: the ‘asparagus’ of a certain region for example.

20.8.1 Preparation of suitable cultivation sites outdoors

Ideally, the air above the mushroom bed is moist year round, shady in summer, and receives extra sunlight in the beginning of spring and late fall. This is the case when intercropping between asparagus. Alternatively, the northern edge of a forest or between trees of a forest are also suitable places. Another way to obtain shade is to plant pumpkins in the middle of the beds; the big leaves keep the environment moist and the mushrooms can still easily be detected. Another suitable place is an orchard; however, if the mushrooms and fruits from the trees have to be harvested at the same time, labour availability may become a problem. An advantage of growing both mushrooms and fruit in an orchard is that the same sprinkler installation can be used for both crops. Strawberry beds can be mulched with inoculated woodchips to suppress weeds. However, the mushrooms are sometimes difficult to find between the strawberry plants. Another alternative is to build simple sheds above the beds; with these, the fruiting season can be extended.

There is some rumour that cultivation on clay gives better results than on sandy soils; in my opinion this is due to the fact that the substrate may dry out quicker on sandy soils. A water permeable foil can be laid out on the ground, preferably 50 cm wider
than the width of the beds. If a non-permeable foil is used, care should be taken that the water doesn’t collect below the beds; this gives rise to foul smell because of anaerobic conditions and the mycelium needs oxygen to grow.

20.8.2 Substrate formulations
Best results have been obtained from woodchips. In general, mainly woodchips from broadleaved trees have to be used; when small amounts of fir or hemlock are included, the Stropharia can overgrow these. Personally I had the best results with fresh poplar chips. A major advantage of fresh chips is that no further substrate treatment is necessary: the chips are sufficiently wet to support the growth of the mycelium.

Tip: When using fresh woodchips, you have to make sure that these contain few leaves. Otherwise a fermentation process starts in the beds, which will increase temperature and the chips may become too dry.

Wheat straw can also be used, but has to be moistened thoroughly, e.g. by immersing the straw bales in water. For outside cultivation, I recommend to use cold water for the immersion. When cultivated indoors, a pasteurisation of some kind can be used. One report states that grass chaff (Lolium spp.) and grass seed would increase the yield by 100%; Lucerne meal or wheat bran did not improve the yield significantly. However, supplementing makes the substrate more susceptible to contamination with weed moulds. These will inevitably attract flies and mites and can spoil the crop.

20.8.3 Preparation of the bed (including spawning and casing)
Most growers prefer straw or woodchip-based spawn. Inoculation with grain spawn,
both in- and outdoors, can lead to serious outbreaks of *Trichoderma* (green moulds) on the grain kernels, which may spread through the substrate. Besides, using grain spawn outside is risky as rodents may consume the grain before the mycelium has established itself. Animals (mice, moles) digging in the substrate can lead to serious crop losses. Using 10% spawn leads to quick results, but is expensive. A 5% inoculation rate may be more profitable: ca. 10 litres = 5 kg of straw spawn per 100 kg of substrate. The density of the wood chips is around 500 kg/m³. The inoculation material doesn’t need to be 100% sterile spawn. One can use colonised straw as inoculation material too, which decreases the costs of spawn considerably. The spawn can be mixed in mechanically (with a hay-turning device on a tractor) or by hand. By hand is laborious for large beds; a simple alternative is to make ridges in the middle (top), and both sides of the bed. Distribute the spawn and cover each ridge with at least five cm of woodchips. If the woodchips are left like that, the top layer will dry out. Below surface however, the mycelium can thrive.

Make the beds not wider than 1,40 m; if they are wider, mushrooms growing in the middle cannot be picked without trampling on the beds. The beds can be made as long as one wishes; the height depends on the moment of spawning, but should never exceed 60 cm. Above that height, fermentation starts in the beds and the temperature rises to 70 or even 80 °C.

The lower the temperature at the time of spawning, the higher the beds can be made. Make them at least 30 cm high and no higher than 60 cm. The mushrooms are easier to pick from the higher beds and these will give a good yield in the following year. Unlike *Agaricus* this mushroom can be cased immediately after spawning, e.g. with a 5 cm layer of peat or (if available) *Agaricus* casing soil (see chapters 15 and 16 for casing soil formulas). Casing will keep the moisture in the beds and supply extra moisture during fruit body formation. Stamets states that casing soil should not be treated for longer than 1 hour at 60 °C; this is true when the casing soil is applied to colonised substrate in growing rooms. Outdoors, heat treated casing soil will become colonised with the favourable microflora during the spawn run if applied right after spawning. The casing soil balances the environment directly above the substrate and creates a suitable environment for fruit bodies. Uncased beds may also give reasonable yields, however.

Whole straw bales (e.g. 20 kg bales) can be spawned by pressing holes 10–20 cm deep in the (moistened) straw and inserting walnut size pieces of straw spawn. The straw doesn’t have to be cut loose in this way and by using this spawning method, inoculation rates can be relatively low at 2.5%.

Alternatively, substrate can be packed in bags and cased. The plastic bag can be loosely closed and some cuts should be made in the bag to allow for water runoff and aeration.

### 20.8.4 Spawn run

The beds can be covered with plastic in order to retain moisture; however, care should be taken that the sun doesn’t heat up the air below the plastic; for this reason, black or transparent plastics should only be used if direct sunshine is very limited.

The beds/bags with substrate ideally have a temperature of 20–25 °C during spawn run in the middle of the bags. The mycelium can stand a temperature rise to 35 °C for a
number of days. The mycelium develops quickly at these temperatures and within two weeks, rhizomorphs (white strands of mycelium) form in woodchips, which colonise the substrate. In heat treated substrates (pasteurised straw), a more even mycelium can be seen. In 4–6 weeks (depending on weather, strain and substrate) the substrate can be fully colonised. Even if climatic conditions are suitable for fruit body formation, no mushrooms will appear yet. It takes at least 8 weeks for straw and 12 weeks for woodchips before any primordia can be formed; possibly the depletion of a specific, as yet unknown substrate component triggers fruit body formation. During spawn run the pH of the substrate drops considerably from 6.5 to 4.5.

20.8.5 Fruiting and harvesting
The fruit bodies appear in groups, but flushes are not as even as in *Agaricus* cultivation. From the same spot, usually three to four flushes can be harvested. A new flush can appear within two weeks after the previous; usually the first two flushes are most important. Try to pick the mushrooms before the caps come free from the stem; open mushrooms are vulnerable and have a shorter shelf life. The older the mushrooms, the tougher the stems. Opened mushrooms release loads of purple-brown spores, which (dis)colour the dish; another reason why they should be picked at the button stage. When the beds are too dry, the mushrooms open too early. Sprinklers are essential to obtain a good quality product. Picking and cleaning the mushrooms can be tedious; it is best to have a cloth at hand during picking and rub the mushrooms clean immediately. Then put them directly in the boxes in which they will be sold to minimise handling. In my experience, an experienced picker can thus harvest 10 kg an hour as a maximum; much less than in White button mushrooms.

![Stropharia](image)

The best yield I have experienced was 15%: 15 kilo’s of fresh cut mushrooms from 10 bags of each 10 kg of woodchips, cased with peat. Individual bags even yielded 20%, so there is certainly potential to increase yields. On straw the yield is usually less, ranging from 5-10%.
20.8.6 Specific pests and diseases for Stropharia

A remarkable trait of this mushroom is that it is more easy to grow under natural conditions compared to growing them in artificial rooms or bags. In bags I have encountered infections with small orange gall midges, whereas these seem to disappear when growing on a bed between plants. Possibly some natural enemies of the gall midges prevent the outburst.

A problem for all outside cultivators are snails and slugs. Check the separate chapter Pests and diseases (26) if you want to know how to avoid slugs feasting on your mushrooms.

Infections with flies and mosquitoes can be encountered with cultivation on straw; on woodchips in outside beds however, I haven’t experienced any problems with insects. Dimilin may help as a preventive measure. Maggots will also grow in fruit bodies which have not been picked in time; yet another reason to harvest only young fruit bodies.

Occasionally, the beds will yield competitor mushrooms, like Inkcaps, Volvariella, Agrocybe, Hypholoma fasciculare, and Crepidotus variabilis. These compete for the same nutrients and thus lower the yields. However, if they appear, their mycelium has already established itself.

<table>
<thead>
<tr>
<th>Substrate temperature</th>
<th>Spawn/casing run</th>
<th>Primordia formation</th>
<th>Cropping</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Air temperature**: 10-20 °C (up to 25 °C, strain-dependent)
- **Relative humidity**: 95-100%
- **CO₂ concentration**: <1000 ppm
- **Watering**: Casing soil should stay moist enough, do not spray water on the small primordia
- **Light**: Diffuse natural light or 8 hours/day fluorescent bulb

### 20.9 Volvariella volvacea cultivation methods

In Volvariella cultivation two methods are employed: indoor cultivation and the traditional outdoor cultivation. The first method is quite similar to the technique for Agaricus, as discussed in the previous chapter. A more simple method uses techniques for making beds in the field.
Comparison between *Volvariella* cultivation methods

<table>
<thead>
<tr>
<th>Indoor cultivation</th>
<th>Outdoor cultivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>first a composting stage, then spawning</td>
<td>moderate fermentation and spawn run in the same phase</td>
</tr>
<tr>
<td>relatively high investment</td>
<td>very limited investment</td>
</tr>
<tr>
<td>higher and stable yields</td>
<td>lower, less predictable yields, strongly influenced by the weather</td>
</tr>
<tr>
<td>climate control possible</td>
<td>natural circumstances</td>
</tr>
<tr>
<td>pest- and disease control</td>
<td>little or no protection against pests possible</td>
</tr>
</tbody>
</table>

As indoor cultivation requires rather high investments, it is less suitable for rural growers unless a trade company can provide loans for the necessary equipment and arrange the collecting and marketing of the product. Outdoor cultivation, on the other hand, frequently leads to low yields because of diseases, leading to vanishing interest of the farmers. Also, marketing the product is more difficult because of uncontrollable fluctuations in supply.

Comparison of yields on different substrates in indoor and outdoor *Volvariella* cultivation (adapted from Chang, 1978):

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Biological efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Outdoor cultivation</em></td>
<td></td>
</tr>
<tr>
<td>paddy straw</td>
<td>4-12%</td>
</tr>
<tr>
<td>banana leaves</td>
<td>10%</td>
</tr>
<tr>
<td>oil palm, pericarp waste</td>
<td>1.5-2.6%</td>
</tr>
<tr>
<td>pericarp waste, shredded paper</td>
<td>7-10%</td>
</tr>
<tr>
<td><em>Indoor cultivation</em></td>
<td></td>
</tr>
<tr>
<td>paddy straw</td>
<td>14-28%</td>
</tr>
<tr>
<td>cotton waste</td>
<td>25-45%</td>
</tr>
<tr>
<td>sugarcane waste</td>
<td>12%</td>
</tr>
<tr>
<td>cotton waste/paddy straw mixture</td>
<td>22%</td>
</tr>
</tbody>
</table>

From these figures it becomes clear that the name Rice straw mushroom is misleading: better yields are obtained from substrates with a higher cellulose content, like shredded paper and, most notably, cotton waste.

Even then, the yield is still much lower than that of Oyster mushrooms and White button mushrooms. On the other hand, *Volvariella* grows much quicker, thus securing a fast return on investment.

Several studies revealed that *Volvariella* mainly utilises cellulose. Lignin is degraded much less or not at all. It seems that the presence of the right kind of carbon (cellulose) is more important than a specific C/N ratio.
20.9.1 Indoor cultivation

**Requirements:**
- plastic mushroom shed with shelves or an industrial building (with water resistant walls and able to withstand the heat),
- electric fan and polyethylene ducts running along the shelves,
- concrete floor for composting phase,
- steam boiler,
- remote reading thermometers,
- a long fork to turn the compost heap,
- a short fork to facilitate spawning,
- spawn,
- compost materials: see formulations below.

Substrate formulas

<table>
<thead>
<tr>
<th>Substrate material</th>
<th>dry weight</th>
<th>Substrate material</th>
<th>dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula 1</strong></td>
<td></td>
<td><strong>Formula 2</strong></td>
<td></td>
</tr>
<tr>
<td>dry cotton waste (short fibre, card fly, gutter fly and a number of other wastes can all be used. Only weaving sweeping has been reported to be unsuitable)</td>
<td>90-92%</td>
<td>Cotton waste</td>
<td>50-75%</td>
</tr>
<tr>
<td>rice bran (supplement)</td>
<td>4%</td>
<td>Rice straw</td>
<td>25-50%</td>
</tr>
<tr>
<td>limestone (pH regulator)</td>
<td>4-6%</td>
<td>Limestone</td>
<td>3-4%</td>
</tr>
<tr>
<td><strong>Formula 3</strong></td>
<td></td>
<td><strong>Formula 4</strong></td>
<td></td>
</tr>
<tr>
<td>Spent substrate from <em>Agaricus</em> cultivation</td>
<td>50%</td>
<td>Chopped water hyacinth</td>
<td>50%</td>
</tr>
<tr>
<td>Cotton waste</td>
<td>50%</td>
<td>Rice straw</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Substrate preparation:** the dry materials have to be moistened thoroughly, for instance by soaking them in water. Cotton waste may become completely saturated with water, thus preventing the access of air. It should therefore always be mixed with another material which secures sufficient aeration, like rice straw. Use the squeeze test to determine whether the substrate materials have absorbed sufficient (or too much) water.

Then form piles of at least 1.5 m³ and cover them with plastic to avoid loss of water and energy (evaporation consumes large amounts of energy). The heaps should be turned once or twice (within a total period of two to four days) to prevent long term anaerobic conditions in the heap. Add rice bran or another supplement during the last turning of the heap. The supplements will increase the temperature of the substrate because they provide easily degradable nutrients for the micro-organisms. Less energy is thus necessary to pasteurise the substrate.

The substrate is now ready for the heat treatment. This can be performed in a tunnel, but is usually done in the growing room itself. Beds are filled with a substrate layer of 10 to 20 cm (about 80 kg wet substrate per m², approximately 22 kg dry substrate material per m²). Steam is blown into the growing room until the substrate (not the air!)
has reached a temperature of 60 °C. The steam inlet is adjusted to stabilise the substrate temperature for about three to four hours.

**Spawning** occurs as soon as the temperature has dropped to 37 °C. Spawning rates differ among producers, depending on the vigour of the strain involved. Usually about 1% is used, with upper and lower ranges of 0.5% and 5% (w:w). *Volvariella* grows very fast, thus often 1% is sufficient.

Spawning techniques and spawn substrate materials differ. Some growers use a short fork to mix the spawn evenly through the substrate, while others make holes with a wooden poke and insert peanut-sized pieces of spawn at a depth of 2 to 2.5 cm at 12 to 15 cm intervals.

An alternative method in Taiwan uses the spent compost of the White button mushroom winter crop to grow a crop of Rice straw mushrooms. The beds with the old compost are emptied and the house is cleaned. Cotton waste is mixed with the old compost and fermented for some
days. Then a heat treatment is applied. Seven to nine days after spawning, the first pinheads appear. Usually two flushes are harvested.

**Spawn run:** Cover the substrate with plastic to keep the temperature high (35 °C) but not above 40 °C. *Volvariella* will colonise the substrate in only a few days. At the same time some Actinomycetes and *Scytalidium* will develop, too. Their growth does not hinder the growth of the mycelium of the Rice straw mushroom. Take the plastic off after three days and ventilate some more after six days. Light is also needed for fruit body formation. Use white light or make sure some daylight can reach the substrate from three days after spawning. Just a little light is sufficient; 15 minutes of sunlight or a day/night cycle of 500 lux have been reported to be sufficient.

Spray a fine water mist to maintain optimum humidity and take care not to damage the delicate mycelium. Pick the mushrooms very young, although this may require two or three pickings per day, when they are still closed. Opened ones are difficult to sell, because they have to be consumed on the same day. The biological efficiency of indoor cultivation has been reported to be as high as 50%, but 25% of the fresh weight in mushrooms compared to the dry weight of the substrate materials is acceptable. Because straw mushrooms can be grown very quickly, a relatively high output can be reached per period although the yield is significantly lower than that of other mushrooms.

<table>
<thead>
<tr>
<th>Spawn run</th>
<th>Primordia formation</th>
<th>Cropping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative humidity</td>
<td>90%</td>
<td>95-100%</td>
</tr>
<tr>
<td>Substrate temperature</td>
<td>35-38 °C</td>
<td>32-35 °C</td>
</tr>
<tr>
<td>Air temperature</td>
<td>adjust to stabilize substrate temperature</td>
<td></td>
</tr>
<tr>
<td>CO₂ level in the air</td>
<td>5000-10,000 ppm or lower</td>
<td>&lt;1000 ppm</td>
</tr>
<tr>
<td>Light requirements</td>
<td>total darkness</td>
<td>diffuse natural light or TL for 12 hours/day</td>
</tr>
<tr>
<td>Watering</td>
<td>not recommended, especially if the substrate is covered with plastic</td>
<td>misting the air regularly to maintain a very high humidity</td>
</tr>
<tr>
<td>Duration of period/flushing interval</td>
<td>4-6 days</td>
<td>4 days</td>
</tr>
<tr>
<td>Picking stage</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In indoor cultivation, more or less the same pests are encountered as in indoor cultivation of *Agaricus*, and measures are similar. Consult the chapter Pests and diseases (26) and their control.

**20.9.2 Traditional outdoor cultivation**

**Requirements:**
- Bed foundation or boxes 60 cm long, 45 cm wide and 20 cm deep,
- Substrate material: e.g. rice straw, banana leaves, cotton waste, sugar cane bagasse or water hyacinth,
- Spawn,
- Covering: plastic on bamboo supports.

The bed foundation can either be made from soil, bamboo or wood. A soil foundation is made by elevating by 5 cm a surface area with a width of 50 to 60 cm and a length of approximately 2 to 3 m. The required soil for elevating can be taken from the surrounding surface in such a way that a channel is created, 15 cm deep and 30 cm wide. The channel can be filled with water afterwards to ease watering and to keep some creeping animals away. A wooden or bamboo foundation should have the same dimensions, but a little higher: 30 cm above the ground. Boxes have the advantage of easier pest management, but the disadvantage is the initial cost of producing them. Several substrates can be used:

**Rice straw:** Only newly harvested and dry stored rice straw can be used. Long rice straw is needed, at least 50 cm but preferably 60 cm (bed-wide). Tie the straw together in bundles with a diameter of 10 cm and cut them all to a uniform length.

**Dead banana leaves:** Use leaves still hanging from the trees, because these have not yet decayed. Cut them to a uniform length preferably as wide as the beds, and make bundles with a diameter of 10 cm.

**Water lily** (water hyacinth): Tear the plants out of the lakes or rivers (where they spoil fishing grounds because of their shade and deprive them of oxygen). Wash the soil out of the roots. The whole plant can be used. Let it dry, preferably on a cement floor or hanging free. Then cut them into a uniform size length (same width as the beds) and make bundles with a diameter of 10 cm. Four to six plants are laid parallel and are subsequently bundled.

**Cotton waste** mixed with rice straw: in a ratio of 1:1 or 1:2.

**Preparing the substrate and building the piles:**
Soak the substrate bundles for four hours in water. Banana leaves should become more transparent after soaking; rice straw becomes darker. Let them drain and cover the foundation with one layer. Moisten the surface of the soil, otherwise it will absorb too much water from the straw. All the butt-ends of the same layer have to be on the same side. Distribute the *Volvariella* spawn evenly over the surface. Use thumb-sized pieces every 10 cm and start about 5 cm from the edge. Now pile the second layer, but put the butt-ends on the other side. Spawn again and repeat the procedure until four to six layers are piled. In the hot season no more than four layers (less than 50
cm high) should be piled, otherwise the temperature will rise too high. In the cool season up to six layers have to be used to get the right temperature of at least 35 °C. Make the bed compact by pressing the bundles down. Make the edge of the bed as even as possible. Otherwise the first mushrooms would appear in deep places and rot before being seen. This would attract fungal contaminants and insects. For this reason, loose strands have to be trimmed after spawning.

**Spawn:** Spawn should be no older than two months, nor too young. Pink spores can be seen if the spawn is still too young. Select a good strain. No blue or green moulds should be present in the spawn.

**Spawn run:** The optimal temperature range is 35-38 °C in the middle of the beds for four to six days. Use plastic or a tent-like construction to prevent the beds from drying out and to prevent rain from damaging the mycelium. A plastic cover over the pile can also help in maintaining the desired temperature range. It will also keep in moisture, so there will be no need for watering for the first ten days. In periods of hot weather, the beds should only be covered loosely, or openings in the upper surface of the plastic should be cut. The beds should be positioned in the shadow, otherwise the temperatures would rise too high during daytime. Alternatively, the beds can also be covered with a bamboo frame stretched with plastic film. In this case, the
plastic should not touch the beds. This tent-like construction can be used until harvest. Fill the channel next to the beds with water to maintain a high humidity and to prevent insects from infesting the beds. Everyday the temperature inside the substrate has to be checked. If no thermometers are available, just feel with your fingers inside the compost. A temperature of 40 °C feels comfortable. Above this temperature the plastic should be loosened to allow more heat to escape.

**Fruiting:** In only eight to ten days after spawning, small white fruit bodies will appear. Stop watering, otherwise the vulnerable pinheads will be damaged. It takes two to three days after their appearance before they can be harvested. Only closed button-stage straw mushrooms can be kept for some time. Harvest two or three times a day for the next three days. Take care not to damage tiny pinheads that grow close to harvest-ready buttons. After a resting period of five or six days the next flush will appear. The total harvest time is only one to two months. Lift the plastic at least once a day for a few minutes to allow fresh air to enter.

A 3 m long and 50 cm wide bed requires 150 to 200 bundles of rice straw (25 kg dry) and six 500 ml bottles of spawn. The yield is 2-3 kilos.

### 20.9.3 Pests and diseases

A common fungal contaminant of outdoor Rice straw mushroom beds is *Coprinus* (Ink caps). It prefers somewhat higher temperatures and will grow faster than *Volvariella*. Spores of *Coprinus* are abundant in the air. If the straw has not been stored properly or if the temperature in the beds rises above 38 °C then *Coprinus* is likely to spoil the crop. If the substrate has been supplemented with urea or another nitrogen-rich material, *Coprinus* is also likely to appear.

Insects may feed on the substrate, especially at the end of the crop. Spraying insecticides is not recommended at the time of bedding. It would increase the costs and is often not necessary. Ants and mites may be controlled by spraying malathion or azodrin if local legislation allows this, but take care not to spray on the developing fruit bodies. Termites are a nuisance in outdoor cultivation, because they will usually appear when the primordia have just appeared. It is then impossible to apply insecticides. Some growers will spray around the beds, but more effective ways should be investigated. The legs of the beds, for example, could be shielded by putting a bowl with soap water under them. A survey on the presence of termites should be performed before outdoor cultivation is started.

### 20.10 Intercropping corn and Volvariella: a case study from mainland China

Various mushrooms can be used for intercropping. They do not compete for nutrients with the plants. *Volvariella* can be grown together with summer corn in the fields. The corn provides shade and a high relative humidity on the ground. The plot is divided into rectangles of 1.5 m x 1.5 m. The corn and the Straw mushrooms are planted on adjacent rectangles, like on a chess board. The distance between the corn rows is 50 cm, so four rows can be planted in a 1.5 m rectangle. The distance of the corn within the rows is approximately 25 cm. As soon as the corn starts to provide shade on the
adjacent plots, the mushroom beds can be prepared. The mushroom plot should have 20 to 25 cm high earth walls, somewhat compacted. Quick lime can be sprinkled to disinfect the plot. Water the plot, but not too heavily. The soil should not become muddy. Prepare the substrate in the following way: soak corn waste or rice straw for 12 hours in boiled water. Drain and apply the squeeze test to determine the moisture content; only a few drops of water should be released from the substrate. Add 3% CaCO$_3$, spread the substrate on the field and compress it slightly. The spawn should be applied to a layer of 20 to 25 cm. About 2.5 kg spawn should be used for 50 kg substrate. Put a piece of spawn on top of the layer every 7 to 10 cm. Add an additional layer of substrate and compress slightly again, and cover the beds with a plastic foil. Remove the foil after two days and water the substrate. A straw mat replaces the plastic sheet. Air can pass through the mat. The straw mat will also provide protection against rain and controls climatic conditions like humidity and temperature. The mycelium will rapidly degrade the cellulose in the substrate materials. Within seven to nine days, the first primordia will occur. After ten to twelve days, the mushrooms are ready for picking. Two or three flushes can be harvested. Per m$^2$ 4.5 to 6 kg of fresh mushrooms can be harvested. Reports state that the corn yield will increase by 2 to 5% because of the vitamins and minerals in the mycelium. No weeds will grow on the mushroom beds, which is also favourable for the corn. Furthermore, the substrate in the beds is loose, nutritious and rather wet and in fact serves as a soil conditioner.
21 Cultivation on sterilised substrates

This chapter discusses the different procedures to grow mushrooms on a more or less sterile substrate in the following paragraphs:
- Different methods of sterile substrate preparation,
- Requirements for substrate production,
- Substrate containers: bags and bottles,
- Filling,
- Sterilisation.

Then the following mushrooms are discussed in detail:
- *Pleurotus* spp. (different kinds of Oyster mushrooms) with a case study from the Philippines,
- *Ganoderma* spp. (different kinds of ‘ling zhi’) with case studies from China and Malaysia,
- *Flammulina velutipes* (Gold needle mushroom) with case studies from China and Taiwan,
- *Tremella fuciformis* (White jelly fungus) with a mixed culture technique and a case study on cotton seed hulls in China,
- *Hericium erinaceus* (Monkey head mushroom) with a case study from a farm near Beijing, China,
- *Auricularia* spp. (Wood ear mushrooms) with case studies of *A. polytricha* in Taiwan and *A. auricula-judae* in China.

The techniques for the cultivation of Shiitake are discussed in the following chapter.

<table>
<thead>
<tr>
<th>Advantages of sterilised substrate production</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>No need for complete wood logs, which can serve other functions like providing energy for cooking</td>
<td>A sterilisation unit of some kind has to be constructed</td>
</tr>
<tr>
<td>Sawdust originating from more types of trees as compared to wood log cultivation can be used</td>
<td>Heat resistant plastic bags are not available everywhere and can be costly</td>
</tr>
<tr>
<td>Handling of the bags is easier than that of wood logs</td>
<td>More energy is consumed compared to growing on woodlogs or pasteurised substrates</td>
</tr>
<tr>
<td>The handling of uniformly sized plastic bags can easily be mechanised</td>
<td>Filling bags by hand is laborious</td>
</tr>
<tr>
<td>The substrate can be enriched with nutrients, thus boosting yields</td>
<td>Filling bags by machines requires high investments</td>
</tr>
<tr>
<td>Spawn run time is much shorter than in wood log cultivation</td>
<td>Impractical to use solar energy as the desired temperatures are near or even above the boiling point</td>
</tr>
<tr>
<td>Pests and diseases can be controlled effectively</td>
<td></td>
</tr>
</tbody>
</table>
21. CULTIVATION ON STERILISED SUBSTRATES

### 21.1 Different methods to produce mushrooms on ‘sterilised’ substrates

<table>
<thead>
<tr>
<th>1 Normal procedure</th>
<th>2 Bulk sterilisation</th>
<th>3 Pre-heated substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Optional: dry heat-treatment of substrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moisten, mixing &amp; spawning</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spawn run</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cropping</td>
</tr>
</tbody>
</table>

- Finely chopped substrate ingredients are moistened, filled in small substrate containers (usually plastic bags) and subsequently sterilised. The bags are spawned and after spawn run (a part of the plastic is removed to allow the mushrooms to fruit. If a contamination occurs, it can usually be separated from the rest of the batch.
- The substrate ingredients are moistened. Sterilisation now takes place in bulk; spawning is easier (because the spawn is added in bulk); the investment is considerably higher, as specially designed equipment is necessary for the bulk sterilisation. The sterility of the spawning process is of utmost importance, as the complete batch can be affected when contaminants are mixed through the substrate during the distribution of the spawn. Completely mature.
- This is the least sterile method as the substrate is prepared under non-sterile conditions; the substrate has either been heat treated under dry conditions or chemically when wet (e.g. with hydrogen peroxide). This method requires the lowest investments, but needs much spawn (8-10%) which acts as a nutrient source too. Adding supplements is very risky as contamination is likely to occur. This method has been used on a limited scale, the technique is not fully mature.

**Cost aspects**

- Limited costs for sterilisation and spawn unit. PP bags must be used
- Costs of HDPE/PP higher than PE
- More labour involved in handling the substrate increases variable costs
- Sterilisation by steam cheaper than by irradiation

- High initial cost of bulk sterilisation unit
- PE bags may be used, and may be cheaper than HDPE or PP
- Lower labour costs because of efficient spawning relatively large bags (15 kg)

- Very low investment level
- Microperforated PE bags, cheapest option
- High percentage of spawn increases variable costs significantly
- Limited handling, also due to the possibility to use
This chapter focuses mainly on the normal procedure (1) but will also cover some aspects of the two other methods; a separate case study is devoted to the Pre-heated substrate method in the next chapter.

Generally speaking, it is more practical to sterilise bags in autoclaves. For larger facilities, with a production of more than 50,000 bags a month, bulk sterilisation is probably the best choice. The cost of a bulk steriliser can be higher than 750,000 US$. This includes the mixing unit with a double wall and cooling facilities, special facilities to open the vessel for inoculation. A 14 metre long autoclave, 1,20 metre diameter autoclave for sterilisation under high pressure from Asia will cost approximately 10,000 US$ (however, check local legislation before acquiring an autoclave from abroad, as the steam regulations may not allow you to work with equipment that is considered safe in other countries). Even cheaper than an autoclave is the equipment needed for sterilisation under low pressure: keeping the substrate a prolonged period at 95–98 °C. The substrate formulations and fruiting conditions may differ per species, but the technique is basically similar. With one and the same technique, many wood-degrading fungi can be cultivated in more or less the same way. Those discussed here are the species which are currently produced most (among wood-degrading mushrooms). In Japan, a number of other species, like *Pholiota nameko*, *Grifola frondosa* and *Lyophyllum decastes*, are also cultivated on sterilised substrates.

21.1.1 Potential for developing countries
Growing mushrooms on sterilised substrates has a great potential for developing countries, as it can be used for many different mushrooms. Therefore mushroom species and strains can be selected that grow at the desired temperature range.
Many different kinds of agricultural wastes can be utilised, as the substrate formulations for the different mushrooms will show. It has also proven to be effective: this cultivation method is widespread throughout South-East Asia. The technology is relatively easy to understand and to master. FAO used this method to provide disabled in South East Asia with an income. The closed bags or bottles provide a barrier to contaminants and insects during spawn run. Especially in (sub) tropical areas with a high contamination pressure, this is an important advantage of the method.

21.2 Requirements for substrate production
The equipment varies depending on the resources of the farmer. On the low investment level only an oil drum and plastic bags are necessary; the high tech approach is to employ complete lines of equipment for preparing the substrate, filling substrate containers, sterilisation and handling of the substrate.
Substrate preparation equipment:
- Substrate mixer (optional, can be performed by hand too),
- Bag filling machines (optional, manual filling is at times more economic),
- A sterilisation unit,
- A steam source (if not incorporated in the sterilisation unit),
- Means of transport, bulldozer, conveyor belts.
For the substrate:
- raw substrate materials, like sawdust, rice bran etc.,
- substrate containers (plastic bags or bottles),
- depending on the type of bags/bottles: additional plugs and plastic rings.

The requirements are further discussed in the paragraphs on the individual stages of this cultivation technique.

21.2.1 Substrate preparation for the normal procedure
Substrate preparation involves the following steps:
- moistening (and sometimes composting) of main substrate materials,
- mixing them with supplements,
- filling the substrate in containers,
- sealing the containers from the air,
- filling the sterilisation unit,
- sterilising the containers.

The sawdust (or other bulk substrate material) has to be stacked on a heap and moistened. By keeping the heap moist, the sawdust will soften. This will ease the absorption of water. Usually the sawdust is stacked for only one or two days, but if fresh sawdust, like sawdust from recently felled trees, is not suitable it should be stacked for a much longer period: up to several weeks. If composting of the sawdust is necessary, the time and procedure to be used are described in the sections on specific mushroom species. In some countries the right kinds of sawdust are not available, and then the available sawdust has to be made more suitable by composting. Composting will remove unfavourable substances, like resins. Composting will result in the removal of volatiles and the most accessible carbohydrates. It will result in a substrate which has (prior to the sterilisation process) many more micro-organisms present compared to substrate from fresh sawdust.

Sawdust substrate should be free of splinters or bigger pieces of wood. These may damage the bags, offering contaminants easy entrance after the sterilisation. On the other hand, several growers feel that a combination of fine and more coarse sawdust or wood chippings provides the best starting material. Very fine sawdust should be avoided as it clogs the airflow when moistened.

21.2.2 Mixing
The aim of mixing is obviously to distribute the different components, including water, as evenly as possible. If one component is added in a rather low concentration, then it is better to mix it first with some of the substrate and only then apply it to the large heap. Otherwise its distribution will probably remain non-uniform. Mixing can be performed by hand or by machines. Of the latter, different types exist: cement mixers, ribbon mixers or auger mixers. Cement mixers consist of a rotating drum with fixed blades inside. Auger mixers consist of a fixed container with two augers running in opposite directions. Ribbon mixers also consist of a fixed container, with mixing blades on a central, rotating shaft. Mixing manually is feasible with a capacity of 2 tons per day for two people, filling should however be done by more people then.

After mixing, the moisture content should be 60–65%. Apply the squeeze test to deter-
mine whether the substrate is moist enough. Highly supplemented substrates can start to ferment within hours after mixing the substrate ingredients. They should therefore be sterilised as quickly as possible and certainly not left overnight for sterilisation.

### 21.3 Substrate containers

Growers can choose between a number of different types of bags and even bottles. The best choice for their situation depends on several factors: local availability, sterilisation method, gas exchange, price, melting point, size, brittleness and transparency. The sterilisation method in particular determines the choice of the plastic bag material:

<table>
<thead>
<tr>
<th>Sterilisation method</th>
<th>Plastic type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilisation of substrate in bags by steam, high pressure (121 °C)</td>
<td>(laminated) PP</td>
</tr>
<tr>
<td>Sterilisation of substrate in bags by steam, low pressure (96-98 °C)</td>
<td>HDPE</td>
</tr>
<tr>
<td>Sterilisation of substrate in bags by gamma radiation</td>
<td>PE</td>
</tr>
<tr>
<td>Bulk sterilisation and spawning</td>
<td>PE*</td>
</tr>
<tr>
<td>Pasteurisation at ca. 60 °C</td>
<td>PE, laminated PE/PP</td>
</tr>
<tr>
<td>Preheated substrate or chemical sterilisation</td>
<td>PE*</td>
</tr>
</tbody>
</table>

* PP can be used but has several unfavourable characteristics

### 21.3.1 Characteristics of different types of plastic

Different types of sterilisable plastic bags.
1. A much used bag type with a diameter of 10-18 cm and a height of 15-30 cm. Closed with a cotton plug in a plastic ring.
2. The same type of bag, now closed with a cheaper option: a cotton plug with a rubber band around it.
3. A filtered bag, with one or two filter patches. It typically holds 2.5 to 5 kg of substrate. These bags are also used for spawn production. A sealing machine is necessary for working with this type of bag, as it is closed by sealing the plastic.
4 and 5. So-called artificial logs.
4-5. closed with rings and plugs; 5. without a plug. If no plugs are used, cuts in the plastic have to be made to provide sufficient aeration.

The general term “plastic bags” can be better specified in two grades of polyolefin, polyethylene (PE) and polypropylene (PP). These two materials are still the least expensive, and the most produced in the world. Cost of PE and PP are about the same, but this may vary depending on the current market situation. PE has a low melting point, and PP has a higher melting point. In general PE starts to melt at temperatures above 90 °C, and PP can with-
stand up to 130 °C. PP disintegrates when sterilised by gamma irradiation. Thus laminated bags of PE/PP can not be used for irradiation, nor for high temperature sterilisation, but only for pasteurisation at low temperatures. A variation of PE is HDPE, or high density polyethylene. This material has a higher melting point than regular PE and can withstand up to 108 degrees °C, but it has the disadvantage of having a heavy haze and is much less transparent than PE, as well as being more brittle. Therefore most growers dislike using this material, as contamination is not easily detected.

A new development is the use of these patented bags of SacO2 which can hold larger amounts of substrate. A sealer is necessary as the bags are closed by heat sealing the top end.

Left: Plastic bottles have the advantage that their handling can be mechanised to a large extent. They are sealed from the air with lids with a paper filter inside. They usually last for about 10 heat treatments after which the plastic becomes weak. Right: Larger amounts of substrate per bag (15 kg) minimise handling, but fruiting of Shiitake takes longer compared to smaller bags. Note the plugs in addition to the filters.
Plastic bottles are used for the cultivation of various Oyster mushrooms (*Pleurotus eryngi* farm in Japan) (left), *Flammulina* (right) and other species.

In general PP is more brittle than PE, but by adding plasticisers in small percentages, PP can be made soft and will not crack at side folds, gussets and low temperatures. As PP has a higher melting temperature than PE, the sealing temperature is also higher. Laminated bags of low temperature melting point PP and high temperature melting point PP reduce chances of breakage due to stress; the high temperature PP is normally at the outside of the bag.

Another consideration when choosing bags, is how the proper gas exchange can be achieved. Several strategies are currently employed:

- bags without filters, gas exchange through cotton of foam plugs,
- bags without filter, with some (limited) air exchange through the plastic, the spawned part is covered with a paper tape and the bags are cut open when the mycelium colonised part of the substrate,
- gas exchange through filters on top of the plastic (*e.g.* Unicorn),
- gas exchange through patented filters in the seams (*e.g.* Mycelia),
- a combination of filters and plugs,
- micro perforated bags, note that contaminants can easily enter the substrate then.

The third consideration is the form of the bag and the volume. The amount of substrate to be filled in each container has increased during the years. The plastic bags used to be rather small, containing 0.5 to 1.25 kg of substrate per bag. Nowadays up to 15 kg of substrate can be filled per bag, thus reducing labour. The substrate has to be rather loose then, otherwise aeration will still be insufficient. A disadvantage of larger amounts is that the internal heat can rise too high for the mycelium; cooling becomes necessary then, especially when the substrate is spawned through as is the case in bulk sterilisation and when farmers shake the spawned bags for faster spawn run. Yet another disadvantage is that the form may be less optimal; for Shiitake, longer cylindrical bags seem to produce better than the same weight of substrate closely packed together.
21.3.2 Filter types

Some commonly available filters and their characteristics are given in the following table:

<table>
<thead>
<tr>
<th>Material</th>
<th>filter type</th>
<th>available pore sizes (micron)</th>
<th>Inch of water permeability</th>
<th>application</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>Non woven</td>
<td>0.05-5</td>
<td>&lt;1-20</td>
<td>general purpose</td>
</tr>
<tr>
<td>PP</td>
<td>Celgard</td>
<td>0.1-0.5</td>
<td>8-12</td>
<td>Shiitake &amp; spawn</td>
</tr>
<tr>
<td>PP</td>
<td>Unicorn M</td>
<td>0.2-0.5</td>
<td>10-15</td>
<td>Shiitake cultivation</td>
</tr>
<tr>
<td>PP</td>
<td>Unicorn T</td>
<td>0.1-0.4</td>
<td>5</td>
<td>spawn production</td>
</tr>
<tr>
<td>PP</td>
<td>Unicorn B</td>
<td>2-5</td>
<td>1-2</td>
<td>spawn production</td>
</tr>
<tr>
<td>PP</td>
<td>Mycelia</td>
<td>0.5 or more</td>
<td>No data</td>
<td>spawn and substrate</td>
</tr>
<tr>
<td>PE</td>
<td>Dupont</td>
<td>1-5</td>
<td>5-10</td>
<td>Shiitake cultivation</td>
</tr>
</tbody>
</table>

Notes: 1. pore sizes of less than 0.05 and more than 5 micron are not generally used in mushroom cultivation-related applications; 2. Permeability is measured in pressure of inch of water when a membrane just under water lets air bubble form above membrane.

Differences in water permeability can be measured with an instrument, consisting of a box with an air inlet and a top clamp. A filter can be clamped between the box and the top frame; the box is pushed under water and air is thus pushed inside. The air pressure can be measured and gives an indication of the water permeability.

Plugging of the filter pores sometimes occurs; usually it is not the substrate itself but the water in the substrate which is responsible for blocking the filter pores. The water may adhere to the bag and may cause contaminants to enter the bag (e.g. small bacteria, which would not be able to enter a dry pore filter). Some growers thus use bags which have filters on top, and no contact between substrate and filters is made. Another consequence of filters, directly in contact with substrate, is that the substrate may dry out.

In the case of heavily supplemented substrates, this leads to very nutritious dead organic material, which can cause infections with green moulds when the bags are opened.

21.3.3 Bags without filter

HDPE cannot withstand sterilisation temperature, therefore substrate in HDPE bags is pasteurised at 95-100 °C, for 8-12 hours. After cooling, holes are punched and the spawn is deposited in the hole and wax or a paper tape is applied to seal the spawn in the hole. This method is laborious but has the lowest material costs, and many Asian countries prefer this method.

21.3.4 Choosing bags or bottles?

The main advantage of bottles is that a complete line of equipment (mainly of Japanese origin) is available to mechanise the process. Picking of Oyster mushrooms from bottles on V-slanted walls is also very efficient at 50 kg/hour. Why then hasn’t bottle cultivation spread around the globe? The main reason is that the required scale of such a plant requires a larger market than exotic mushrooms have up till now in other parts of the world. Of the three most consumed mushrooms in the western market, White buttons,
Shiitake and Oyster mushrooms, it is only the Oyster mushroom which can be cultivated on the bottles.

Most Japanese growers focus on one type of mushroom. In Nagano county alone there are ca. 2000 growers; the market for special mushrooms like Flammulina velutipes, Pholiota nameko, Hypsizygus marmoreus is much larger in Japan than in the western markets. The bottle size is optimised for one type of mushroom; the machines are adapted to that particular bottle size, which decreases flexibility to switch to other mushroom species. A bag grower is much more flexible to the size of his substrate containers.

Another disadvantage is the high initial investment necessary for mechanisation. In order to use the machines efficiently, there has to be continuous use of them. Lou Hsu from Unicorn calculated the investment and came up with the following figures:

- Cost of caps: ca. 0.15 US$; cost of bottles: 0.65 US$
- Cost of substrate container per run: 0.80 US$ for ten times (bottles can be reused)
  0.500 kg of substrate = 0.16 US$/ kg of substrate
- Capacity of spawning machine: 4500 bottles/hour, at 2 hours a day 2.34 million bottles have to be filled.

The initially required number of bottles depends on the total cycle period, e.g. three months for Pleurotus. Thus 580,000 bottles have to be bought at the start of the company. Machinery will cost (depending on the capacity) ca. 350,000 US$. Additionally trays have to be acquired, and the costs for the real estate have to be added. All in all ca. 1–2 million US$ is required to set up a continuously working mushroom company on bottles. For growers outside the Japanese and Taiwanese area, growing on bags is safer as it offers more flexibility and the cost of substrate container per kg of substrate is lower.

### 21.4 Filling

The substrate has to be filled in the containers and is usually compressed (unless large bags are used). Often a hole is pressed in the middle of the substrate with a metal stick. The hole will allow faster mycelial growth during spawn run. All the work can be performed by hand, but this is rather laborious. The tools shown in the figures can decrease labour input and at the same time ensure a standardised production.

For bottles, special equipment has been developed in Japan. Consult the paragraph Bags or bottles in this chapter, as well as the case study on Flammulina.
This machine is in use in Thailand. After bags are filled by hand, a machine compresses the substrate and presses a hole in the middle of it, plugs are put on the bags by hand (courtesy TARJ).

Special bagging machines have been developed in Taiwan; it depends on the wages whether it is profitable to fill by hand or machines. The machines have a capacity of about 500 bags per hour. Three people are necessary to put new bags in the machine, take filled bags out and put plugs on top of the bags.

A special machine has been developed by Unicorn which uses a sheet of plastic with filters, prepares a bag, and subsequently uses the film to hold the substrate. The bags can either be closed at the bottom only (e.g. for facilities which sterilise in a double door autoclave) or both top and bottom (for bulk sterilisation and the use of PE bags). Bottom picture, same machine (a form-fill and seal machine), with an auger for accurate filling.

Sterilised substrates in bags can easily be stacked and handled.

Filling manually. The sawdust substrate is tightly compressed in the bags. A ring and a plug seal the inside from contaminants, but still allow aeration.

Maitake growing in PE (polyethylene) bags; the substrate was sterilised in bulk, spawned and subsequently filled in the bags (courtesy L. Hsu).
21.5 Sterilisation

The objective of any heat treatment is to decrease the number of contaminants in the substrate to a level that allows a good spawn run. A complete sterilisation, where each and every organism is killed by the heat treatment, is often difficult to achieve. Two types of heat treatment can be distinguished: sterilisation under pressure at temperatures around 121°C and semi-sterilisation below 100°C at ambient pressure. The heat treatment depends on the mushroom species and local traditions. Sterilisation at or around 121°C requires vessels which can stand the overpressure of 1 atmosphere. Semi-sterilisation can be achieved in cheaper vessels or tent-like constructions. The substrate can be sterilised in bulk, or already packed in bags, which is normally the case.

There are some indications that bags sterilised under pressure at a temperature of 121°C give a shorter yielding period, because the organic substances are more degraded by the high temperature. Thus complete sterilisation is used when only a few flushes are expected: Shiitake cultivation in Thailand (contamination pressure is very high and climate is only fit during a short period, therefore there is usually only one flush) and Ganoderma tsugae (only two flushes) and Flammulina velutipes (only one flush gives good quality fruit bodies) in Japan and Taiwan. When more flushes are expected, farmers will apply semi-sterilisation; for example, Shiitake cultivation in Taiwan, Pleurotus cystidiosus and Auricularia in the Philippines and Taiwan. HDPE bags can be used when sterilising under low pressure.

During the heat treatment most contaminants in the substrate will be killed. It is necessary that the temperature is high enough in each bag, so packing the bags in the autoclave must allow the steam to reach each bag properly.

The duration of the treatment varies with the equipment used and how the sterilisation units are packed. Densely packed rooms take much longer to heat up than loosely stacked rooms. The following table gives an indication of the duration.

Different heat treatments for 1.2 kg bags

<table>
<thead>
<tr>
<th>Country</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taiwan</td>
<td>4 hours at 121°C, or: 4 hours at 96-98°C and even up to 10 hours for heavily supplemented substrate</td>
</tr>
<tr>
<td>Thailand</td>
<td>2 hours at 121°C, next day again 2 hours at 121°C</td>
</tr>
<tr>
<td>Philippines</td>
<td>1.5 hours under pressure at 121°C or 4-6 hours in an oil drum</td>
</tr>
<tr>
<td>Europe</td>
<td>2.5 hours at 121°C or threefold treatment of 8 hours with dry heat at 70°C within two days.</td>
</tr>
</tbody>
</table>

To prevent condensation from wetting the cotton plug, the bags often have to be covered with a plastic sheet. Wet plugs clog the air and allow easy access of contaminants to the sterilised substrate. The heat treatment makes the plastic weaker, and it will crack easily if not handled with care. Each crack will lead to contamination. Neurospora ("Agent Orange") is a common contaminant if the plug sealing has become too wet during sterilisation.
21.5.1 Sterilisation units
The equipment varies, depending on the available resources. Only for developing countries oil drums can be feasible. Bigger companies prefer big tent-like constructions or use autoclaves.

A simple oil drum can be used in the following way: put a wooden rack on the bottom with a height of around 20 cm. Fill water in the drum up to the height of the rack. Take a big polypropylene bag that allows steam to pass through and pack the small bags with the substrate inside. Put the lid on the drum and steam for four to six hours by heating the drum with either wood or gas. Allow the steam to escape by a few small holes. Each time about 75 bags can be steamed in this way. Take care not to boil away all the water.

21.5.2 Walk-in sterilisation chamber
The sterilisation chamber is constructed from cement mortar and brickwork, measuring 1.5 x 2.3 x 2.3 m high. The inner walls are lined with heat resistant cement. Heat is supplied by two pressurised kerosene furnaces. The water level in the container underneath the sterilisation chamber is maintained automatically by a water reservoir outside the chamber. Two pipes run into the sterilisation chamber. The bags are stacked in wooden crates with ample space around them for efficient steam circulation. 1300 bags of 700 g substrate can be steamed at the same time. The cost for this kind of sterilisation chamber in Malaysia was US$ 1000 (reported from Malaysia).

21.5.3 Steaming unit
Relatively simple tent-like constructions can also be used to semi-sterilise the bags. Prolonged heating at around 96-98 °C will sterilise the substrate sufficiently. Obviously the used materials should be able to stand the temperatures. Insulation panels can keep energy costs down.

21.5.4 Autoclaves
Autoclaves are double-walled steel containers, which are able to withstand an over-
pressure of 1 atmosphere. Thus the temperature inside can be raised to 121 °C. Check technical features of autoclaves in the appropriate section in the chapter Spawn production.

21.6 Spawning and spawn run

The substrate should be sterile after the heat treatment. It is thus very easily contaminated. The so-called biological vacuum will be filled rapidly by all kinds of microbial life, if it gets the chance. At spawning the bags have to be opened to put the spawn in. This is the moment at which contamination is most likely to occur. So keep the time the bags are open as short as possible. Contamination can be reduced if liquid spawn is used; check the case study on exotic mushroom cultivation in Finland in the next chapter. Also very important is the air in the room: an inoculation room with ultraviolet lights on during the night and filtered air would be best. Consult the sections on clean rooms in the chapter Spawn production. The following measures can be taken in controlling contamination during inoculation:

- Put on clean clothes,
- Put the hot bags in a special room with UV lights. Let the bags cool down without ventilation, or ventilate with filtered air. Inoculate next day (do not forget to put the UV light out),
- Hold both substrate and spawn containers in a horizontal position to prevent spores falling in,
- Use a flame near the mouths of the bottles of spawn and plastic bags to keep the environment more or less sterile,
- Spawn at night when the contamination in the air is less,
- Fumigate with chemicals: chlorox, formalin, alcohol. Be careful not to come into contact with these chemicals. The use of chemicals can affect both health and environment; hygienic measures should be considered first. Misting with $H_2O_2$ is an environment-friendly way to obtain a clean room for spawning, as its end products are oxygen and water.

Spawning is performed by lifting the plugs from the bags and putting a small amount of spawn inside. When bags were used which have to be sealed (bag type 3 and 6) spawning is slightly different. If a two door autoclave is available, with one door opening in a clean room, the bags can be stacked in the autoclave with the plastic end folded downwards after filling. They are sealed after spawning. If only one door autoclaves are available, the bags have to be sealed before they are autoclaved and have to be opened for spawning. They have to be sealed once again after spawning.

21.6.1 Spawn through or top spawn?

Growers who use the normal procedure and pack the substrate in bags before sterilisation, can choose between top spawning and through spawning. Through spawned bags have to be shaken or kneaded after spawning to distribute the spawn evenly; this takes somewhat more time and increases the chances for contamination. Bulk sterilised and bulk spawned substrates are obviously always through spawned. Through spawning leads to a quicker spawn run and the mycelium has the same age everywhere. However, the fast
mycelial growth also means that much heat is generated; the bags of substrate have to be packed loose from each other, otherwise the temperature would go up too much. This means that the spawn run room cannot be packed as dense compared to the top spawning method.

Top spawning is easier to perform; however it leads to a different age of the mycelium. This can be helpful, as in the Taiwanese case study the mushrooms will emerge from the oldest (top) part of the bag only. Both during spawn run and fruiting this means that the blocks/bags can cover the complete surface of the shelves or rooms, because they don’t need any space separating the bags.

21.6.2 Spawn run

During this stage the mycelium will grow through the substrate. Spawn run time is different for each species and depends on the size of the bag, amount of spawn, the strain used and the temperature.

21.6.3 Determine causes of contamination

An observation of the distribution of fungal contaminants may reveal how they entered the substrate, so that appropriate measures can be taken.

- If the contaminants immediately spread all over the bag or a large proportion of the substrate, the reason is probably an insufficient heat treatment.
- Colonies of moulds around each particle of grain spawn are an indication of improper sterilisation, but may also indicate contaminated spawn.
- If the contaminants spread from the inoculation site, contamination is most likely to have occurred during spawning. Check the measures mentioned above.
- If there are a few (but possibly large) colonies of contaminants, then the bags may have cracks or holes. Examine the plastic near the centre of a colony.

The causes for a bacterial infestation are more difficult to trace. The spawn may have been contaminated, or the substrate may have contained a large population of anaerobic bacteria of which some survived the heat treatment. Anaerobic bacteria often pro-
duce a foul smell. A sudden change in pH and high CO₂ levels within a week after spawning can also indicate bacterial contamination.

21.7 Oyster mushrooms

Oyster mushroom cultivation in the western hemisphere is mainly performed on pasteurised substrates. In South-East Asia sterilised plastic bag cultivation is widely used for many kinds of Oyster mushrooms. The infection pressure in (sub)tropical countries is much higher than in moderate climates, therefore the use of small bags that cannot be entered by contaminants and insects has advantages. Still, pasteurisation by immersion is another interesting option for cultivation of Oyster mushrooms. The pasteurised substrate could also be filled in small plastic bags, which keep away contaminants. Substrate formulations differ according to availability of materials. The formulas given show that quite a few agricultural wastes can be used.

21.7.1 Substrate formulations

The basic formula is:
- Sawdust (80-95%),
- Supplements (e.g. 5-20% rice bran, or 5-10% wheat bran, or 5% soy bean meal),
- CaCO₃ (1-2%).

Sawdust from many different broad-leaved trees can be used fresh. Sawdust from coniferous trees needs to be composted, similar to the sawdust in Shiitake cultivation in Taiwan. The water content has to be adjusted to around 60%. Squeeze to test the moisture content. The sawdust has to be moistened for at least one day to soften it and to enable it to absorb the required amount of water. The more supplements are used, the higher the yield; the risk of contamination, however, will increase too. Substrate formulas used in several parts of the world are the following:

From experiments on Filipino strain of *Pleurotus* in Peru:
- 40% sawdust originating from predominantly Catalhua (*Hura crepitans*), Cedro (*Cedrella spp.*) and Tornillo (*Cedrelino spp.*),
- 40% parts of corn straw,
- 20% part of wheat bran (all dry weight).

From commercial cultivation of *Pleurotus sajor-caju* in Peru:
- 47% of sawdust,
- 35% of coffee hulls,
- 17% wheat bran.

From *Pleurotus sajor-caju* cultivation in the Philippines:
- 78% sawdust,
- 11% rice bran, – 11% part Ipil-ipil meal.

From *Pleurotus sajor-caju* cultivation in the Philippines:
- composted cotton and rice straw in a ratio of 1 : 1 or 1 : 2.

Soak in water first. This mixture has to be put on heaps of 1.5 x 1.5 x 1.5 m and has to ferment for two to four days. Then add 20% rice bran and 1% lime. It is also possible to use only water hyacinth for composting, then add the same amounts of rice bran and lime.
From *Pleurotus* cultivation in the Philippines:
- 98% sawdust,
- 1% urea,
- 1% lime.
(Mix the three components well, then moisten and make a heap of not more than 1.5 m high and ferment for 30 to 40 days. Turn the heap every week, cover with plastic to avoid loss of water and energy. After fermenting, the sawdust mixture should not smell rancid or foul, and the sawdust will have softened. Add 10% rice bran before filling the bags. Sometimes composting can be performed in only seven to ten days, if fresh softwood sawdust is used).

From *Pleurotus* cultivation in India:
- 50% cotton waste (both short fibre and cotton seed hulls).
- 50% wheat straw in a 1:1 ratio (dry weight).
(Cotton waste only gives rather low yields. Soak the cotton waste in tap water for two days, then drain. Fill the bags and steam according to the volume of the bags for two to four hours, or steam in bulk at 90 °C for one hour).

From *Pleurotus* cultivation in Pakistan:
- corncobs and broken pith.
Other corn industry wastes like after shelling dust or after cleaning dust can also be used but gives lower yields. If supplemented they may be used as bulk substrate materials. Corncobs must be crushed in pieces of 1 to 2 cm, other types of corn industry waste only need to be wetted. The corncobs have to be soaked in water for two days. Other reported substrate materials are:
- Coconut shell: flakes usually have a size of about 1.4 x 0.6 cm. These absorb water easily. Use 60 litres of water for 40 kg of dry coconut shells. Adjust the pH to 6.5 by adding CaCO₃. When filling the bags, take care not to rupture them with the flakes. Use wide-mouthed glass or plastic bottles. (Until now, only experiments with moderate results are reported.)
- Coconut soil: some experiments indicate that coconut soil can be used when mixed with wheat straw (pasteurised) or sterilised with supplements. The high water holding capacity may clog the airflow, however.

### 21.7.2 Filling and heat treatment
Use one of the techniques discussed previously. Heat treatments should be adapted to local resources. The most secure sterilisation method employs expensive autoclaves, satisfactory results can be achieved even with the simplest means (an oil drum as sterilisation unit).

### 21.7.3 Spawning and spawn run
Usually grain or sawdust spawn is used in *Pleurotus* cultivation. Other substrate materials can also be used. The strain is most important. It should be vigorous and fruiting at the right (local ambient) temperature. The mycelium will cover the substrate in two to eight weeks, depending on the amount of spawn, the type of substrate and the employed strain. Optimal temperature for mycelial growth for all *Pleurotus* species (except *P. tuberregium*) is about 25 °C.
The following table gives a comparison of complete colonisation time and fruiting
temperatures of different *Pleurotus* strains in 1.2 kg bags in Taiwan, at 25 °C on standard sawdust/rice bran substrate, top spawned with approximately 10 g of sawdust spawn.

<table>
<thead>
<tr>
<th>Species</th>
<th>Colonisation time</th>
<th>Fruiting temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pleurotus sajor-caju</em>**</td>
<td>3 weeks</td>
<td>18 to 30 °C</td>
</tr>
<tr>
<td><em>Pleurotus cystidiosus</em></td>
<td>5 to 6 weeks</td>
<td>25 to 28 °C</td>
</tr>
<tr>
<td><em>Pleurotus ostreatus</em></td>
<td>4 to 5 weeks</td>
<td>10 to 20 °C</td>
</tr>
<tr>
<td><em>Pleurotus flabellatus</em></td>
<td>4 to 5 weeks</td>
<td>20 to 28 °C</td>
</tr>
<tr>
<td><em>Pleurotus eryngii</em></td>
<td>6 to 7 weeks</td>
<td>18 to 22 °C</td>
</tr>
<tr>
<td><em>Pleurotus pulmonarius</em></td>
<td>3 to 5 weeks</td>
<td>13 to 20 °C</td>
</tr>
<tr>
<td><em>Pleurotus cornucopine</em></td>
<td>4 to 5 weeks</td>
<td>15 to 25 °C</td>
</tr>
<tr>
<td><em>Pleurotus djamor</em> (<em>Pleurotus salmoneostreminus, Pleurotus incarnatus</em>)</td>
<td>4 to 5 weeks</td>
<td>18 to 22 °C</td>
</tr>
<tr>
<td><em>Pleurotus ostreatus var ‘colombianus’</em></td>
<td>3 weeks</td>
<td>12 to 20 °C</td>
</tr>
<tr>
<td><em>Pleurotus abalonus</em></td>
<td>4 to 5 weeks</td>
<td>25 to 30 °C</td>
</tr>
</tbody>
</table>

* *Pleurotus flabellatus* is a previously much used synonym of *P. djamor*; ** *Pleurotus sajor-caju* is a previously much (ab)used name for strains of *P. pulmonarius*.

21.7.4 Fruiting

The bags are opened when the mycelium has covered the substrate completely. Then conditions in the growing room have to change, or the bags are moved from the inoculation room to the fruiting room. The mushroom house needs ventilation channels that may also provide light. Oyster mushrooms are very sensitive to insufficient aeration and light. Required light (colour and intensity) depends on the strains. Some growers adhere to the rule of thumb that light should be sufficient to read a newspaper everywhere in the growing room. In general, the mushrooms themselves will show when the CO₂ level is too high or lighting is insufficient. A light requirement of ‘cool white’ fluorescent bulbs for two to four days is mentioned in the FAO (Food and Agricultural Organization) manual. In the complete absence of light, Oyster mushrooms will form no cap but only stipes. Then they will look like a coral.

Several techniques are employed in filling the mushroom house and making the bags ready for fruiting. A common practice is to make bamboo or wooden frames and stack the bags on them to form a wall of plastic bags. The plugs are taken off and the other side will be cut open as well. Another method is to slash each bag length-wise with a razor and hang them from the ceiling for fruiting. Take care not to cut too deep, otherwise you damage the mycelium. If small mushrooms are demanded, a larger surface should be exposed to the open air. The substrate will dry more rapidly then, however. It takes three to four days after opening the bags before the primordia will form.

The ambient temperature has to fit the chosen strain. Humidity at the time of primordia induction must be high: 90%. If the plastic is left around the bags and holes are punched in the plastic, it will be easier to maintain the right humidity near the holes. In fact a gradient of 100% to the RH of the growing room will form, thus providing optimal conditions somewhere along the gradient. If the complete upper surface is exposed to
the air and mushrooms only appear under the plastic at the sides, then the relative humidity of the air can easily become too low. When the fruit bodies start to develop, the humidity should be lowered to 80-85%. Otherwise the mushrooms will have difficulty in evaporating water and the nutrient-flow into the fruit bodies may slow down.
In some countries, like the Philippines and Nepal, growers take off all or almost all the plastic from the bags. This leads to fast drying out of the substrate and lower yields and should be discouraged. Another disadvantage of removing plastic is that more but smaller fruit bodies are formed, which increases the picking costs considerably.
If the temperature in the mushroom house is too high for the chosen strain, the house must be frequently misted and doors and windows should be opened to lower the temperature. Especially opening the doors at night helps in keeping the temperature down.
In five days (if the temperature is between 15 and 20 °C) or two to three days (at higher temperatures) the mushrooms are ready for harvesting. There is so much variability among strains and substrates used that it is difficult to give periods for fruiting. Typically, it will take about one week before new primordia are formed, but much depends on the local climatic conditions. Harvesting is performed by gently pulling or twisting the mushrooms from the substrate. Only very little substrate should be pulled out. Some growers in the Philippines scrape off some of the substrate to free it from small undeveloped primordia. These would easily become infected and have to be removed, but scraping the substrate will also retard the formation of new primordia. Rubbing the surface of the sawdust bags is a better method to remove the small and already dead fruit bodies without causing harm to the mycelium. Harvesting can continue as long as the mycelium remains white and firm.
In the Philippines typically four flushes of the locally cultivated Oyster mushroom are harvested with a yield around 20% of the wet weight of the substrate. When the substrate becomes soft and colourless, it is time to remove it from the house. Do not throw the spent substrate near the mushroom houses. Pests present in the used substrate can too easily spread to the fresh substrate.
21.7.5 *Pleurotus* cultivation in Thailand

Thai techniques differ slightly from those discussed above. In the northern part of Thailand, mainly two kinds of Oyster mushrooms are grown: *Pleurotus sajor-caju* (possibly a mislabeled strain of *P. pulmonarius*) in the winter and *Pleurotus ostreatus* var. *florida* in summer. In the hotter Bangkok region *Pleurotus djamor* is also cultivated, as well as *Pleurotus abalonus* and *P. cystidiosus*.

**Substrate materials:** Lumber sawdust can be used without fermenting, while rice straw is fermented for eight days.

**Fruiting:** For the first flush only the plug is removed, for the second and later flushes the plastic on top, and sometimes the bottom has to be removed. The market in Thailand demands big mushrooms. Less mushrooms, but bigger ones, will form when only the plug is removed. Some farms used to apply a casing soil on top of the bags. The loamy casing soil that was commonly used for this purpose became very expensive, because of the costs of transport. In contrast to *Agaricus* production, the casing soil is not essential for fruiting, but merely a means to create a favourable micro-climate.

21.8 *Ganoderma lucidum* and *G. tsugae*

The shiny *Ganoderma*’s can easily be cultivated, but the market is more difficult to handle. In literature on medicinal mushrooms, usually *Ganoderma lucidum* is mentioned. However, *Ganoderma lucidum* is probably a parasite on trees and could infect
trees in the vicinity of the farm. *Ganoderma tsugae* has a more saprophytic nature and will not harm neighbouring trees.

**Substrate:** The sawdust may originate from many more trees than for Shiitake, as *Ganoderma* is less selective. Stacking time can therefore be much shorter than for Shiitake. The preparation of the substrate is very similar to that of substrate for Shiitake cultivation on small sterilised plastic bags.

**Heat treatment:** Usually sterilisation is applied as only two flushes are harvested.

**Spawn run and fruiting:** Take the plug or cap from the mouth of the bag when the mycelium has grown half-way through the bags. Now the humidity has to be maintained at a high level of 85–95%. Fruiting temperature is quite high, 22-30 °C. Watering has to be carried out at least twice a day. The primordia will appear after about one week. It takes, however, at least two more months before they can be harvested. It is important to open the bags in time, when the mycelium has grown only half-way, because the mycelium needs increasingly more aeration.

The mushrooms are ready to pick when the fruit bodies become red and the white margin disappears. Some growers use a knife to cut the stalks, but it is also possible to break the mushrooms from the substrate. Right after picking, the next flush will follow. Harvest the mushrooms after about eight to ten weeks and do not leave them on the substrate under moist conditions. Store the harvested mushrooms in a cool, dry place.

**Pests and diseases:** The same contaminants may occur in all mushrooms grown on sterilised sawdust. These are *Neurospora* (‘Agent Orange’), *Trichoderma* and other moulds, which occur when the bags are contaminated due to breakage or incorrect spawning. Mites can also carry fungal contaminants to fresh substrates. The *Ganoderma* fruit bodies grow very slowly. If they are not picked in time, *Trichoderma* can also attack the mushrooms themselves.

**Post harvest:** The *Ganoderma* fruit bodies are sold at US$ 50-60 in Taiwan and around half that price in mainland China. The growing costs in Taiwan were said to be US$ 35-45. It is far more profitable for the farmers to make the medications from the mush-
room themselves and sell these to the Chinese medical profession all over the world, than to sell the mushrooms in a dried state.

**Preparation of Ganoderma extract and pills:** Boil the mushrooms in water. The medicinal extract is then dissolved in the water. It can be drunk as a very bitter tea. A second extraction can be carried out with ethanol. For pills the (water-)broth has to be centrifuged until a sticky fluid remains. The boiled fruiting bodies are dried and ground. The powder is used to absorb the sticky fluid. This product is sold in the form of pills or capsules. Other products include bitter soft drinks and tea bags.

### 21.8.1 Cultivation of Ganoderma lucidum in Malaysia

A number of products prepared from *Ganoderma* fruit bodies have been imported to Malaysia in the 80’s. Some farmers thus became interested in cultivating it. The published report mentions *Ganoderma lucidum*, the parasitic species, but since the different species of *Ganoderma* are difficult to distinguish and the taxonomy of this genus is confusing, it may concern a less parasitic species with a similar appearance.

**Substrate preparation:** Polypropylene bags measuring 10 x 22 cm are used. Substrate consisted of sawdust, 10% rice bran and 3% lime. These are mixed first and then filled in the plastic bags. A plastic ring is slipped through the open end of the bag and sealed with a cotton plug. The bags are covered with newspapers or plastic sheets. Each bag weighs around 700 g.

**Heat treatment:** The bags are sterilised under low pressure at 95 to 100 °C for five hours. Spawning and spawn run: The bags are cooled overnight and are then aseptically spawned with grain spawn in a laminar flow cabinet. The production cost of each bag is about US$ 0.12. The spawned bags are kept in the dark until the mycelium has colonised the substrate completely in three to four weeks.

**Mushroom house:** Refer to Mushroom farms for a description of the concrete mushroom house in Malaysia and its advantages over the commonly used attap barn.

**Fruiting:** The bags are placed on vertical racks to fruit. The plug will be removed and fruiting would commence in one to two weeks. Humidity is kept high all the time by spraying water at least twice a day (85 to 95%).

**Harvesting:** The mushrooms are ready to pick when the white margin disappears in two to three months. Bags will yield two or three flushes in total. The average yield of one bag is reported to be 28 g (dry) mushrooms.

**Post harvest:** The harvested mushrooms are dried in the sun for a few days, then they are packed in plastic bags and stored. Alternatively, some mushrooms are cut in slices for further drying.

*(Adapted from C.C. Tong and Z.C. Chen, in Mushroom Journal for the Tropics, 1990, Vol. 10)*

### 21.9 Flammulina velutipes (Velvet stem collybia or Gold needle mushroom)

This mushroom can be found all over the world in temperate areas, from Australia to China, Africa, Europe and America. It is sometimes called the Winter mushroom, because it usually fruits in winter at temperatures below 10 °C. *Flammulina* is collected in the wild in some areas in Europe and northern America. Only the cap is considered
suitable for consumption; the stalk is discarded. The wild specimens look quite different from the cultivated mushrooms, because of light deprivation and the collar around the culture bottles, which forces the cultivated mushrooms to form long stems. It is discussed in this book, because the marketing potential is considerable and the cultivation technique is similar to that of other wood-degrading fungi. Substantial commercial cultivation can only be found in Taiwan, China and Japan. Flammulina ranked fourth in the total worldwide production statistics in 1983/1984 and sixth in the 1991 statistics, after the White button mushroom, Oyster mushrooms, Shiitake, Rice straw mushrooms and Auricularia. The cultivated winter mushrooms have a very long stalk and a much lighter colour than the wild ones. The Asians favour this beautiful product and gave it the poetic name Gold needle mushroom. The stalk forms the main portion of the mushroom, and is artificially grown that long. It is not as tough as the stalk of the wild ones, and can therefore be eaten. It can be grown as a winter crop, or year-round if air conditioning is available.

Materials: Most Flammulina are cultivated in bottles, though plastic bags can also be used. The polypropylene bottles (800 to 1000 ml) usually contain 540 g wet substrate. These bottles can stand about ten heat treatments. Glass bottles were used in the past. These break more easily, but can be reused many times. The bottles are sealed by plastic caps, providing aeration through filter paper. These caps can also be reused, but the paper filter has to be checked for breakage regularly. Special machines have been developed to handle the bottles economically. Flammulina cultivation in Taiwan actually derived from spawn makers, who used such machines for filling spawn bottles. As the spawn production was concentrated in a three-month period each year, they started thinking on how to use the machines more efficiently around the year. If plastic bags are used, then about 500 g of substrate should be filled and the bags should be cylindrical.

Substrate materials: In nature Flammulina only grows on broad-leaved trees. In Japan and Taiwan conifer sawdust is used as well, but it has to be stacked to ferment for one year. Resins and phenols will leach out and the sawdust will decompose during this time, thus rendering a more suitable material. The conifer sawdust is then mixed with fresh hardwood sawdust in a 1:1 ratio. Mixtures of Cryptomeria japonica, Chamaecyparis obtusa, Pinus spp. (conifer sawdust) with Fagus crenata or Quercus serrata (hardwood sawdust) can be used.

The most commonly used supplement is rice bran, but other carbohydrate sources can also be used. Proteins and ammonium compounds have been recorded as suitable. Some trace elements like Fe, Zn, Mn, Cu, Co, Mo, Ca and thiamine are necessary for mycelial growth and fruit body formation.

Substrate formulation: conifer tree sawdust (decomposed, 6 to 12 months old) 45%; hard
wood sawdust (properly moistened for at least several days) 45%; rice bran 10 to 20%; water content adjusted to 58 to 60%; CaCO$_3$ 1 to 3%. If hardwood sawdust is readily available, *Flammulina* can be grown on a medium of 80 to 90% fresh hardwood sawdust, 10 to 20% rice bran, and some chalk to regulate the pH. No fermentation of the substrate is necessary then.

**Filling, holing and capping:** In Taiwan and Japan specially designed machines are available to fill and hole the bottles. The most advanced even cap fully automatically. Machines for spawn preparation can also be used. Make sure the substrate is compressed in the bottles or bags. The caps have to be pressed firmly on the bottles. No airflow is allowed through seams or cracks in bottle or cap.

**Sterilisation:** There will be quite a few microbes thriving in the old sawdust. *Flammulina* is not a strong competitor (unlike, for example, Oyster mushrooms), therefore the substrate has to be sterilised and not just steamed. Three hours steaming at 95 °C and one hour at 121 °C underpressure are sufficient to sterilise the bottles. The procedures are in fact very similar to spawn production. Notice that the cylindrical form of the bottles allows the steam to reach each container.

**Spawning:** The bottles can be spawned as soon as they have cooled down to 20 °C. The substrate is sterile and therefore very susceptible to contamination at this stage. Perform spawning preferably in a clean room. The large-scale producers use machines that lift the bottles out of the trays and inoculate them one by one. The cap is lifted off, a small piece of spawn is laid inside and the cap is immediately replaced. Take precautions to prevent from contamination (refer to Spawn production).

**Spawn run:** The mycelium will cover the substrate in the bottles in 25 to 30 days. The optimal temperature for mycelial growth is 22 to 25 °C, in commercial production the temperature in the densely packed spawn run rooms is kept somewhat lower: 18-20 °C. After spawn run, the bottles are placed in a different room for the induction of primordia. All subsequent rooms are kept dark to obtain a white or yellow mushroom.

**Primordia induction:** The cap is taken off and the old spawn is removed when the mycelium has colonised 90% of the substrate in the bottles. The surface is smoothened with a machine to get an even distribution of fruit bodies. The bottles are kept at 10-12 °C in the dark to promote primordia formation. The relative humidity should be 80-85%. The moisture level is very important: too dry will lead to uneven fruiting, too wet will cause amber-coloured drops that reduce the quality of the crop. Primordia are formed within 10 to 14 days.

**Controlling:** A temperature of 10-15 °C is efficient for primordia formation, but the temperature has to be lower during subsequent fruit body development to obtain a high-quality product with a firm texture. To this end, the temperature is lowered to 3-5 °C.
and some air flow is introduced within the room: 3-5 m/s. This will ensure firm, white and dry mushrooms. This stage (named ‘control stage’) lasts for five to seven days, until the stems reach a length of 2 cm.

**Growing:** Now the temperature has to be raised a little to 5-8 °C and the humidity should be 75-80%. This will stimulate development. A waxed paper or a plastic film is wrapped around the bottles to obtain long stems (Gold needles). If plastic bags are used as containers, then the upper part of the bag should be rolled upward to support the long stems.

**Harvesting:** When the stems are 13 to 14 cm long, the mushrooms can be picked. The plastic can be picked together with the mushrooms and will be sorted out later. The first flush usually brings 100 to 140 g per bottle. A second flush will take yet another 50 days, and yield only 60 to 80 g per bottle but the quality is less. If air conditioning is expensive, then it is more profitable to grow only one flush. Up to four flushes can be harvested from plastic bags if the quality is less important. The quality is mostly determined by humidity and temperature, and by the number of fruit bodies growing in a bundle. This number declines as flushes proceed.

**Pests and diseases:** *Cladobotryum variospermum* (a cobweb mould) is at times very damaging to the cultivation of *Flammulina*. The pesticide PY-101 (panmush) can control this and other harmful fungi. Other regular contaminants are the ones to be found in plastic bag cultivation of all other wood-degrading fungi: *Trichoderma* spp., *Penicillium* (if bags with plugs are used) and *Neurospora*.

**Post-harvest:** The bundles are cleaned by cutting off the part which was attached to the sawdust substrate. Most *Flammulina* are sold fresh, some are canned. The mushrooms are remarkably robust, which is caused by the low temperature at which they grow; they are sometimes handled rather rough without visible damage to the fruit bodies.

### 21.10 Bag cultivation of Tremella fuciformis (‘Silver ear’)

Pure cultures of *Tremella* gave low yields when inoculated on sawdust substrate. Most *Tremella* species are parasitic and have limited saprophytic potential. Therefore they need another organism to degrade the wood. Wood log growers noticed that the *Tremella* needs a ‘biological factor’ as they called it, for increased yields. Chinese scientists managed to isolate this ‘friend of the mycelium’ and found that it belonged to the Ascomycete genus *Hypoxylon*. The particular species was identified as *Hypoxylon archeri*. A mixed culture technique from mainland China gives the best yields and bigger fruit bodies. In wood log cultivation it is only *Tremella* that has to be inoculated.
Hypoxylon will enter by germinating spores at the surface of the bark. When growing in closed plastic bags, Hypoxylon has to be inoculated together with the Tremella culture.

**Pure culture techniques:** Pure cultures of both *Tremella fuciformis* and *Hypoxylon archeri* are needed for plastic bag cultivation. It is not simple to obtain a pure tissue culture of *Tremella* from the fruit bodies. These are gelatinous, and many bacteria and spores of other fungi are present on them. The fruit bodies have to be cleaned with alcohol very well. One recommendation is to use the non-gelatinous tissue from under the base of the fruit body. The mycelium from under the base, however, is often slow growing and not vigorous. The usual method in mainland China is to isolate mycelium from wood logs. The bark and the base of the mushroom are removed from the wood and the wood is cleaned with 70% alcohol. The wood is sliced on the spot where the mushroom was attached and a small piece of wood is taken from the inside of this spot. This is inoculated on agar and carefully observed. It is important to combine feather-like mycelium and *Tremella* that grow on the same wood logs, because the feather-like mycelium is specific for wood species and *Tremella* strains.

The colour of *Tremella* mycelium is between white and yellow. It has erect aerial hyphae, and both surface and submerged mycelium. The diameter of hyphae is 1.5 to 3 μm, and clamp connections are present in the dikaryotic mycelium. The mycelium grows rather slow in comparison to other edible fungi. The feather-like mycelium is white and has long, thin main hyphae with side branches. The culture medium will turn from yellow (PDA medium) to black or very dark green. The different strains of *Tremella* have specific ‘friends of the mycelium’. Both strains have to be isolated from the same wood log.

**Spawn production:** Since two cultures are involved, one of which grows much faster than the other, spawn production is slightly different from the other mushrooms. The mother culture spawn has to be prepared in the following way:

Inoculate *Tremella* on a number of test tubes at 25 °C.

When the culture has a diameter of 1 cm, the *Hypoxylon* is added.

As soon as the two mycelia have grown together, the culture is ready to inoculate the spawn.

To produce substrate for spawn production and final production, use:

- sawdust of selected broad-leaved trees 78%
- rice bran 20%
- CaCO₃ 2%

or:

- bagasse (dry) 79%
- rice bran 19%
- soy bean powder 1%
- CaCO₃ 1%

Mix well and moisten until the optimal moisture content of 62-68% is reached. Fill the bags and sterilise. Inoculate the culture. The spawn has to be used within 45 days from full mycelial growth, otherwise it degenerates.

**The final substrate** consists of the same materials. It may be somewhat moister than the spawn (65 to 70%), because a higher moisture content will promote formation of
yeast-like conidia by the *Hypoxylon* that penetrate deeper into the substrate. A drier substrate is more suitable for *Tremella* than for the feather-like mycelium. The substrate is packed in different bags: 50 cm length, 10 cm diameter. Ropes on each end keep the bag together.

**Heat treatment:** The substrate is sterilised for six to eight hours and allowed to cool down to ambient temperatures.

**Inoculation:** The spawn is inserted by cutting four to six holes in the bag and putting 1 cm³ pieces of spawn inside the sawdust mixture. The holes have a diameter of 1 cm and are 0.5 to 1.2 cm deep.

**Spawn run:** The inoculated bags are placed in an incubation room at a temperature of 28-30 °C. When the mycelium is developing well, the bags can be transferred to a somewhat cooler place: 25-28 °C. This is the optimal temperature range for vegetative growth.

**Fruiting:** The mycelium will cover the substrate in about one month. Then the bags are transferred to a mushroom house. The plastic is carefully removed. Some ventilation is necessary. There has to be very little light in the mushroom house. If one can barely see, it is just enough. The humidity should be kept constant at 85 to 90%. The primordia will form within seven to ten days. It takes the mushrooms an additional five days to mature. The yield is reported to be 35 to 50 g dry mushroom weight per bag. The bags can contain 15 litres of substrate, approximately 12 kg. The Silver ear is exclusively sold dried. It may lose up to 92% of its weight on drying.

21.10.1 *Cultivation of* *Tremella on cotton seed hulls in Gugien, China*

Cotton seed hulls are agricultural wastes that can readily be utilised for mushroom production. About 15% of the farmers in Gugien is engaged in *Tremella* production. The production in 1985 was more than 2000 MT, with a value of more than US$ 5 million. Since then, the prices have fallen somewhat, but *Tremella* remains one of the cash crops in Gugien county. The following substrates give a higher yield than the common sawdust substrates:

- cotton seed hulls 100 kg
- wheat bran 20 to 25 kg
- gypsum 4 kg
- magnesium sulphate 0.5 kg
- water 100 to 120 litres

or:

- cotton seed hulls 50 kg
- corn cobs 50 kg
- wheat bran 25 kg
- gypsum 4 kg
- urea 0.4 kg
- water 100 to 120 litres

Magnesium sulphate and urea should first be dissolved in water. The cotton seed hulls and corn cobs have to be wetted beforehand with some of the water. Then mix in the wheat bran and gypsum. Add the rest of the water and mix thoroughly. The pH should be between 5.8 and 6.2.
Filling: The substrate has to be filled in bags right after preparing. Otherwise it might start to ferment and metabolites would be formed, which inhibit the growth of the desired fungi. From the beginning of mixing to the end of filling should not take more than six hours. The plastic bags, used in Göttingen, are 50 to 55 cm long and have a diameter of 12 cm (these are often referred to as ‘artificial logs’). These bags are cut out of plastic tubes. One end is sealed with a rope, while the other end is sealed after filling. The substrate is filled tightly, commonly by using horizontal bag-filling machines.

Holes are made in the bag for later spawning. (Some growers will make the holes before sterilisation, others after.) Four or five holes per bag, with a diameter of 15 mm and 2 cm deep, are sealed with medical adhesive tape squares (33 x 33 mm).

Heat treatment: The bags are semi-sterilised at a temperature of just below 100 °C for eight to ten hours.

Spawning: The tape squares are taken off as soon as the substrate has cooled down to below 28 °C. A piece of spawn is put inside, then the tape is immediately put back. If no holes were made prior to the sterilisation, the bags are wiped with 70% alcohol, holes are made and the spawn is put inside. Tape squares are immediately put over the cut to prevent contamination.

Spawn run: The spawned bags should be kept at a temperature of 28-30 °C for one to four days in an incubation room. They may be stacked during this period. When the mycelium is seen growing into the substrate after five to ten days, the temperature should be lowered to 26 °C and the relative humidity should be lowered to below 70%. The bags can now be placed on beds, with just a few centimetres between them.

Fruiting: After 10 days the mycelium can be seen colonising the substrate radially. Now the tape should be loosened, but still covering the opening. Some air exchange is possible then. The room temperature should be lowered to between 20 and 25 °C, and the relative humidity raised to about 82%. The windows or doors should be opened for 15-20 minutes, four or five times a day. Yellow-red drops will appear at the cuts. They indicate that fruit bodies will be formed.

Usually it takes only 16 days before the primordia appear. They are white and a few millimetres in size. Mushrooms will appear 18 days after inoculation. The tape squares should now be removed entirely. After two days the cuts should be enlarged by cutting 1 cm more from the plastic. This facilitates the growth of the fruit bodies and permits better aeration. The temperature should be 23 to 25 °C. If the temperature is higher, newspapers covering the bags and foam mats placed in the room can be sprinkled with water to lower the temperature. Do not water the artificial logs, as this will result in lower-quality fruit bodies.

Harvesting: The mushrooms can normally be harvested 35-40 days after spawning. Their diameter is 8 to 12 cm. They should be harvested when:

- the ‘ears’ are completely open,
- the colour changes from translucent to white,
- the outer part of the fruit body starts to soften.

If they are harvested too early, the yield will be low. If they are harvested too late, the quality is affected. The colour of the base may have turned dark and start to decay. Watering should be stopped one day before harvesting. It is best to harvest the mush-
rooms on a dry day. If it rains harvesting can be delayed by one more day. The mushrooms are cut from the bags with a knife. Yellowish tissue should be removed from the mushrooms, but take care not to cut too deep, or else the petals will fall of the fruit body.

**Climatic conditions:**
- Temperature between 20 and 27 °C.
- A relative humidity of 85 to 95%.
- Only little light is necessary. The farms in Taiwan are almost completely dark.

**Yields:** 100 kg of cotton seed hulls will produce 160 to 180 kg of fresh mushrooms.

### 21.11 Hericium erinaceus: Monkey head mushroom

The typical feature of the Monkey head mushroom is the spines that cover the rudimentary stipe on all sides. The fruit body ranges in size from 5 to 30 cm, has white spores and is usually white, with a tendency to turn yellow. It is known to be edible both in the Orient and in Europe. Collectors in Europe will seldom find it, as it has become relatively rare and is presently mentioned in a number of ‘red lists’ of threatened fungi. Scientists at the Shanghai Agricultural Academy of Science were the first to domesticate it. Although it is a famous edible mushroom in China, few Taiwan people have heard about the Monkey head mushroom. Its cultivation is relatively easy and a large variety of substrate materials is suitable. Marketing this product will often be less easy, as many people are unfamiliar with it. For medicinal use, it is best to use fresh mushrooms. Refer to chapter 1, Beneficial aspects of mushrooms, for medical aspects.

**Substrate materials:** Bulk materials like sawdust, sugarcane, bagasse, corncobs, residues from alcohol distilleries, cotton seed hulls and chopped paddy straw can be used. As supplements wheat or rice bran and sucrose are used. Gypsum is added to stabilise the pH and improve the structure. A commonly used formula for both spawn and final production is:
- sawdust 78 kg
- rice bran 20 kg
- sucrose 1 kg
- gypsum 1 kg

The water content should be 65%.

**Heat treatment:** Some method of sterilisation is applied, for example, three hours at 95-98 °C under ambient pressure and one hour at 121 °C under high pressure for a 1 kg bag. Smaller bags can be sterilised in a shorter period.
Spawning and spawn run: Both sawdust and grain spawn can be used. For a 1 kg bag, 10 g of sawdust spawn is sufficient. The spawn run is quite fast. Bottles of 500 ml will be colonised in 20 days, 1.2 kg bags in Taiwan were colonised in four to six weeks. The optimal temperature during the spawn run is between 20 to 30 °C. Mycelial growth will stop above 35 °C.  

Fruiting: After the substrate is colonised, the temperature in the room should be lowered to stimulate fruiting. Fruiting temperatures are somewhat strain dependent. In Taiwan, for example, one strain would fruit at 20-28 °C, another at 15-25 °C. No other shock is required to start the formation of pinheads. Little light is required and strong light even inhibits primordia formation. As soon as the first primordia form, the plug should be removed from the bottles. Humidity should be kept high (85 to 90%). The pinheads will grow into mature fruit bodies in about ten days. The mushrooms should be picked when they are still white and start to discharge spores. Yellow fruit bodies have a lower market value. The stalk should be removed gently and no pieces of the stalk should be left in the bottles/bags, since they will rot. Take care not to break the spines. Bags of 1.2 kg need two to three weeks before they start to give the next flush. Smaller bottles will give new primordia in eight to ten days. The yield of a 1.2 kg bag in Taiwan is 20 g of dry mushrooms, approximately 200 g fresh mushrooms.  

Post harvest: Mushrooms for the fresh market have to be handled with care to avoid bruising. Drying is possible, too.  

Another conservation method is to boil the mushrooms and preserve them in brine (a very salty solution).  

Pests and diseases: The usual contaminants of sterilised bag cultivation are encountered in Hericium growing, too. In addition, irregularly formed fruit bodies may appear, in which the spines are very thick, irregular or absent, or show an abnormal colour. Insufficient control of factors like temperature and a too high CO₂ concentration have been found to lead to these irregularities.  

21.11.1 Case study: Monkey head mushroom farm near Beijing, China  
Near the famous Ming dynasty tombs north of Beijing a mushroom growing unit was established in 1989 with support from Prof. Shen of the Chinese Academy of Agricultural Sciences. Twelve workers, one a graduate in agriculture, produce four kinds of mushrooms: Volvariella and Ganoderma in summer, Flammulina in winter and Hericium erinaceus year-round. The spawn is also produced at this farm, using the common Chinese inoculating cabinet, in wide-mouthed bottles on sawdust. This case study is concerned with the cultivation of the Monkey head mushroom, Hericium erinaceus.
Substrate: The substrate for the Monkey heads consists of alcohol distillation waste. After fermentation of grains a residue is left that proved to be very suitable for this mushroom. The residue has to dry first and is then supplemented with 15% rice bran and wetted to a water content of about 70%. The latter is tested by the squeeze test. The substrate is thoroughly mixed by two subsequent machines, of which the last is used for filling the bags (with a screw jack below the mixing device to force the substrate out). The pH of the substrate is rather low: 4.5 to 5.5.

Filling: The bags are made out of a plastic tube, cut in appropriate lengths. The ends are tightened with a rope, one before and one after filling the bags with 250 g wet substrate each. Then the bags are put in an autoclave for two hours (because of the small bag size, two hours is sufficient).

Spawning and spawn run: After cooling down the bags are spawned by three people: two of them opening and closing the bags after the spawn has been put in, and one putting the spawn in the bags with a spoon. Using this method, 1000 bags can be inoculated per day. To avoid contamination the spoon is flame-sterilised after each inoculation and the interior of the mouth of the spawn bottles was kept absolutely clean. One bottle of 250 ml was enough to spawn 50 bags. Spawn run takes place in a separate room at 25 °C for 15 days. The small size of the bags allows a fast colonisation of the substrate. It must be noted that Flammulina and Ganoderma are cultivated in larger bags of 500 g and Volvariella on plastic dishes on the same farm.

Fruiting: The bags are then moved to the cropping room kept at a very high humidity (90 to 95%) and opened to permit the mushrooms to grow out. In 10 to 14 days after opening the bags, the first flush of Monkey heads could be harvested. The second flush would follow the first one immediately.

Yield: The total yield is up to 70 g per bag on average base (28%). Because of the small size of the bags, the mushrooms are also rather small. The price of uniformly-sized small Monkey head mushrooms is higher than of bigger ones. Most are sold fresh as they will lose nutritional value after drying, according to Prof. Shen. Dry Monkey head mushrooms typically weigh 9-10% of fresh ones.

21.12 Auricularia cultivation on sterilised substrate

Wood ears (Auricularia spp.) are commonly cultivated in the Far East, and exported all over the world, even though taste and appearance may hamper its popularity for Western people. Plastic bag cultivation is gaining popularity, due to the scarceness of suitable logs and the ease with which different species of Auricularia can be cultivated on sawdust. Spreading of the technology can be expected in the near future.
Three species are commonly cultivated: *Auricularia polytricha* (the Woolly wood ear), *Auricularia fuscouscinea* and *Auricularia auricula-judae*. There are many more edible *Auricularia* species, of which *Auricularia delicata*, *Auricularia tennis*, and *Auricularia emini* have been reported from the tropics.

**Substrate preparation:** Sawdust is fermented for a longer time than for Shiitake, depending on the type of sawdust. To enrich the substrate, 2 to 20% rice bran is added. The preparation of the bags is the same as for Shiitake, and the same equipment (mixers, filling machines) will be useful. The utilised bags have a length of 15 to 18 cm and a diameter of 10 to 12 cm.

**Heat treatment:** The filled bags are steamed at around 95 °C for 90 minutes.

**Spawning and spawn run:** Usually sawdust spawn is employed. 10 ml of spawn per bag is sufficient. During spawn run, the temperature should be 25 to 28 °C. The mycelium will cover the substrate in about four weeks.

**Fruiting:** Cuts in the bags are made so the mushrooms can emerge. Sometimes both ends of the bags are cut off. Take care in handling the bags, because the texture of the substrate will stay soft even after the mycelium has colonised it. The mycelium is very sensitive to breakage (similar to the mycelium of many other wood-degrading fungi).

Only little light should be present in the mushroom house. Three to four flushes can be expected. Per bag 300 to 500 g mushrooms can be harvested.

21.12.1 Case study: *Auricularia* (Hirneola) *auricula-judae*

*cultivation on cotton seed hulls in Hebei province, China*

The province of Hebei (around Beijing) is one of the main producers of the ‘Black wood ear’ *Auricularia auricula-judae*. The strains of this species differ in yields. Some perform better on wood logs, others better on a sawdust- or cotton seed hull-based substrate.

The spawn substrate contains the following ingredients (weight percentages):

- wood chips 70 kg
- cotton seed hulls 10 kg
- wheat bran 18 kg
- cane sugar 1 kg
- gypsum 1 kg

The final substrate contains (weight percentages):

- cotton seed hulls 93 kg
- wheat bran 5 kg
- cane sugar 1 kg
- gypsum 1 kg

Mix the ingredients thoroughly, then add water in a ratio of 1:1.3 or 1:1.4.
Plastic bags: Flat bags are used of 35 x 17 cm, and a thickness of 0.05 to 0.06 mm. The bags are two-thirds filled, which is approximately 250 to 300 g dry material per bag. The material is slightly compressed by hand, and a stick is inserted to obtain an aeration hole with a 2 cm diameter. Then a plastic ring with a diameter of 3.5 cm and a length of 3 cm is slipped around the opening of the bag and a cotton plug is added in the ring. Wrap the neck of the bag with waterproof paper.

Heat treatment: Sterilise under a pressure of 1.4 kg/cm² for one hour.

Spawning and spawn run: Open the bags by taking the plug off and add a small quantity of spawn (1 to 2%) when the bags have cooled down. This operation should preferably be performed in a clean room or inoculation cabinet. Then incubate at 24-26 °C for 30 days, after which the mycelium should have colonised the substrate. The light level should stay below 500 lux during the spawn run, otherwise primordia will form prematurely.

Fruiting: Give the bags a few days of diffuse sunlight to promote primordia formation, then remove the plastic ring and the cotton plug. The open ends are closed by wrapping the bags tightly. Cut eight holes with a diameter of 3 cm in the bags, at regular distances. A larger number of holes will result in too small fruit bodies (at least with this size of bag). Too many small mushrooms also decreases the yield. Hang the bags with an S-shaped iron wire in a mushroom house and keep the temperature between 18 and 22 °C, maintain a relative humidity of more than 85%, and allow 2000 lux of diffuse daylight. If the temperature is 12-15 °C primordia will form, but they cannot mature. Between 18 and 22 °C the mushrooms grow thicker. Above 25 °C the mushrooms will be thinner and larger, but will often develop atypical fruit bodies.

Picking: Care has to be taken to pick only the ripe mushrooms and not to damage the small primordia. Around 250 g of fresh mushrooms can be harvested per bag of around 700 g initial wet weight. Four flushes can be expected, of which the second and third are biggest.

Yield: About 8.5 kg dry *Auricularia* per 100 kg dry seed hulls can be harvested. Light influences the colour, lustre and thickness of the mushrooms. If the light intensity is below 500 lux, the mushrooms will be yellowish white. At 750 lux, the colour will become black-brown, the thickness of the mushroom will become very small, 0.14 cm. At 3500 lux they will become shiny black, the thickness of the mushroom will be 0.18 cm. (From: Mushroom Journal for the Tropics, 1988, Vol. 8, No. 4)

21.12.2 *Auricularia polytricha* cultivation in the Philippines

The market for the smaller *Auricularia auricula-judae* (the Black wood ear) is better than that for *A. polytricha*. The temperature range of *A. polytricha*, however, is more suited for the Philippines. Only in relatively cooler areas, like Baguio, can *A. auricula-judae* be grown.

Substrate preparation (weight percentages):
- sun-dried white or light brown sawdust 78 kg
- fine rice bran (first class) 20 kg
- sugar 1 kg
- CaCO₃ 1 kg

The sawdust has to be dried until it has a moisture content of 15-18%. The rice bran has
to be sifted to break bigger particles into small pieces. The bigger particles would be the first to become contaminated. Weigh the substrate ingredients and mix CaCO₃, rice bran and sugar well before mixing them with the sawdust. The weight of the dry substrate material should be at least 100 kg, but may be increased proportionally. Add water slowly until the moisture content is 65-75% (check with squeeze test).

**Fermentation:** Pile the substrate in pyramids and cover with plastic to keep in its moisture. Let the heap ferment for five days and turn the heap on the third day. Sieve through 1.5 mm mesh to remove bigger particles and to break the clumps that have formed during fermentation. The bigger particles might damage the plastic bags.

**Filling:** Pack about 1 kg per 12 x 30 cm bag and at the ring and plug.

**Heat treatment:** Sterilise the filled bags for 1.5 hours at 121 °C or semi-sterilise for 10 hours at a temperature just below 100 °C.

**Spawning and spawn run:** Use one 500 ml bottle of spawn for 50 bags. Spawn run takes about one month at 25 to 30 °C. Now place the bags in rows, as shown in the figure.

*Auricularia polytricha* tolerates higher temperatures than *Hericium erinaceus* or *Lentinula edodes*. *Auricularia* can thus be cultivated on shelves, but *Lentinula* has to be kept at a lower, cooler level in Taiwan and the Philippines.

A mushroom house (5 m wide, 12 m long and 4 m high) can hold 2640 bags. Each row has 55 bags per layer. Four layers per row can be placed. Four rows with 220 bags each can hold 880 bags. Three shelves may hold 2640 bags.

**Fruiting:** The optimum fruiting temperature of *Auricularia polytricha* is 23 to 28 °C. To promote primordia formation, the cotton plugs should be removed from the bags and holes cut in the bottom. Try to keep the temperature below 30 °C by spraying water and opening the mushroom house at night. The primordia will develop in fruiting bodies in seven to ten days. Twist the fruit bodies with the hands from the substrate, leaving no pieces left.
22 Lentinula edodes (Shiitake) cultivation on sterilised substrates

22.1 Introduction

As Shiitake cultivation has developed much, a special chapter is devoted in this third edition to their cultivation on substrates in different parts of the world. The cultivation of Shiitake in sterilised plastic bags is rapidly gaining popularity. Mushrooms can be harvested faster and the yield is higher compared to growing on wood logs. However, the quality is sometimes lower than that of Shiitake from wood logs. Filling the bags and sterilising them is labour-intensive and energy-consuming. The big advantage is that many types of organic waste can be used. The method is practised in Taiwan, mainland China, Singapore, New Zealand, the USA, Finland, France, The Netherlands, Germany, the Philippines, Sri Lanka and Thailand. In some countries sawdust spawn is commonly available, and the preparation of the final substrate is quite similar to spawn preparation. In Europe no sawdust spawn is available and grain is the usual substrate for spawn makers.

The incubation times differ from country to country. If the substrate has been compressed and only little spawn has been used, the incubation period is three to four months. If the substrate is loosely filled in bags and spawned with 2 to 5% grain spawn, which is mixed through the substrate, the incubation will take only one to two months. Typical practices are given in the case studies. Some general aspects are discussed first.

**Substrate preparation:** The most commonly used substrate formulations are:

| Sawdust, 3 to 4% rice bran, 1% corn meal or wheat bran, 1% CaCO₂ | Sawdust, 10 to 25% corn waste, 1 to 2% CaCO₂ |

Fresh sawdust from the trees rating excellent and very good (refer to the list in the section on wood log cultivation) can be used without prior fermentation. Sawdust of other trees can also be used, but then the sawdust has to ferment for a number of months. Much depends on the kind of sawdust used. How to perform the fermentation is explained in the case study on Taiwan.

The C/N ratio of the substrate must be around 25 at inoculation, later it will rise to 30 as more nitrogen than carbon is used by the mycelium. A very high carbon content may result in a shorter spawn run period, but the mycelium will be less dense and the mushrooms will have a lower quality.

When the sawdust is moist enough it has to be mixed with the supplements and the chalk. Mix the chalk first with the rice bran, as it will be easier to get an even distribu-
tion. The moisture content (usually between 56-63% moisture at the time of preparation, apply the squeeze test) of the substrate increases during incubation; take care to compare the right data (e.g. always measure before sterilisation). Some reports indicate that a high water holding capacity of the substrate combined with good aeration will give better results. If tea leaves are mixed with the above mentioned substrate, substantially higher yields have been reported. If the substrate is too moist, the air flow will be clogged and even a long spawn run period will not deliver a high quality substrate. If water collects at the bottom of the bags, the substrate is certainly too wet. Some farmers prefer relatively dry substrate (56%).

**Filling and sterilisation:** Several types of bags are in use. Check the previous chapter for a discussion on the pros and cons of each bag type. Some plastics will produce substances which inhibit mycelial growth when heated. These should therefore not be used. Fill the bags with the substrate according to one of the techniques discussed in the previous chapter.

### 22.1.1 Heat treatment

In Taiwan steaming at a temperature of 96-98 °C showed better results than sterilisation under pressure, but both methods can be used. If only one or two flushes are expected than it is better to sterilise the substrate under pressure. Steaming under low pressure is appropriate if more flushes are expected. Take care cotton plugs stay dry, otherwise a common contaminant called *Neurospora* (‘Agent Orange’) will start to develop orange fruit bodies on top of the bags. Ample space between the crates and bags should provide sufficient steam circulation. There should be an air outlet, too.

### 22.1.2 Spawning

Let the bags cool down and spawn them the next day. 10 g of sawdust spawn is sufficient to spawn one bag of 1.2 kg, so one bottle of 550 ml is sufficient for about 50 bags. Grain spawn is best introduced at 2 to 5%. The strain for sawdust cultivation should be carefully checked. Some serious losses in yield have occurred because spawn makers would sell new strains that produced well on wood logs, but gave very low yields on sawdust. Some strains will perform better on a substrate of corn cobs, others better on a sawdust substrate.

Take the usual precautions when spawning. Use the measures for spawn making if extreme levels of contamination occur. Not more than 5% of the bags should become contaminated. Try to determine when contamination occurs and where it starts. If con-
tamination is close to the top of the substrate, the contaminants are likely to have entered after filling and sealing. If the contamination occurs at the bottom, check the bottom for bad bottom seal. If they occur all over the substrate, the substrate may suffer from insufficient sterilisation or the spawn was not pure enough.

22.1.3 Spawn run and mycelial development
It will take one to four months for the mycelium to colonise the substrate and mature, depending on the type and the amount of spawn (refer to the case studies). Some light should be present during at least the end of the spawn run for fruiting. Some growers have completely dark spawn run rooms; they should illuminate with a day/night cycle at the end of spawn run. Problems can be avoided if a little light is present during all stages of the growth. All strains show optimal mycelial growth at 25 °C. The temperature inside the bags is usually a few or even ten degrees higher than the ambient room temperature. If many bags are packed in a room, extensive cooling may be necessary. Five different stages of the mycelial growth of *Lentinula* can be distinguished for all strains. The first phase is the normal spawn run as it occurs in all fungi. When the substrate has turned white, it is not ready to fruit, however. It has to mature first. The following stages can be recognised:

1. Mycelial running: The spawn will give rise to white hyphae, which produce enzymes to degrade complex substances like cellulose, lignin and hemicellulose into smaller fragments. The fragments will be consumed at later stages of mycelial growth. As soon as the complete substrate is colonised, the next phase is entered.
2. Mycelial coat formation: A thick, white mycelial sheet will develop on the surface of the substrate. This will occur in two to four weeks after inoculation. If the CO₂ level is high, the sheet will be thicker.
3. Mycelial bump formation: Bumps are clumps of mycelium, commonly formed on the surface by most strains. These bumps can turn into primordia at a later stage, but most of them abort. Bump formation is promoted by fluctuating temperatures and a high CO₂ level. Lower the CO₂ level if many bumps are formed by slitting the plastic open. The bumps may form a problem at a later stage of the cultivation. They can easily become contaminated by green moulds.
4. Pigmentation phase: Some aeration should be provided when the bumps have formed. The mycelium will turn reddish-brown. If the plugs are removed entirely, however, the substrate may dry out too much.
5. Coat hardening phase: Remove the plastic when bags have partially (half or for one third) turned brown. The coat will gradually become hard. The outside of the substrate should be hard, the inside should be softer and more moist. The moisture content of the substrate core can be as high as 80%. If the outside is relatively wet, contaminants will have easy access to the substrate. The brown hard skin acts like the bark in wood log production: it protects against contaminants and keeps the humidity in the substrate. It is important to regulate climate conditions to obtain a mycelial coat of the right thickness.

22.1.4 Fruiting
The same factors that promote fruiting in Shiitake cultivation on wood logs are used in manipulating the flushes in plastic bag cultivation. These are:
- temperature fluctuation,
- high humidity,
- soaking,
- removal of CO₂,
- physical shocks.

Different stages of mycelial growth of Shiitake. From left to right: mycelial running, bump formation, coat hardening and pigmentation (courtesy TARI).


<table>
<thead>
<tr>
<th>Stage/activity</th>
<th>Days (°C)</th>
<th>Temperature intensity</th>
<th>Light humidity</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation</td>
<td>30-120</td>
<td>20-30</td>
<td>none</td>
<td>65-70%</td>
</tr>
<tr>
<td>induction of fruit bodies</td>
<td>2-4</td>
<td>10-20¹</td>
<td>500-1000</td>
<td>85-96%</td>
</tr>
<tr>
<td>fruiting</td>
<td>7-14</td>
<td>12-18¹</td>
<td>500-1000</td>
<td>60-80%</td>
</tr>
<tr>
<td>rest</td>
<td>7-21</td>
<td>20-30</td>
<td>none</td>
<td>65-70%²</td>
</tr>
<tr>
<td>induction of fruit bodies</td>
<td>2-4</td>
<td>10-20</td>
<td>500-1000</td>
<td>85-95%</td>
</tr>
</tbody>
</table>

¹ The temperature range for fruiting is strain-dependent; ² A dry period after harvesting will prevent contaminants from spoiling the substrate at the scars where the mushrooms have been picked. The artificial logs may be given a water bath to restore a high moisture content of the substrate. The substrate blocks do not need to be watered during incubation.

Keep ambient humidity low (60 to 70%) to prevent contamination. If the plastic is removed too early or too late, yields will be affected. Deformed fruit bodies during the first flush are a sign of a too short spawn run or a too high CO₂ level during incubation. Strains differ in mycelial growth rate.
For one strain 60 days is sufficient to mature, whereas another strain would yield many deformed mushrooms after the same period of maturing. If the temperatures are rather low and a suitable strain has been used, high-quality ‘donko’ mushrooms can be harvested. If humidity is also relatively low (60 to 70%), then cracks may appear in the caps of the most expensive quality in the Far East, which is called ‘flower winter mushroom’ (hua dong gu) in Chinese.

**Harvesting:** Take the mushrooms by their stalks and break them from the substrate. Do not tear them from the surface, otherwise too much substrate will be torn loose. Harvest the mushrooms at an early stage, according to the quality requested by the buyers. Do not water the scars left for three or four days. White mycelium growing on the scar is a sign of recovery. Completely opened mushrooms have a much lower value in Asia; in Europe buyers are less critical. Normal yields are 15 to 35% of the wet substrate weight.

Picking and sorting costs can be high as the picking rate is often limited to 15 kg per hour. According to some American mushroom growers, a vibrating mushroom sorter did not give good results as the fresh mushrooms became bruised and released a sticky fluid. If Shiitake are grown under rather dry circumstances during the last days of their maturation, the dam-
age will be less. Shiitake cultivation has not spread as much as Agaricus; even there mushrooms for the fresh market are all manually picked. The picking process is completely different however, as the button mushrooms grow on flat beds. Some of the equipment which has been developed to ease picking in the button mushroom industry may be used however, such as height-adjustable picking platforms, conveyor belts to improve logistics and automatic cutters which allow the pickers to pick with both hands (beware of cutting fingers!). Possibly a conveyor belt with holes could do some pre-sorting, but manual control will still be needed as Shiitake are often odd-shaped compared to the round Agaricus.

Another strategy would be to choose for strains with relatively heavy fruit bodies and adapt culture techniques to obtain fewer but heavier mushrooms. Much depends of course on the local market situation and prices for the different grades. Even if smaller mushrooms fetch a higher price, the profitability of larger mushrooms can be better because the picking costs are significantly lower.

**Pests and diseases:** Green moulds are the most common contaminants at the moment of spawning. They will also grow if there are any cracks in the bags. The use of ‘panmush’, a Japanese fungicide, may control Trichoderma, but most important are hygienic measures. The substrate should be kept dry in between the flushes; moist conditions promote contamination, and contamination attracts flies which spread contamination even further. Normally the Shiitake mycelium will form a crust below the Trichoderma colony; it is then best to spray the green moulds after the harvest with a strong flush of water. However, if the substrate is too soft (because of too high moisture content), the block would be damaged; it is more difficult to obtain a good second flush then.

### 22.2 Introduction to Shiitake cultivation in the USA

*This paragraph is a contribution of Lou Hsu, Unicorn, Texas, USA*

There are about 250 serious exotic mushroom growers in the USA, most of whom employ the traditional method of sterilising plastic bags in autoclaves of some kind. Only a few use bulk sterilisation.

Two cultivation processes are generally followed by most growers in the USA.

a. Brown in bag

b. Brown out of bag

As the name implies, the first method lets the sawdust block incubate, and forms its brown skin in the bag. With the second process the bags are opened earlier in the growing process. When the block completes its incubation and is white, the bag is stripped from the block and “curing” of the block (browning) of the skin is formed in a curing room. After about 30 days the sawdust block is soaked in cold water and fruiting occurs after 3–4 days in the fruiting room.

The ‘brown in bag’ procedure generally gives a more meaty, darker, thicker mushroom cap. The down side of this process is a longer cycle time, and thus more shelves are required. Browning outside the bags will give a shorter growing cycle. It depends on the local market and farmers’ skills which method is preferred. Note the high supple-
mentation ratio in the brown out of bag method; occasionally farmers use even more than 45% of extra nutrients. Special strains from China have been introduced for these farmers.

<table>
<thead>
<tr>
<th></th>
<th>Brown in bag</th>
<th>Brown out of bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bags used</td>
<td>Unicorn type 3T or 14</td>
<td>Unicorn type 14</td>
</tr>
<tr>
<td>Block weight</td>
<td>5-6 lbs</td>
<td>5-6 lbs</td>
</tr>
<tr>
<td>Substrate mix</td>
<td>75-80% sawdust</td>
<td>55-65% sawdust</td>
</tr>
<tr>
<td></td>
<td>20-25% nutrient</td>
<td>35-45% nutrient</td>
</tr>
<tr>
<td>sawdust size (typical)</td>
<td>30-60 mesh 70%</td>
<td>30-60 mesh 70%</td>
</tr>
<tr>
<td>sawdust chips (typical)</td>
<td>30% 5-10 mm</td>
<td>30% 5-10 mm</td>
</tr>
<tr>
<td>Type of sawdust</td>
<td>Oak preferred</td>
<td>Oak preferred</td>
</tr>
<tr>
<td>Nutrients</td>
<td>locally available</td>
<td>locally available</td>
</tr>
<tr>
<td>(e.g. wheat bran, rice bran, millet, corn waste, or other grain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gypsum &amp; Calcium Sulphate</td>
<td>0.5-2%</td>
<td>0.5-2%</td>
</tr>
<tr>
<td>Temperature incubation</td>
<td>70 °F 1 month</td>
<td>70 °F 15-25 days</td>
</tr>
<tr>
<td></td>
<td>65 °F 1 month</td>
<td>strip bag</td>
</tr>
<tr>
<td>Browning temperature</td>
<td>65 °F 1 month</td>
<td>65 °F 30 days</td>
</tr>
<tr>
<td>Fruiting initiation</td>
<td>strip bag, water spray</td>
<td>immersion in cold water</td>
</tr>
<tr>
<td>CO₂ at incubation</td>
<td>1000 ppm and less</td>
<td>1000 ppm and less</td>
</tr>
<tr>
<td>CO₂ at curing</td>
<td>8000 ppm</td>
<td></td>
</tr>
<tr>
<td>Lighting</td>
<td>50-100 lux</td>
<td>500-1000 lux</td>
</tr>
<tr>
<td>Type of strain</td>
<td>low temp</td>
<td>fast growing</td>
</tr>
<tr>
<td>Type of mushroom</td>
<td>large cap meaty</td>
<td>less thick cap</td>
</tr>
<tr>
<td>Diameter</td>
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</tr>
<tr>
<td>Number of flushes</td>
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<td></td>
</tr>
<tr>
<td>Average weight of 1st flush</td>
<td>4-8%</td>
<td>8-17%</td>
</tr>
<tr>
<td>Average weight of 2nd flush</td>
<td>4-8%</td>
<td>4-8%</td>
</tr>
</tbody>
</table>

22.3 Case study: Shiitake cultivation in Gutien, Fujian province, China

Fujian is famous for its many mushroom farms and Gutien is the centre of Shiitake production in this province. The production technique differs from that in other places: long bags are used, creating artificial 'logs' (see 21.3.1). Many agricultural wastes have been found to give satisfactory yields.

To these formulas 65 to 72 litres of water have to be added to obtain an optimal moisture content of 60-65%. The pH should be stabilised by the gypsum and the CaCO₃ at 5.5 to 6.0. Ingredients in small amounts should be mixed with a small part of the mixture first, and only then mixed with the rest of the substrate. Soluble ingredients (citric acid, sugar and sulphates) are usually dissolved in water before applying. Sawdust has to be soaked for at least two days, to soften and absorb more water. Rice straw should be soaked for three hours in clean water. Apply the squeeze test to determine whether the moisture content is high enough.
Substrate formulations from Gutien county, Fujian province, mainland China.

<table>
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<tr>
<th></th>
<th>1 sawdust</th>
<th>5 corncobs</th>
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<tr>
<td></td>
<td>wheat bran</td>
<td>sawdust</td>
</tr>
<tr>
<td></td>
<td>1.5kg</td>
<td>10kg</td>
</tr>
<tr>
<td></td>
<td>corn meal or cassava powder</td>
<td>wheat bran</td>
</tr>
<tr>
<td></td>
<td>1.0kg</td>
<td>12.5kg</td>
</tr>
<tr>
<td></td>
<td>cane sugar</td>
<td>cane sugar</td>
</tr>
<tr>
<td></td>
<td>0.6kg</td>
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<tr>
<td></td>
<td>gypsum</td>
<td>pectin</td>
</tr>
<tr>
<td></td>
<td>1.5kg</td>
<td>15g</td>
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<tr>
<td></td>
<td>ammonium sulphate</td>
<td>urea</td>
</tr>
<tr>
<td></td>
<td>20 g</td>
<td>20 g</td>
</tr>
<tr>
<td></td>
<td>calcium superphosphate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 g</td>
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<thead>
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<th>6 rice straw</th>
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<td>20kg</td>
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<tr>
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<td>citric acid</td>
</tr>
<tr>
<td></td>
<td>20kg</td>
<td>0.2kg</td>
</tr>
<tr>
<td></td>
<td>CaCO₃</td>
<td>CaSO₄</td>
</tr>
<tr>
<td></td>
<td>30 g</td>
<td>0.5kg</td>
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<table>
<thead>
<tr>
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<th>3 cotton seed hulls</th>
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<td>CaCO₃</td>
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<tr>
<td></td>
<td>cane sugar</td>
<td>citric acid</td>
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<td>0.2kg</td>
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<table>
<thead>
<tr>
<th></th>
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<td></td>
<td>bagasse</td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>rice bran</td>
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</tr>
<tr>
<td></td>
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<td></td>
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22.3.1 Filling the bags

Fill the bags immediately after mixing and wetting of the substrate; otherwise fermentation and contamination will render a less suitable substrate. Two types of bags are used: high temperature resistant bags of 500 x 160 mm with a thickness of 0.09 mm, and low temperature resistant bags measuring 500 x 150 mm with a thickness of 0.04 mm. Usually a horizontal bagging machine is used: a mixer which has a horizontal screw below the bottom plate. After mixing, the bottom plate is opened and the screw presses the substrate out. Workers keep the bag around the end of the pipe until the bag is full. The bags are kept together by a rope before filling at one end. After filling, the other end will be closed with a rope too. The ends of the bags are sealed in a flame. The bags are first loosely filled, later a pressure of 20 kg is applied. This results in cylindrical bags. The time between mixing the supplements and sterilisation should be less than six hours to avoid fermentation of the substrate. During fermentation, toxic metabolites would be formed.

Some growers make holes for later inoculation before semi-sterilisation, others make holes after the heat treatment. In any case, two 15 mm diameter 20 mm deep holes are punched on opposite sides with an auger and covered with 33 mm square adhesive medical tape.
22.3.2 Sterilisation
A home-made boiler provides the steam for the heat treatment. The bags are placed in
an oven-like building and then kept at just below 100 °C for 12 hours. Then they are
allowed to cool down for two hours and dry by means of (natural) ventilation. An
alternative way is to heat the bags in a dry oven with hot air of 140 °C. The heat
treatment determines the type of plastic which has to be chosen.

22.3.3 Spawning
If no holes were made before sterilisation, the bags are cleaned with 70% alcohol and
forceps are used to make the holes as described before. The tape is put back or applied
directly after the spawn has been inserted and the substrate is compressed at the inocu-
lation site. The spawn amount per hole is about 1 cm³ or a little less. A 750 g bottle of
spawn can inoculate 25 to 30 bags. One of the much used strains used in Gutien is
available at the culture collection of the CBS: CR-02. The plastic can be removed from
the bags after only 50 days when this strain is used.

22.3.4 Spawn run
The farmers in Gutien keep the bags indoors for the first stage of mycelial growth. It is
thus possible to adjust the temperature, and Shiitake favours an even temperature dur-
ing spawn run. The bags are stacked in a criss-cross pattern or next to each other with
bags at right angles on top after spawning: three or four bags per layer, 10 layers per
stack. The temperature should be kept rather high: 25-29 °C. Some mycelium should
be seen growing from the spawn in four or five days. Then the temperature should be
lowered to 25-27 °C and the windows opened for aeration. Coal-heated rooms in par-
cular suffer from a high CO₂ concentration. The tape should be loosened at one cor-
ner to allow some aeration as soon as the mycelium has grown 10 cm from the inocula-
tion site. The room temperature should then be lowered to 23-25 °C and relative hu-
midity should be 80%. Normally two sprinklings a day is sufficient to keep the desired
humidity. If the temperature rises above 30 °C, then sprinkling has to be performed
more often and the windows have to be opened. Spray the floor heavily in combination
with ventilation, as this will lower the temperature.

22.3.5 Fruiting
The bags can be placed outdoors when the mycelium has formed clumps and has turned brown at
some spots. Now the plastic can be removed entirely. The tempera-
ture during the indoor spawn run
was more or less constant, now the
substrate is subjected to fluctua-
tions in temperature. This will en-
hance fruit body formation.
It is important to remove the plas-
tic at the right time. If the plastic
is removed before the mycelium has reached its physiological maturity, the yields will be low and many fruit bodies will be deformed. The chance of contamination is also much higher if the dense mycelial coat has not yet formed. It depends on the strain how long it takes before the plastic can be removed. Early maturing strains will show brown mycelium in 50 to 60 days, average and late maturing strains need 80 to 100 days. It is therefore not enough to count the days after inoculation. Some knowledge of the physiology should be present, too. The bags are generally inoculated in August or September and the plastic can be removed in October or November.

The bags are placed in arched sheds of bamboo and covered with plastic. The sheds are 1.2 to 1.3 m wide, thus permitting easy access to the artificial logs in the middle. Twelve to fifteen bags can be put per m². The artificial logs lean against bamboo sticks, about 25 cm above the ground, at an angle of 60 to 70 °C. The distance between the blocks of substrate within one row is 2 to 3 cm.

22.3.6  Harvest

From 100 kg of dry sawdust or cotton seed hulls, 5 to 6 kg of dry Shiitake can be harvested (45 to 55 kg wet mushrooms from 240 kg of wet substrate). The yields from the indoor brick method of Shanghai have been reported to be 20% lower. Shape, flavour and colour of Shiitake from Gutien were also reported to be better than those from Shanghai.

22.4  Shiitake cultivation in Thailand

Only the hills of northern Thailand have a suitable climate in winter to grow one or two flushes of Shiitake. The infection pressure is also very high in Thailand, another reason why only a few flushes can be harvested per bag.

Sawdust from commonly used logs in Shiitake cultivation, like oak, is unavailable. Therefore research has been performed to adapt sawdust from other trees. Experiments by the late prof. Triratana with the trees *Dipterocarpus alatus*, *D. obtusifolius* and *Pentacme suavis*, all common in northern Thailand, showed that their sawdust had to be stacked for 10 weeks. Each week the heaps were turned over. Still their yields were lower than when using sawdust from the Para rubber tree, *Hevea brasiliensis*.

**Substrate formulation:**
- Fermented sawdust 93.5%
- Rice bran 5%
- Corn starch 0.4%
- Magnesium sulphate 0.1%
- Gypsum 1%

The pH of the substrate was 5 to 5.5, moisture content 50%. Particle size ranged from 50 to 80 mesh.

**Heat treatment:** The bags were sterilised in an autoclave, allowed to cool and sterilised again the next day. A double autoclave treatment thus ensured the death of all microorganisms in the substrate.

**Spawning and spawn run:** Grain spawn (Sorghum) was used in the experiments. The spawn run took two to three months. The same criteria to determine maturation, as
discussed above, were used: dense patches of mycelium, leathery texture and browning of the coat. The yield has been reported to be 30% (wet weight of mushrooms versus wet weight of substrate).

22.5 Shiitake cultivation on sterilised plastic bags in Taiwan

Taiwan, like Thailand, lacks sufficiently suitable trees for Shiitake cultivation, too. Years of experiments have led to the following technique, adapted to the locally available agricultural waste products. Sawdust originating from trees that cannot be used in wood log cultivation can be adapted by a fermentation procedure. Usually the substrate consists of mixed softwood sawdust. If hardwood sawdust is available, it will be more expensive, but on the other hand, the fermentation time is much shorter.

The sawdust has to be stacked on a heap of 2 to 4 m high and moistened. The temperature in the heap will rise and then it has to be turned over. The unfavourable alkaloids and resins in the sawdust will be leached out by the water, otherwise they would hinder the growth of the mycelium. In addition, fermentation will have the following effects on the substrate:
- a temperature rise at the beginning of the fermentation,
- microbial activity is lowered at the end of the fermentation period,
- the pH increases,
- it will smell better (ammonia disappears).

As fermented sawdust contains less nutrients than the fresh material, it has to be supplemented with rice bran (5%) and wheat bran or corn meal (2 to 3%). This rich medium is steamed for up to 10 hours to break down or evaporate all fatty acids. If sawdust from hardwood can be obtained, the usual supplementation is:

3–4% rice bran,
1% corn meal or wheat bran,
1% CaCO₃.

22.5.1 Substrate preparation and filling

Several machines are employed in mixing and filling the substrate. Rice bran and chalk are added to the substrate in the mixer, a conveyor belt will bring the substrate to a sieve, then another conveyor belt will feed the bag-filling machine. If the fungicide panmush is used, then 48 g of it should be mixed with 24 kg of rice bran and only then be added to the bulk substrate. The bags used vary from 15 to 18 cm in length and from 10 to 12 cm in diameter.
Heat treatment: Tent-like constructions or steel containers are employed to steam the bags at 96-98 °C for four hours. If microbial activity is high (in case a rich medium is used) the steaming should be prolonged to maximally ten hours.

Spawn run: Spawning techniques are described above. The mycelium will grow down to the bottom of the bags in four weeks if the temperature is kept at 25 °C. Sudden changes in temperature will result in mycelial knots on the surface. Inexperienced growers will then open the bags because they think these are the first pinheads. However, the mycelium has not matured enough to start fruiting. Only when the upper part of the mycelium starts to turn brown, the plug has to be taken off (usually after 60 to 110 days). The texture of the mycelium on the surface will now become hard. Then it is ready for fruiting.

22.5.2 Fruiting
The upper part of the plastic is removed when half of the mycelium has turned brown. The usual method is to put the bags upside down on a moistened floor, and leave them that way for two days and then turn them back. The mycelium will absorb the water and get a physical shock: both enhance fruit body formation. Sometimes this shock treatment is not necessary for the first flush, but is used for the subsequent flushes. Watering is stopped when the pinheads start to grow.

The bags get a three week rest and then receive the same treatment. There are five to seven flushes, of which the first two give the highest yields. The total yield is about 300 to 400 g per 1.5 kg substrate bag. This equals 20 to 27% of the wet weight of the substrate in fresh mushrooms.

22.6 Shiitake cultivation in Europe
The market for fresh Shiitake is still small in Europe because of the high price of the product and high production costs. There are only two significant producers of substrate in The Netherlands, for instance. One large grower, Pleunis, which cultivated both White button mushrooms and Shiitake, went out of business after the company
was sold. His technique was different from all others: he grew on a pasteurised substrate in beds and used the same equipment as is normally used for Agaricus cultivation in The Netherlands.

The technique cannot easily be transferred to other countries, therefore it is not discussed here. Most growers have limited resources. They produce a few hundred kilograms per week, using the sterilised plastic bag technique. Recent developments include the cultivation on bags filled with 15 kg of substrate. The specially developed bags for this purpose have been discussed above. Special spawning techniques have been developed to ease inoculation and to obtain a faster spawn run. The growing cycle is usually shorter than in South-East Asia, but not as short as can be found in the USA. A new trend is that substrate block producers tend to sterilise in bulk to save labour.

22.6.1 Substrate preparation and filling

Suitable substrate materials are readily available. Most used are oak (Quercus spp.) and beech (Fagus sylvatica) sawdust. Supplements include 10 to 25% shredded corn (used to feed animals), rice bran or corn flour. Mixing is generally performed by ribbon or cement mixers. So far, few machines are used in filling the bags. Some growers use a screw jack to transport the substrate from mixing machine to filling device. They will keep the bags under the flow of substrate and one person can fill 350 bags per hour. This does not include putting the plastic ring and the plug on the bags.

Heat treatment: Sterilisation methods differ from grower to grower, actually depending on their resources. Some use an autoclave that can actually reach 121 °C. others heat the substrate three times to 75 °C with dry heat and can produce only limited amounts of substrate. The latest trend is bulk sterilisation, quite similar to the way Sylvan produces spawn. The substrate is heated in bulk under pressure, and cooled down by a cooling liquid which flows through the double wall of the substrate vessel. The most difficult part is how to obtain sterility when the vessel is opened and the substrate has to be spawned.

22.6.2 Spawning and spawn run

Several methods are employed:

- Grain spawn is laid on top of the substrate. A pipe, cut in halves, serves as the ‘spoon’ to distribute spawn evenly over the large bags, so-called top spawning.
- Grain spawn is mixed through the substrate in a clean room, so-called through spawning.

If the first is employed, the spawn run is longer and the substrate does not have the same age everywhere. If the grain is dispersed (method 2), then the spawn run is two weeks faster. The amount of spawn differs from only 0.5% to 10% for the pre-heated substrate method.

Fruiting: Up to five flushes can be harvested. The substrate needs to be soaked if the moisture content inside becomes too low. This will happen after two to three flushes. The minimal period between flushes is 16 days: picking, one week rest, soaking one day, eight days later picking, one week rest, etc. Many growers pick only two flushes to use their growing rooms efficiently.
22.6.3 Harvest

The yield can be as high as 800 g of fresh mushrooms per 3 kg wet substrate. A typical yield is 600 g, about 20% (wet/wet). The market for fresh Shiitake is difficult to handle. For example, in Germany the price had to be as high as 8 € per kg fresh Shiitake (1995), otherwise Shiitake cultivation was hardly profitable.

22.7 Shiitake cultivation on pre-heated substrate

Is it possible to produce a stable crop of Shiitake without expensive equipment like autoclaves, bag filling machines and clear air rooms? Experiences with the so-called pre-heating method indicate that only a mixer, bags and sufficient spawn are needed. The method was first developed by Blaak Specialty Mushrooms Consultancy. The method has also been tested on a pilot scale with Pleurotus ostreatus, Pleurotus eryngii and Pholiota nameko and gave satisfactory yields.

22.7.1 Substrate production procedure

Dry pre-heated beech sawdust (fibres with a length of ca. 2-4 mm and ca. 1-2 mm thick) is moistened with a small amount of gypsum and vermiculite. Per ton of substrate, 380 kg of sawdust is used. This type of beech sawdust is normally used by the large fish-smoking companies. Alternatively, other formats of wood chips can be used (up to a size of 2 x 2 cm). Care has to be taken then that the wood chips are completely saturated with water and that they are sufficiently clean. Dry wood chips may need up to 2 days under water before they contain sufficient water. If the wood has not been preheated, it is advisable to steam it or use $\text{H}_2\text{O}_2$ during the substrate production process (however, this is a different procedure than the method developed by Blaak, which works entirely without any chemicals). When using the fine material, the sawdust moistening takes only 5 minutes. 10% spawn is added to the sawdust/wood chips and mixed thoroughly for ca. 10 minutes, as well as a small amount of gypsum.

Note that only a selected number of strains perform well with this method, e.g. Mycelia 3776 (Shiitake), Pleurotus 2191, Pholiota nameko 4140 and Sylvan 4080.

The mixing can be done with a standard cement mixer. The substrate is then filled in perforated bags, 12–15 kg per bag. The currently used bags are 20 cm in diameter, but possibly thinner artificial ‘logs’ would give better results. It is important not to make the blocks thicker as the internal heat would kill the Shiitake mycelium in the core of the bags.
A simple cement mixer in a relatively clean environment has shown to be effective for substrate preparation. Right: incubation of the bags.

22.7.2 Contamination prevention
The mixing procedure is not difficult, but it is important to use equipment which can easily be cleaned and to maintain strict hygienic measures. Also important is to choose a mixer which does not crush the spawn during the mixing procedure. Misting of \( \text{H}_2\text{O}_2 \) in the substrate preparation room prior to the mixing process (and/or the addition of 3% \( \text{H}_2\text{O}_2 \) to the substrate) can help in keeping green moulds away. Ideally, a room under overpressure with filtered air blown in, is available for the substrate preparation process.

Note that a low concentration of \( \text{H}_2\text{O}_2 \) does not kill mycelia of fungi which produce enzymes which neutralise the oxidation effect of \( \text{H}_2\text{O}_2 \). Spores in general will be killed, however. Keep in mind that plastic bags are often static and thus spores of unwanted green moulds can be present on them. Therefore store the bags under clean conditions; otherwise a clean substrate is put in contaminated bags!

22.7.3 Spawn run
As this method is typically a spawn through method, much internal heat is generated in the substrate. The bags should not touch one another, otherwise the heat cannot dissipate. Some growers hang the bags, others put them in special crates. The heat dissipation should not be underestimated. Especially in summer cooling has to be present to keep the temperature of the air down to ca. 20 °C; the temperature inside can rise to 35 °C; preferably, the temperature should stay below 30 °C. Again, thinner bags would help in lowering the internal temperature.

The spawn run takes 6-8 weeks; if everything runs normal, no bulbs or only a few should be present on the substrate. Otherwise the aeration has been limited. When a brown crust has formed, the plastic is taken off and the bags are placed in a growing room.

22.7.4 Growing and picking
The temperature is forced down and the environment is kept very moist. The first flush
will appear without any shock treatment. The advantage of this system is that it is very easy to pick the mushrooms. One grower normally harvested more than 30 kg/hour. He would cut the mushrooms directly from the substrate blocks and keep the stems on them until the end of the flush. Only then would he remove all the left stems. After the flush the temperature will be heightened to 24 °C for two weeks, then a cold shock is given to the substrate. Water is injected in the bags until they have regained their original weight with an irrigation system. This can simply be monitored by weighing a block during injection and turning the water off when the bag is back to its original weight. The temperature is then lowered to 16–18 °C and the floor is kept moist all the time during primordial formation. When the buds mature, humidity can be lowered slightly.

A 12-week cycle will yield four flushes; the total yield can be as high as 25%. Some growers have managed to grow 20% on average for over a year. Stability of the used mushroom spawn is of utmost importance; weak spawn will lead to spots where green moulds will colonise part of the substrate. Usually the mycelium overgrows green moulds, but according to the grower the bag will show a lower yield. According to Blaak the incubation time has to be one or two weeks longer then to obtain similar yields.

### 22.7.5 Economy of pre-heating method

The ingredients are more expensive compared to a standard sterilised plastic bag cultivation method. However, the costs of the substrate containers are much lower. Other
advantages are that no expensive system is necessary and labour is kept to a minimum. Whether this method is economical depends on the local price of spawn and labour and the availability of pre-heated substrate. If the method can be refined and spawn rate can be lowered without obtaining more contamination, then this method is certainly competitive. However, it should be noted that the spawn serves not just as an inoculant but also as a source of nutrient. If feather meal or other supplements would be used in this substrate, the chances of contamination become very high.

22.7.6 Growing room requirements
The Shiitake are less sensible to CO₂ than other mushrooms like Agaricus and Oyster mushrooms. A simple heat radiator was placed at the back of the growing room and would heat the incoming air flow if needed. A curtain between the radiator and the rest of the growing room could be moistened. A steady air flow would run through the growing room. No shelves are required and the growing rooms are very easy to clean.

22.8 Case study: exotic mushroom cultivation in Finland
By Hannu Mäkelä, Mauri Lamminsalo and Ari Pappinen

22.8.1 General situation in Finland
Specialty mushroom cultivation in Finland is a relatively new business; the first commercially functional small-scale enterprises were founded in the mid 1980's. At that time, new alternatives for traditional crop and animal production were welcomed with great enthusiasm. Approximately 90 Shiitake farms were built in this first wave of interest. Most of these farms proved economically less viable than expected and in 2001 only a handful of this wave of mushroom farms were still in business. The specialty mushroom production in Finland in 2001 was 263 metric tons (fresh weight) for Shiitake and 7 tons (fresh weight) for Oyster mushroom. Shiitake was cultivated in 4-5 farms of which the biggest one produced nearly half of the annual production. There is only one small-scale commercial Oyster mushroom farm in Finland. One of the largest Shiitake farms used to import the ready-to-use cultivation blocks from Japan by air, because their Japanese associates insisted they use substrate delivered by them. For obvious reasons this farm is no longer in business. At the moment the largest Shiitake farm is Polar Shiitake Ltd located in Eastern Finland. Twenty years back the knowledge of mushroom cultivation was concentrated mostly in
Japan, China and to some extent in a few European countries like The Netherlands and France. In addition, the markets for new products were limited, logistical problems were significant and labour costs high. Nowadays lack of knowledge and information is no longer a limiting factor in mushroom business, although only a few people in Finland have an adequate practical experience in mushroom cultivation. The technology has become highly developed since the beginning.

22.8.2 Liquid spawn use

The technical research institution (VTT) was conducting developmental research activity in specialty mushrooms between the late 1970’s and the early 1990’s. As an outcome of this activity the liquid spawn utilisation was widely adopted for specialty mushroom production and most of the farms still existing use it. However, the utilisation of the liquid spawn in commercial specialty mushroom cultivation has not spread much beyond Finland (some North American growers use it also). In the beginning, the use of liquid spawn was seen as an innovative and labour-saving method with small risks. This presumption has proved to be too optimistic.

Experiences gathered from general microbiology, where these techniques have been used successfully for decades, were not fully tested for large-scale mycelia production. The liquid spawn method requires high investments and specially trained personnel is also needed for the quality control of spawn. Mushroom mycelium is much more sensitive to mechanical stress than bacterial cells and their lag phase in the beginning of liquid cultivation is longer. Due to these factors harmful genetic changes are frequently seen in mushroom liquid spawn cultures. In solid spawn systems the spawn can readily be evaluated visually but the liquid spawn has to be checked separately in petri dishes. In some cases farmers reported that the fungal strain used for inoculation had degenerated and no successful production had occurred at all.

The following description aims to show the general situation in liquid spawn production. Producers tend to have recipes and methods of their own which are reluctantly revealed.

The mushroom strains are stored by using various techniques as described in the appendix ‘Conservation of strains and culture collections’. The main supplier of Finnish liquid spawn is the VTT-research centre where the strains are kept in liquid nitrogen and gradually acclimatised to normal growing temperatures in a laboratory. Some farmers prefer to make their spawn in their own laboratories where they usually keep a stock of strains cooled at +4 °C in nutrient-poor agar slants. Often the main stock or back up stock is deposited in Finnish or international strain banks where the facilities are appropriate for long-term storing. The mother spawn is normally prepared by applying small pieces of stored mycelia to petri dishes with a growing medium, under strictly aseptic conditions. Personnel responsible for this task should be trained in this aspect. VTT grows the spawn in fermentors where the factors influencing the growth can readily be optimised. They are equipped with thermostats, inlets and outlets for acquiring proper gas concentrations in the liquid and mixers to assure even blending.

The time needed for mycelia to grow from the beginning to inoculation of production blocks varies depending on the method used. When fermentors are used, it is possible to obtain the spawn in 5–7 days. Obviously the longer the time needed, the higher the
risk of contamination. The purity of the spawn is sometimes hard to guarantee, even under laboratory conditions, if growth takes over a week. The mutation rate increases greatly at the same time and these changes in DNA are usually hard to spot in shaken liquids. Mutations are detected by cultivating samples of mycelia in petri dishes where anomalies are relatively easy to see. The signs of unwanted adaptation of mycelia to nutrient-rich liquids are regularly seen particularly when extended growth times are used.

Adaptation to normal production substrates can be guided by applying either particles or extracts made from wood chips, straw etc. to the liquid. Obviously, this also lessens the risk of mycelia to adapt solely to the use of easy nutrients. However, this is not always done as it provides a limited advantage. Mycelium adapted to liquid grows very slowly in production blocks during the first days after inoculation. Fast spawn run is essential, as the substrate is not sterilised under high pressure. Increasing the inoculation rate and assuring an even distribution of the spawn minimises the risk of slow growth and contamination. There are no exact marks by which to decide when the spawn is ready to be inoculated. It is not recommended to grow a lot of biomass for this usually means too long a time to grow in liquid phase. After expansion, the mycelia are broken down with a laboratory homogeniser in order to get an evenly distributed cell suspension, which passes easily through needles used in inoculation. Shearing the mycelia contributes a lot of mechanical stress to cells and the risk of mutations increases and the viability of the mycelium decreases. Broken mycelium requires 2-4 days in normal room temperature to recover from stress, after which the spawn should be inoculated. The spawn can be stored under cooled conditions after the recovery

A laboratory to make liquid spawn. Notice the bottles with sterilised nutrient medium in the laminar flow. The bags are injected with the liquid spawn (courtesy Hannu Mäkelä).
period, for a maximum of one week but this is not recommended. Even at +4 °C the mycelium continues to grow slowly. Spawn which is too old for inoculation should be rejected and not used for any purposes.

22.8.3 Polar Log Ltd and Polar Shiitake Ltd

Polar Shiitake Ltd and its sister company Polar Log Ltd was founded in 1999 by entrepreneurs who were one of the first cultivators in Finland. After 14 years of practical experience they had to choose: either close their laborious business or expand and modernise. Plans were made for a semi-automated factory with the capacity of 500 metric tons of fresh Shiitake per annum in Polar Shiitake Ltd. Polar Log Ltd has a capacity for producing more than 2000 tons of ready-to-use blocks per year. Both of these companies use very high-tech solutions in many phases of the production although some crucial points like harvesting still have to be done manually. The facilities for these companies were in use in spring 1999. Technical problems have emerged since and some of these problems still need to be solved.

The two Polar companies employ around 30 people. The main product is fresh Shiitake; only a small fraction of mushrooms is processed further to canned mushrooms and dried mushroom powders. The majority of the mushrooms are sold to a marketing company called Mykori Ltd. More information (in Finnish) can be obtained from www.polarshiitake.com. The production system is organic i.e. no pesticides or artificial nutrients are used. The markets in Europe demand pollution-free high quality products and the popularity of organic mushrooms is rising.

In spite of this the price paid to the farmer is the same regardless of the certification. Polar Log Ltd used to buy spawn from VTT but now has a laboratory of its own to ensure a continuous flow of fresh spawn. The laboratory is equipped with normal lab devices needed in spawn production. The spawn production is based on fermentor technology. The company sells small amounts of spawn to mushroom producers around Finland. The company aims to form an integrated, closed farming system with the least possible environmental disturbance. Alder is collected from areas were this species is removed for forestry anyway. Electricity is bought from sources producing it in an environmentally sound way. The company supports research on suitable uses of used substrate.

22.8.4 Process at the Polar companies

A subcontractor delivers freshly cut logs to the farm. The logs are stored only briefly to avoid the formation of moulds, which could increase the infection pressure on the farm. The logs are fed to the chipper, the organic cereal grain is added to wood chips of a homogeneous and defined particle size and the mixture is conveyed to the storage silo and further to the bulk pasteurisation chamber.

The raw materials are fresh alder (Alnus) sawdust supplemented with organically cultivated cereal corn. The mean water content of blocks is between 60-65 %. The exact recipe for the substrate is kept confidential. The computerised heating unit controls heating and cooling. The substrate batch is heated up, mixed and cooled down during the phase. Temperatures below 100 °C are used and the process takes altogether approximately one day, heating and cooling included. This stage destroys most of the
harmful competitive organisms but some thermo-tolerant bacteria may survive. The cylindrical blocks used for production contain on average one kilogram of substrate. Similar blocks can be seen for instance in China but rarely in European farms where bigger units are preferred. Blocks are made with semi-automated machinery with a high capacity, but some technical problems still have to be solved. The blocks are kept in boxes with holing, which ensure the proper gas flow and easy handling when transferring the blocks. The boxes are stacked to columns by a robot, with free space between columns to minimise the risk of overheating and to ease the visual controlling of the phase.

The incoming fresh air is filtered, cooled or heated and blown into the growing rooms. The humidity of rooms is maintained by vaporising water from nozzles. The vegetative phase takes place in darkness. The spawn run generally lasts about three months. **Fruiting:** After the vegetative phase, the substrate blocks are conveyed to rooms designed for production. This phase takes about four weeks consisting of one to two separate crop cycles. Normally, just the first crop is picked and the blocks are transported to smaller farms where more crops are produced. Mean yield is approximately 250 g of fresh mushrooms per 1 kg of substrate. The patemoster-type production shelves facilitate easy loading, picking and unloading by making it possible to transfer the shelf wanted to a height optimal for working. During this phase, the operations necessary for induction like watering are made manually. The rooms have controlled lighting. Only few pickers are needed for cropping one production room with 8 tons of substrate on shelves. The used substrate is removed manually. The disadvantages of these shelves are the high price (100 000 €/unit), problems in cleaning and structural instabilities. The movable part of the shelves puts considerable pressure on ball bearings. The general structure of shelves is rather complicated and some places are difficult to clean. Cleaning is done with steam and pressurised water.
Post harvest: After cropping mushrooms are cooled down as fast as possible to maintain the quality of the product. The temperature in the storing room is +2 °C and in the working room +8 °C. The crop is divided in classes after visual inspection and packed manually in boxes containing 100 g to 2 kg of fresh mushrooms. Mushrooms are transported to the client twice a week on standard pallets in trucks with adequate cooling. Even though some mushrooms are 4 days old when transported from the farm, quality is still good because the mushrooms are picked timely (before the caps open) and because of the efficient cooling shortly after harvesting.
Cultivation on wood logs

Some mushrooms have been grown on wood logs for hundreds of years. This chapter discusses the cultivation on wood logs of the following species: Shiitake (*Lentinula edodes*), *Tremella fuciformis* and two *Auricularia* species. Other wood-inhabiting species, like Oyster mushrooms (*Pleurotus* spp.), *Flammulina velutipes* and *Kuehneromyces mutabilis* can be grown in a similar way. These are rarely grown commercially on wood logs, however, and only *Pleurotus* is therefore discussed in detail. General aspects of wood log cultivation are discussed in the first paragraphs, specific information concerning the species is given in the relevant sections.

- introduction,
- requirements,
- Shiitake: *Lentinula edodes* on wood logs,
- suitable wood logs for Shiitake,
- spawning procedures,
- spawn run: temporary and permanent laying,
- fruiting,
- case study: traditional Shiitake cultivation in Japan,
- case study: Shiitake cultivation in a subtropical climate in Taiwan,
- *Auricularia* spp.,
- Oyster mushrooms on wood logs and stumps.

**23.1 Introduction**

In former days, people would put a freshly cut mushroom on wood logs. The spores would germinate and the mycelium would colonise the substrate. This cultivation method was very similar to nature, but provided very unstable yields, however. The technique has been improved: for more than 75 years pure cultures of the mycelium have been inoculated in wood logs. The investment can be kept low, because the method is still quite similar to nature.

Cultivation in this way can be profitable if the following conditions are met:

- high quality sawdust or wood prop spawn,
- sufficient and cheap labour,
- sufficient suitable logs,
- suitable climatic conditions.

If wood log cultivation is promoted in a region, then reforestation should also be introduced at the same time. Farmers otherwise might increase deforestation. Moreover, timber can be used for other purposes, like heating, carpentry and cooking. Growing
Shiitake on wood logs is still quite popular in Japan, but in most other countries plastic bag cultivation using sawdust waste is preferred, because of lack of suitable wood logs. In developed countries cultivation of Shiitake on wood logs is a marginal business because of high labour costs.

23.2 Substrate requirements

Suitable wood logs for *Tremella, Auricularia* and *Lentinula* cultivation are given at the beginning of the sections dealing with each species. Other trees might yield mushrooms as well, but they have not yet been reported to do so. Especially in regions other than the Far East, there may be more suitable hosts. In subtropical and moderate climates logs are inoculated in winter, because labour is more easily available then. Another advantage of inoculation in winter is that the logs contain more sugars when the leaves start to fall or have just fallen, thus contributing to fast mycelial growth. In Japan, *e.g.* *Quercus acutissima* is felled when the leaves start to turn red.

For all wood-inhabiting mushroom species it is important not to damage the bark of their wood logs. The bark is a natural shield against unwanted invaders and it prevents evaporation. Shiitake is no exception, and also needs the bark for fruiting. To avoid bark damage, logs should be handled with care and preferably be felled in winter. Straight logs are easier to handle; forest practices can be adjusted to obtain these straight logs, *e.g.* by cutting a relatively dense oak forest every ten years.

23.3 Requirements

The following must be available:
- (chain) saws: for felling the trees and sawing the logs,
- equipment to spawn (depending on the spawning techniques),
- steady water supply,
- optional tanks or ponds to immerse the logs in after mycelial growth and/or a spraying installation,
- means of transport of the logs,
- suitable site.

23.4 Spawning techniques

Several spawning techniques have been tried and some methods have proved to be better than others. The goal of spawning is of course to obtain a completely colonised wood log, without any colonies of competing fungi. Mostly employed nowadays is
inserting the spawn in holes evenly through the surface of the wood log. Inserting spawn in cuttings made in the wood logs has shown to lead to poor results, as the logs tend to break where they were spawned.

Five different types of spawn can be distinguished, as the following table shows.

<table>
<thead>
<tr>
<th>Type of spawn</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Necessary equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>wood plugs</td>
<td>fast and efficient inoculation</td>
<td>electricity should be available to drill the holes, spawn relatively expensive inoculation is more laborious (the spawn has to be pressed into the holes, which should be sealed with wax)</td>
<td>high speed drills with special drill bits</td>
</tr>
<tr>
<td>sawdust spawn</td>
<td>is easily available in a region which also produces mushrooms on plastic bags; cheap substrate for the spawn</td>
<td>higher cost, limited lifetime of spawn once pressed into ‘tablets’, ‘tablet’ (plugs) tool if spawn is produced this type of spawn is not available everywhere, special equipment is necessary to produce it</td>
<td>high speed drills with special drill bits; sawp plungers or a stick, wax applicators</td>
</tr>
<tr>
<td>sawdust spawn plugs</td>
<td>fast and efficient inoculation (plugs)</td>
<td></td>
<td>high speed drills with special drill bits; sawp hammer with sharp end</td>
</tr>
<tr>
<td>wood wedges</td>
<td>no need for electricity; low investment</td>
<td>can only be used for thick logs, see last paragraph</td>
<td>none</td>
</tr>
<tr>
<td>straw-based spawn</td>
<td>fast and efficient inoculation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sawdust spawn grows much quicker through the wood logs than wood dowels or wood wedges: the spawn run time can be decreased from 24 to 12 months or even 6 months in subtropical climates with heavy spawning.

The traditional method to inoculate with sawdust spawn is more tedious than the wood dowels: an extra inoculator is needed and the logs have to be covered with wax to prevent the spawn from drying out. The latest developments from Korea combine the ease of plug spawning with the speed of mycelial growth of sawdust: sawdust spawn plugs. The sawdust plug has to be made out of fine sawdust, which is sterilised in special bags which allow for aeration. Normally no extra nutrients are applied to the sawdust, unlike the cultivation of Shiitake on plastic bags. The sterilised bags are spawned with the desired strain. When the sawdust is completely colonised, it can be used to produce the sawdust plugs. The sawdust is pressed with a special tool in special sheets and covered with a Styrofoam cap. The sheets have to be kept at room temperature for about a week and can then be used for spawning. Each sheet contains 520 plugs, sufficient to spawn at least 10 logs at an average inoculation rate of 40-50 holes per log. There is only one aspect to take care of with this kind of spawn: it has to be used within six weeks after production, as the sawdust dries out. Wood dowels spawn can be kept for 12 months without loss of vigour. Professional growers who calculate labour and capital expenses will choose for the sawdust plugs; growers who don’t count their hours may keep using the standard sawdust spawn or dowels.
Inoculation with sawdust spawn. A stick is used to compress the spawn in the holes (courtesy TARI).

Covering the spawn with hot wax to prevent the spawn from drying out (courtesy TARI).

Appropriate instruments for wood wedge inoculation. If wood wedges are used, a special hammer is convenient to make holes and insert the wedges. The holes should be deep enough to hold the wedges completely; no parts should be sticking out.

Sawdust inoculation: the surface of the spawned hole should be even. No spawn should stick out or be lacking nor should an indentation remain. The left drawing shows the correct way to inoculate with sawdust spawn; the other three ways are all wrong.
23.5  Shiitake: Lentinula edodes

The Shiitake (or black Chinese mushroom) is the second most cultivated mushroom in the world. It is mainly cultivated in the Far East: Japan and China are the biggest exporters. Chinese communities all over the world import dried Shiitake. The cultivation on wood logs is adapted to the available wood species and climatic conditions in different regions. The latter is accomplished by selecting strains with an appropriate fruiting range. Some strains fruit around 10 °C, others between 10 and 18 °C, and the high temperature strains fruit at or above 20 °C. A combination of different strains is necessary to achieve a year-round production of Lentinula in Japan. If the temperature is continually high, then it is difficult to obtain good quality fruit bodies. The same strain can yield high quality donko Shiitake at low temperatures and thin-capped, less valuable mushrooms at higher temperatures.

23.5.1 Suitable wood logs for Lentinula edodes

Extensive testing of the suitability of different tree species has mainly been performed in Japan, China, the USA and Taiwan. Probably more tree species are suitable to grow Shiitake, but they are either not present or rather scarce in the Orient. From Argentina successful trials with Red acacia have been reported. Trees from the following lists have been tried for Shiitake cultivation:

Trees suitable for wood log cultivation of Shiitake. The rating means: very poor (0), poor (1), fair (2), good (3), and excellent (4) substrate. Poor and very poor-rating trees can be used in sawdust cultivation, but give too low yields when used in wood log cultivation (FAO Manual 106).

<table>
<thead>
<tr>
<th>Species</th>
<th>Description</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer nigrum</td>
<td>Black maple</td>
<td>2</td>
</tr>
<tr>
<td>Acer pensylvanicum</td>
<td>Striped maple, moose wood</td>
<td>2</td>
</tr>
<tr>
<td>Acer pictum</td>
<td>Japanese maple</td>
<td>2</td>
</tr>
<tr>
<td>Acer platanoides</td>
<td>Norway maple</td>
<td>2</td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>Red maple</td>
<td>2</td>
</tr>
<tr>
<td>Acer saccharinum</td>
<td>Silver maple, River maple</td>
<td>2</td>
</tr>
<tr>
<td>Acer saccharum</td>
<td>Sugar maple</td>
<td>2</td>
</tr>
<tr>
<td>Alnus firma</td>
<td>Alder</td>
<td>3</td>
</tr>
<tr>
<td>Alnus japonica</td>
<td>Japanese alder</td>
<td>2</td>
</tr>
<tr>
<td>Alnus rubra</td>
<td>Oregon or Red alder</td>
<td>2</td>
</tr>
<tr>
<td>Alnus serrulata</td>
<td>Hazel alder</td>
<td>2</td>
</tr>
<tr>
<td>Alnus tinctoria</td>
<td>Alder</td>
<td>2</td>
</tr>
<tr>
<td>Betula lutea</td>
<td>Sweet/Black/Cherry birch</td>
<td>2</td>
</tr>
<tr>
<td>Betula nigra</td>
<td>Yellow/Silver/Scamp birch</td>
<td>3</td>
</tr>
<tr>
<td>Betula papyrifera</td>
<td>Red/River/Water birch</td>
<td>3</td>
</tr>
<tr>
<td>Betula populifolia</td>
<td>Paper/Canoe/White birch</td>
<td>3</td>
</tr>
<tr>
<td>Carpinus caroliniana</td>
<td>American hornbeam, Blue beech,</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Ironwood, Water beech</td>
<td></td>
</tr>
<tr>
<td>Carpinus laxiflora</td>
<td>Hornbeam</td>
<td>4</td>
</tr>
<tr>
<td>Carpinus tschonoskii</td>
<td>Hornbeam</td>
<td>4</td>
</tr>
<tr>
<td>Species</td>
<td>Common Names</td>
<td>Number</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td><em>Carya cordiformis</em></td>
<td>Bitternut hickory, Swamp hickory</td>
<td>3</td>
</tr>
<tr>
<td><em>Carya glabra</em></td>
<td>Pignut hickory, Oval pignut, Sweet hickory, Red hickory, Red heart hickory</td>
<td>1</td>
</tr>
<tr>
<td><em>Carya illinoinensis</em></td>
<td>Pecan</td>
<td>2</td>
</tr>
<tr>
<td><em>Carya ovata</em></td>
<td>Shake bark hickory</td>
<td>1</td>
</tr>
<tr>
<td><em>Carya tomentosa</em></td>
<td>Mockernut hickory, Bullnut</td>
<td>2</td>
</tr>
<tr>
<td><em>Castanea crenata</em></td>
<td>Japanese chestnut</td>
<td>4</td>
</tr>
<tr>
<td><em>Castanea dentata</em></td>
<td>American chestnut</td>
<td>3</td>
</tr>
<tr>
<td><em>Castanopsis cuspidata</em></td>
<td>Shii</td>
<td>4</td>
</tr>
<tr>
<td><em>Castanopsis sieboldii</em></td>
<td>Shii</td>
<td>4</td>
</tr>
<tr>
<td><em>Cornus florida</em></td>
<td>(flowering) Dogwood</td>
<td>1</td>
</tr>
<tr>
<td><em>Cyclobalanopsis ocuta</em></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><em>Cyclobalanopsis glauca</em></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><em>Cyclobalanopsis myrsinifolia</em></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><em>Diospyros virginiana</em></td>
<td>Common persimmon</td>
<td>2</td>
</tr>
<tr>
<td><em>Fagus grandifolia</em></td>
<td>American beech</td>
<td>3</td>
</tr>
<tr>
<td><em>Fagus sylvesteris</em></td>
<td>Beech</td>
<td>4</td>
</tr>
<tr>
<td><em>Liquidambar styraciflua</em></td>
<td>Sweetgum</td>
<td>2</td>
</tr>
<tr>
<td><em>Liriodendron tulipifera</em></td>
<td>Yellow poplar</td>
<td>1</td>
</tr>
<tr>
<td><em>Lithocarpus densiflorus</em></td>
<td>Tan oak</td>
<td>3</td>
</tr>
<tr>
<td><em>Malus sylvestris</em></td>
<td>Apple</td>
<td>0</td>
</tr>
<tr>
<td><em>Ostrya virginiana</em></td>
<td>Eastern hop hornbeam, Ironwood</td>
<td>4</td>
</tr>
<tr>
<td><em>Pinus virginiana</em></td>
<td>Virginia pine, Scrub pine</td>
<td>0</td>
</tr>
<tr>
<td><em>Platanus occidentalis</em></td>
<td>American sycamore</td>
<td>0</td>
</tr>
<tr>
<td><em>Platycarya strobilacea</em></td>
<td>Walnut</td>
<td>3,4</td>
</tr>
<tr>
<td><em>Populus balsamifera</em></td>
<td>Balsam poplar</td>
<td>2</td>
</tr>
<tr>
<td><em>Populus grandidentata</em></td>
<td>Big tooth aspen</td>
<td>1</td>
</tr>
<tr>
<td><em>Populus tremuloides</em></td>
<td>Quaking aspen</td>
<td>1</td>
</tr>
<tr>
<td><em>Populus trichocarpa</em></td>
<td>Black cotton wood</td>
<td>1</td>
</tr>
<tr>
<td><em>Quercus acutissima</em></td>
<td>Oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus alba</em></td>
<td>White oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus bicolor</em></td>
<td>Swamp white oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus borealis</em></td>
<td>Northern red oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus chrysolepis</em></td>
<td>Canyon live oak, Maul oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus cocinea</em></td>
<td>Scarlet oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus crispula</em></td>
<td>Oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus dentata</em></td>
<td>Toothed oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus fulcata</em></td>
<td>Spanish red oak, Southern red oak</td>
<td>3</td>
</tr>
<tr>
<td><em>Quercus fulcata var. pagodae folia</em></td>
<td>Swamp Spanish oak, Swamp red oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus garryana</em></td>
<td>Garry oak, Oregon oak, Oregon white oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus imbricaria</em></td>
<td>Shingle oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus kelloggii</em></td>
<td>Black oak, California black oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus laurifolia</em></td>
<td>(Swamp) Laurel oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus lobata</em></td>
<td>California white oak, Valley oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus lyrata</em></td>
<td>Overcup oak, Swamp post oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus macrocarpa</em></td>
<td>Bur oak, Blue oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus marilandica</em></td>
<td>Black jack oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus michauxii</em></td>
<td>Swamp chestnut</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus muehlenbergii</em></td>
<td>Chinkapin oak</td>
<td>2</td>
</tr>
<tr>
<td>Species</td>
<td>Description</td>
<td>Yield</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------</td>
<td>-------</td>
</tr>
<tr>
<td><em>Quercus nigra</em></td>
<td>Water oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus palustris</em></td>
<td>Pin oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus phellos</em></td>
<td>Willow oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus prinus</em></td>
<td>Chestnut oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus rubra</em></td>
<td>Northern red oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus serrata</em></td>
<td>Oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus shumardii</em></td>
<td>Shumard oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus stellata</em></td>
<td>Post oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus variabilis</em></td>
<td>Oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus velutina</em></td>
<td>Black oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Quercus virginiana</em></td>
<td>Ivy oak</td>
<td>4</td>
</tr>
<tr>
<td><em>Salix nigra</em></td>
<td>Black willow</td>
<td>4</td>
</tr>
<tr>
<td><em>Ulmus americana</em></td>
<td>American elm</td>
<td>3</td>
</tr>
<tr>
<td><em>Ulmus rubra</em></td>
<td>Slippery elm</td>
<td>3</td>
</tr>
<tr>
<td><em>Ulmus thomasii</em></td>
<td>Rock elm</td>
<td>2</td>
</tr>
</tbody>
</table>

In Taiwan, *Liquidambar formosana* (Formosan sweetgum) was most used. The following trees from Taiwan have been reported to give similar or higher yields than *Liquidambar*:

<table>
<thead>
<tr>
<th>Species</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia magnus</em></td>
<td>Formosan alder</td>
</tr>
<tr>
<td><em>Aleurites Montana</em></td>
<td></td>
</tr>
<tr>
<td><em>Alnus formosana</em></td>
<td></td>
</tr>
<tr>
<td><em>Bredelia balansae</em></td>
<td></td>
</tr>
<tr>
<td><em>Carpinus minutiserrata/seki</em></td>
<td></td>
</tr>
<tr>
<td><em>Castanea crenata</em></td>
<td>Taiwan hornbeam</td>
</tr>
<tr>
<td><em>Castanopsis carlesii</em></td>
<td>Japanese chestnut</td>
</tr>
<tr>
<td><em>Castanopsis hystric</em></td>
<td>Long-leaf evergreen chinkapin</td>
</tr>
<tr>
<td><em>Castanopsis indica</em></td>
<td>Red chinkapin</td>
</tr>
<tr>
<td><em>Castanopsis kawakamii</em></td>
<td></td>
</tr>
<tr>
<td><em>Castanopsis stipitata</em></td>
<td>Borneo evergreen chinkapin</td>
</tr>
<tr>
<td><em>Cunninghamia lanceolata</em></td>
<td>Sharp-spine evergreen chinkapin</td>
</tr>
<tr>
<td><em>Cyclobalanopsis gliva</em></td>
<td>Red bark oak</td>
</tr>
<tr>
<td><em>Cyclobalanopsis glauca</em></td>
<td>Blue Japanese oak</td>
</tr>
<tr>
<td><em>Elaeocarpus decipiens</em></td>
<td>Common elaeocarpus</td>
</tr>
<tr>
<td><em>Elaeocarpus japonicus</em></td>
<td>Japanese elaeocarpus</td>
</tr>
<tr>
<td><em>Elaeocarpus sylvestris</em></td>
<td></td>
</tr>
<tr>
<td><em>Engelhardtia roxurghiana</em></td>
<td>Yellow basket willow</td>
</tr>
<tr>
<td><em>Lagerstroemia subcostata</em></td>
<td>Subcostate crape myrtle</td>
</tr>
<tr>
<td><em>Lithocarpus amygdalifolius</em></td>
<td>Almond-leaved tan oak</td>
</tr>
<tr>
<td><em>Mallotus paniculatus</em></td>
<td></td>
</tr>
<tr>
<td><em>Mangifera indica</em></td>
<td>Common mango</td>
</tr>
<tr>
<td><em>Pasania brevicaudata</em></td>
<td>Short-tail leaf tan oak</td>
</tr>
<tr>
<td><em>Pasania konishi</em></td>
<td>Konishi tan oak</td>
</tr>
<tr>
<td><em>Prunus phaeosticta</em></td>
<td></td>
</tr>
<tr>
<td><em>Quercus acutissima</em></td>
<td>Low level oak</td>
</tr>
<tr>
<td><em>Quercus variabilis</em></td>
<td>Chinese cork oak</td>
</tr>
</tbody>
</table>

* suitable but gives lower yield
period, for a maximum of one week but this is not recommended. Even at +4 °C the mycelium continues to grow slowly. Spawn which is too old for inoculation should be rejected and not used for any purposes.

22.8.3 Polar Log Ltd and Polar Shiitake Ltd
Polar Shiitake Ltd and its sister company Polar Log Ltd was founded in 1999 by entrepreneurs who were one of the first cultivators in Finland. After 14 years of practical experience they had to choose: either close their laborious business or expand and modernise. Plans were made for a semi-automated factory with the capacity of 500 metric tons of fresh Shiitake per annum in Polar Shiitake Ltd. Polar Log Ltd has a capacity for producing more than 2000 tons of ready-to-use blocks per year. Both of these companies use very high-tech solutions in many phases of the production although some crucial points like harvesting still have to be done manually. The facilities for these companies were in use in spring 1999. Technical problems have emerged since and some of these problems still need to be solved.
The two Polar companies employ around 30 people. The main product is fresh Shiitake; only a small fraction of mushrooms is processed further to canned mushrooms and dried mushroom powders. The majority of the mushrooms are sold to a marketing company called Mykora Ltd. More information (in Finnish) can be obtained from www.polarshiitake.com. The production system is organic i.e. no pesticides or artificial nutrients are used. The markets in Europe demand pollution-free high quality products and the popularity of organic mushrooms is rising.
In spite of this the price paid to the farmer is the same regardless of the certification. Polar Log Ltd used to buy spawn from VTT but now has a laboratory of its own to ensure a continuous flow of fresh spawn. The laboratory is equipped with normal lab devices needed in spawn production. The spawn production is based on fermentor technology. The company sells small amounts of spawn to mushroom producers around Finland. The company aims to form an integrated, closed farming system with the least possible environmental disturbance. Alder is collected from areas were this species is removed for forestry anyway. Electricity is bought from sources producing it in an environmentally sound way. The company supports research on suitable uses of used substrate.

22.8.4 Process at the Polar companies
A subcontractor delivers freshly cut logs to the farm. The logs are stored only briefly to avoid the formation of moulds, which could increase the infection pressure on the farm. The logs are fed to the chipper, the organic cereal grain is added to wood chips of a homogeneous and defined particle size and the mixture is conveyed to the storage silo and further to the bulk pasteurisation chamber.
The raw materials are fresh alder (Alnus) sawdust supplemented with organically cultivated cereal corn. The mean water content of blocks is between 60-65 %. The exact recipe for the substrate is kept confidential. The computerised heating unit controls heating and cooling. The substrate batch is heated up, mixed and cooled down during the phase. Temperatures below 100 °C are used and the process takes altogether approximately one day, heating and cooling included. This stage destroys most of the
23.5.5 Spawn run

Spawn run is divided into two periods: temporary laying and permanent laying. The first stage promotes fast growth of the mycelium, but its conditions are favourable for moulds and contaminants, too. For temporary laying, the logs are stacked horizontally, closely together in 1 m high stacks. The logs rest on two non-inoculated logs, placed at right angles to the spawned logs. Alternatively, logs can be stacked vertically: 50 to 80 logs under a shady canopy. The logs touch each other and on the bottom a plastic sheet or straw should be used to prevent moulds from the soil contaminating the logs. The conditions during temporary stacking also favour the growth of competitor fungi, therefore the logs are moved to a more permanent site after one to two months. It is not advisable to water the logs during spawn run. Only if they have dried excessively, some watering can be applied. Consult the appropriate section in the chapter Mushroom farms about the conditions for a permanent yard.

23.5.6 Fruiting

Experienced growers can ‘feel’ whether the logs are ready to fruit. More objective ways to check the mycelial growth are the following:

- Measure the pH: if it decreased from 5.5-6 at the time of inoculation to 3.8-4, the wood log is ready for fruiting (to measure pH, put 10 g of inside wood in 100 cc distilled water, then measure pH).
- Stain a cross section of wood with ferro chloride (FeCl₃) the mycelial area will turn white, the rest will turn brown, because the Shiitake has degraded tannin. If more than 75% of the surface of the cross section turns white, the log is ready to fruit.
- Cut a section of the wood and put it in a plastic bag. Some distilled water should be added to prevent the sample from drying out. After one week the mycelium should have turned the cut ends of the wood white.

Primordia initiation requires the right temperature for the specific strain and a high humidity. A temperature difference of 8 to 10 °C between day and night, a physical shock, a high moisture content in the logs all stimulate fruit body formation. Often, a
water bath is applied to the logs. The water will rapidly replace the CO₂ in the logs and provide sufficient moisture for one or two flushes of mushrooms. The logs can also be slammed against the ground, or thrown on the ground and watered heavily. Afterwards the logs are replaced upside down with the previous back facing front.

**Pests and diseases.** Most moulds occur during spawn run, when moisture conditions favour other organisms such as *Trichoderma* species and *Stemonitis splendidens* on willow, *Stemonitis fusca* on maple. *Trichoderma viride* is the most feared contaminant, as it can parasitise on the Shiitake mycelium and contaminate already colonised logs. Other unwanted fungi include *Poria versipora*, *Hypoxylon truncatum*, *Hypoxylon coccineum*, *Merulius tremellosus*, *Cryptoderma citrinum*, *Lenzites betulina*, *Schizophyllum commune*, *Trametes sanguinea*, *Poria vaporaria*, *Porodiscus pendulus*, *Panellus stypticus*, *Bulgaria polymorpha*. *Stemonitis* typically develops at a later stage in growth, when the logs have already yielded some flushes.

Bacteria may develop if the humidity is too high: *Pseudomonas fluorescens* may cause bacterial blotch on caps of maturing mushrooms, if the caps are in contact with the wood or touch each other. Pick the mushrooms before they touch each other.

### 23.6 Case studies: Japan and Taiwan

Two case studies are given: the traditional cultivation method on hardwood in Japan, and its adaptation to a warmer climate and different kinds of trees in Taiwan. By comparing these two, many features of wood log cultivation will become clear.

#### 23.6.1 General comparison

The quality of Shiitake grown in Japan is usually better because of the lower temperatures and optimal types of trees, like oak. The Japanese conditions sustain growth of better quality strains than the Taiwanese conditions. The wood plug spawn in Japan is slightly more expensive, but more convenient to use. The higher costs of labour in Japan demand a less time-consuming spawning technique.
23. CULTIVATION ON WOOD LOGS

Relative cost of growing in Taiwan and Japan (US$, 1991)

<table>
<thead>
<tr>
<th></th>
<th>Wood</th>
<th>Spawn</th>
<th>Labour</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>52</td>
<td>23</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Taiwan</td>
<td>57</td>
<td>21</td>
<td>17</td>
<td>5</td>
</tr>
</tbody>
</table>

- traditional Japanese techniques on hardwood in a temperate climate
- Taiwanese method on softwood in a subtropical climate

log life: 4-6 years
spawn run time: 1-1.5 years
yield: 10-20%

23.6.2 Case study: traditional Shiitake cultivation in Japan

Shiitake cultivation on sawdust in plastic bags has not become very popular in Japan. The traditional way is most used, although modernised to some extent. Both tree species and climatic conditions (temperature, humidity in spring and autumn), are favourable for the cultivation of Shiitake. Although the name Shiitake derives from the Shii-tree (*Castanopsis*), different kinds of oak (*Quercus*) are mostly used nowadays. The number of flushes and the lifespan of the logs depend much on the type of strain, the diameter of the wood logs, and the average temperature. Thick logs need a longer spawn run period, but will generally yield high quality Shiitake. They will last for up to six years.

<table>
<thead>
<tr>
<th>Diameter of logs</th>
<th>6cm</th>
<th>8cm</th>
<th>10cm</th>
<th>12cm</th>
<th>15cm</th>
<th>18cm</th>
<th>21cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of rows</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>number of holes per log of 1.2m</td>
<td>12</td>
<td>16</td>
<td>20</td>
<td>24</td>
<td>28</td>
<td>36</td>
<td>44</td>
</tr>
</tbody>
</table>

*Note: rows run parallel to the length of the log*

**Spawning:** The logs are best spawned when the temperature is just above 10 °C. The Shiitake mycelium grows vigorously above 10 °C, whereas competitors prefer higher temperatures. A temperature of 20 °C is actually more suitable for mycelial growth of Shiitake, but it is even more suitable for competing micro-organisms. Wood plugs and wedges are most used for inoculation. Check the table for the required number of holes.

**Spawn run:** This usually takes 12 to 18 months, depending on the strain,
wood species, temperature, and number of holes per log. Temporary laying takes one to two months. The logs are stacked as discussed before (horizontal or vertical) under rather moist conditions. Moulds favour a high humidity too, so as soon as the mycelium has established itself (in one to two months) the logs are unstacked and moved to the permanent laying yard. Choose a way of stacking that suits the local conditions best. After 1 to 1.5 years the mycelium will have fully colonised the logs. Use one of the above mentioned techniques to determine whether colonisation is complete.

**Raising and fruit body induction:** If the weather is suitable for the strain that has grown through the logs, the logs are removed from the permanent laying yard and receive a water bath. The water temperature should be 13 to 18 °C. In the winter the logs are soaked for 16 to 48 hours, in the summer for 6 to 18 hours. Special soaking racks can contain pallets with wood logs. A steel tube at the top of the pallet prevents floating of the logs in the soaking tank. During or shortly after soaking, the logs are vibrated to allow trapped CO₂ to escape. A simpler method is slamming the logs on the ground. The logs are covered with plastic for four to six days to maintain a very high humidity. They can be kept in an empty soaking tank for this period, or brought to the raising yard and covered there. Pinheads (primordia) will grow from the logs within one week after soaking. Usually the logs are stacked according to the X-frame stacking method. Horizontal stacking requires expensive racks. Moreover, mushrooms emerging from the bottom of horizontally stacked logs will be deformed and more difficult to detect and therefore horizontal racks are seldom used.

**Harvest:** One to four flushes per year can be harvested under the growth conditions prevailing in Japan. Many farmers nowadays raise the mushrooms indoors in winter and summer, because the price of the fresh product is much higher then. The mushrooms should be picked under relatively dry conditions. Do not touch the gills when harvesting. In total (after five-six years) about 25 kg of dried mushrooms can be harvested per m³ of wood logs. The first two years after colonisation give higher yields than later years.

### 23.7 Cultivation of Shiitake on wood logs in Taiwan

The Chinese in Taiwan consume large amounts of Shiitake. The local subtropical climate, however, is not very well suited for its cultivation. Wood log cultivation only takes place in the mountains, where the outside conditions are better suited for Shiitake. The Taiwan Agricultural Research Institute has performed many experiments on how to adapt the traditional cultivation methods from Japan to the warmer Taiwanese climate. The higher temperatures mean that more spawn has to be inoculated to ensure a
fast spawn run, countering the higher contamination pressure. The logs are ready for fruiting sooner, but give lower quality fruit bodies than in Japan because of (usually) higher temperatures. Hardwood logs are only available in limited quantities in Taiwan. Therefore many kinds of other (softwood) trees have been tested and some were suitable to grow Shiitake, too. The treatments of hardwood and softwood are somewhat different during fruiting management.

<table>
<thead>
<tr>
<th></th>
<th>Hardwood</th>
<th>Softwood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spawn run</td>
<td>6 months</td>
<td>6 months</td>
</tr>
<tr>
<td>Fruit body induction</td>
<td>2 days</td>
<td>2 days</td>
</tr>
<tr>
<td>Growth of mushrooms</td>
<td>5-9 days</td>
<td>5-9 days</td>
</tr>
<tr>
<td>Picking</td>
<td>2 days</td>
<td>2 days</td>
</tr>
<tr>
<td>Rest</td>
<td>1 months</td>
<td>2-3 weeks</td>
</tr>
<tr>
<td>Fruit body induction</td>
<td>2 days</td>
<td>2 days</td>
</tr>
<tr>
<td>Growth of mushrooms</td>
<td>Etc.</td>
<td>Etc.</td>
</tr>
</tbody>
</table>

**Inoculation with sawdust:** In Taiwan there is no manufacturer of wood plugs or wood wedge spawn. Therefore, the use of sawdust spawn is standard. The mycelial coat of the spawn (if older this will have a brownish colour) is not suited for spawning and should be discarded. Many more holes are drilled in the logs than in Japan with its lower contamination pressure. Holes with a diameter of 15 to 18 mm are drilled with a distance of only 10 cm and filled with spawn. The depth of the holes should be 3 to 4 cm when the logs have a diameter of around 12 cm, and 5 to 6 cm deep if the log has a diameter of 20 cm. A metal stick is used to compress the spawn in the holes, which are then covered with hot wax to prevent evaporation.

The temperatures in Taiwan are higher than in Japan, and since also more holes are drilled per log, this results in a fast spawn run; six to eight months are sufficient to colonise the wood logs. The high temperature also favours many pests and diseases. This is another reason why so many holes are drilled per log. Try to inoculate near branching twigs, establishing a fast spawn run near spots where contamination is most likely to occur. There are also some extra holes drilled at the end of the logs: the so-called 'strong points'.

After temporary stacking, the logs are brought to the laying and raising yard. In Taiwan, laying and raising take place at the same spot.
The logs are moved to the raising yard after one month of mycelial growth (fresh mycelium can be seen at the cross cuts). The mycelium has to grow for another five months before it is ready to fruit. In practice it is the farmer’s experience that tells him when to induce fruit bodies. If the mycelium has been growing well and the surface of the logs is smooth, if the weight of the logs has decreased a little and when knocking on the wood sounds solid, he will start the water treatment. The polyethylene foil is removed from the roof, so that rain can enter. Heavy spraying for two days and a temperature difference between day and night all stimulate fruit body formation. Turning the logs upside down also promotes pinheads.

A comparison of growing under subtropical conditions on softwood or on hardwood in a colder climate.

Small mushrooms will appear within a week. A polyethylene sheet should be reinstalled to prevent rain from deteriorating the growing mushrooms. The mushrooms will be ready to harvest in five to seven days. Their caps have to be strongly convex, and traces of the veil should still be visible.
The logs get a three to four weeks rest after picking, then they are thrown on the ground or turned upside down and watered heavily again. These methods are less labour-intensive than a water bath. About a week prior to this treatment some light watering is applied.

**Yield:** About 13 to 16% of the initial weight of the wood logs can be obtained in fresh mushrooms in this way. It takes quite a lot of time to select suitable logs for Shiitake cultivation in Taiwan. Labour is getting more and more expensive. The farmers also have to compete with growers from mainland China. More than half of the production is currently being produced by a different technique: on sterilised sawdust in plastic bags.

### 23.8 Wood log cultivation of Auricularia

Several kinds of *Auricularia*-species can be grown on both wood logs and small plastic bags. Two species are discussed in this section: *Auricularia auricula*, the small Jew’s ear (or Black wood ear in Chinese), and the larger *Auricularia polytricha* (Woolly wood ear in Chinese). The small species fruits at a lower temperature than the big Woolly ears. Check the strain for its fruiting temperature range when ordering or manufacturing spawn. Typical is 15 to 25 °C for the small and 23 to 28 °C for the big, woolly wood ear species.

**Suitable wood logs:** These typically have a diameter of 3 to 6 cm and are about six to ten years old. Many kinds of trees can be used because *Auricularia* is not as selective as Shiitake. Members of the oak family (Fagaceae) are preferred, but the trunks and branches of the Ipil-ipil tree (Wonder tree, *Leucaena glauca*) can also be used, as well as most other kinds of both soft- and hardwood.

Trees for *Auricularia* cultivation, reported from Taiwan:

<table>
<thead>
<tr>
<th>Species</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia confusa</em></td>
<td>Taiwan acacia</td>
</tr>
<tr>
<td><em>Acer palmatum</em></td>
<td>Maple tree</td>
</tr>
<tr>
<td><em>Aleurites fordii</em></td>
<td>Tung oil tree</td>
</tr>
<tr>
<td><em>Alnus japonica</em></td>
<td>Alder</td>
</tr>
<tr>
<td><em>Betula japonica</em></td>
<td>Birch</td>
</tr>
<tr>
<td><em>Bombax ceiba</em></td>
<td>Silk cotton tree</td>
</tr>
<tr>
<td><em>Broussonetia papyrifera</em></td>
<td>Common paper mulberry</td>
</tr>
<tr>
<td><em>Cetis sinensis</em></td>
<td></td>
</tr>
<tr>
<td><em>Elaeagnus pungens</em></td>
<td>Elaeagnus</td>
</tr>
<tr>
<td><em>Fagus crenata</em></td>
<td>Beech</td>
</tr>
<tr>
<td><em>Ficus retusa</em></td>
<td>Small-leaved banyan</td>
</tr>
</tbody>
</table>
Ficus stipulosa           Large-leaved fig
Gardenia jasminoides     Cape jasmine
Laportea pierastigma     Poisonous wood nettle
Macaranga tenarius       Macaranga
Magnolia obovata          Small-leaved mulberry
Morus australis           Poongamia
Pongamia pinnata          Varnish tree
Rhus verniciflua          Chinese tallow tree

The time of felling is similar to that of Shiitake: when the leaves have dropped from the trees and the sugar content in the logs is highest. Always cut logs of the same length, for example 1 m. This facilitates handling. Like in Shiitake cultivation, avoid bruising the bark. It will strip off, after which contaminants can easily enter. Soak the logs in water for 24 hours and rinse. Freshly cut wood logs do not need soaking. The water content should be rather high: 50% to 80%. When cracks appear on the cross section, the wood is too dry. To check the water content, saw a piece and split it in small 5 to 10 g pieces. Weigh the pieces, dry them in an oven and weigh again.

**Spawning and spawn run:** Usually sawdust spawn is available, check the paragraph inoculation with sawdust spawn in the case study on Shiitake in Taiwan.

**Spawn run:** The most important parameter to monitor is the humidity of the logs. Pack them in plastic and take care to keep them very moist all the time. It takes the mycelium one to two months to grow all the way through the wood logs. The logs should be rotated a few times to distribute mycelial growth more evenly. Check whether the mycelium has grown properly by using one of the methods described before (refer to Spawn run of Shiitake). The optimal temperature range for spawn run is between 20–26 °C.

**Fruiting:** Slant the logs against wooden bars and water heavily. This will stimulate fruit body formation. The second way to stimulate fruiting is to give the logs a dry resting period of two to three weeks. Afterwards they should be soaked in water for 12 to 20 hours and placed against bars. The first primordia will form within a week. In the beginning they will be located near the spawned holes, later they will emerge all over the log. Optimal temperature range is 14–22 °C. The Chinese distinguish five stages in the development of the fruit bodies:

- rice grain: 1.5 days
- coral: 1.5 days
- wood ear appearance: 2 days
- wood ear unfolding: 1.5 days
- maturation: 2 days

The duration of each stage is given assuming a temperature of 24 °C and a relative humidity of 95%.

Light is important in fruit body formation. The mycelium not only requires an amount of diffuse light, but also some directly radiated light (according to Chinese scientists). A light intensity of 500 lux is reported to be optimal for fruit body formation. The mushrooms become thicker and blacker when cultivated at the lower end of their fruiting temperature range. An air humidity of at least 80% is required to prevent the Wood
ears from shrivelling. Stagnant air will cause fruit bodies to rot and to become deformed.

**Harvesting:** After harvesting, the logs should be cleaned and all the remaining small fruit bodies should be removed. Stop watering and give the logs a two to three week rest. Then, repeat the treatment of watering or soak in water again. Logs typically last one to two years and give a yield of 1 kg fresh mushrooms per log of 1 m length with an average diameter of 10 cm or 10 to 15% of the weight of the freshly cut logs. Under relatively stable conditions in the tropics, harvesting can continue for some months before a resting period should be given. In temperate or subtropical zones, there will be no fruiting in winter when the temperature drops below the fruiting range, again depending on the strain used.

**Post harvest:** Wash the mushrooms thoroughly and dry them in the sun before packing. For the fresh market they can be kept in water for at least a week.

**Pests and diseases:** Some wood fungi may compete with *Auricularia, Schizophyllum commune*, many polypores and some myxomycetes. If during spawn run proper conditions were met, less than 10% of the logs will become contaminated.

### 23.9 Growing Oyster mushrooms on thick wood logs and stumps

In nature, Oyster mushrooms grow on many types of broadleaved trees. Most of them are saprophytic, but sometimes they can become parasitic. Living poplars (*Populus* spp.) sometimes host large bundles of Oyster mushrooms: the tree is seriously affected by the mycelium then. When the tree is located beside the road it has to be cut down to avoid any damage: the tree could easily break in a storm. Commercial cultivation of Oyster mushrooms is normally on some kind of ligno-cellulose-rich waste material, like straw, sawdust or cotton waste. Cultivation on stumps can easily be done, but usually most mushrooms emerge at the same time, when the weather is most favourable for fruiting. The irregular harvest makes marketing more difficult.

#### 23.9.1 Preparing a growing site and substrate material

Ideally, the site should be shady and have irrigation facilities. Either thick (more than 20 cm in diameter) logs or stumps, with their roots still in the ground, can be used. The logs should be fresh and be spawned within one month after cutting. There is no need to let the logs rest for a while to kill off the living wood cells with the method of spawning described below. When using logs, they should be placed on a piece of plastic or weather-resistant cloth. If the sheet is not water permeable, you have to take care that water will collect at the lower spots; cuts have to be made to make sure that the water can drain. Otherwise anaerobic conditions would arise. The cloth/plastic sheet will keep weeds at bay. Gritty material (fir needles, oyster shell grit) can be placed around the logs and stumps after spawning; this will discourage snails to feed on your mushrooms. Logs should not be longer than 75 cm in order to have them colonised within one season.

#### 23.9.2 Spawning and spawn run

It is best not to spawn with dowels or sawdust in holes. Especially the cambium of fast
regenerating trees (Populus, Salix, Ulmus) can overgrow that kind of spawn with new wood cells. It is better to cover the complete cut surface with straw-based spawn: the dead wood cells on the inner side of the cambium cannot defend themselves. Growing outdoors, the use of grain spawn should be avoided as it would attract rodents and insects. In case of thick trunks, the cut surface on the ground can also be spawned. Just lift the wood block, put a layer of 5 cm straw spawn under it, and then put the piece of wood on the spawn. Straw based spawn (or even non-sterile substrate, which is much cheaper) should be put in a 5 cm thick layer on top of the stumps, covered with a piece of plastic or newspaper, and subsequently covered with 5 cm of soil. The plastic/paper on top of the wood should not be tightened with an elastic, as these are not weather-resistant. A simple piece of rope is sufficient. The soil on top of the paper/plastic prevents the spawn from getting too hot. The mycelium will grow through the wood in 6 to 18 months, depending on the ambient temperature.

23.9.3 Fruiting
Fruiting will occur after heavy rains or spraying water; preferably in combination with a decreasing temperature. By spraying only a limited number of stumps/wood logs weekly, the harvest can be controlled somewhat. The first mushrooms usually appear from near the

Some Oyster mushrooms even grow at temperatures around 0 °C notice the ice on this Pleurotus ostreatus HK 35 strain.
spawned edges. Always pick complete bundles, do not try to cut away only the most mature mushrooms. All remaining pieces of the stems on the wood should be removed after picking, otherwise the left stems attract insects. Later flushes can appear all over the stumps; hardwood like oak can give a harvest for up to five years. The yield (fresh mushrooms/wet weight of wood) can be as high as 20%.

23.9.4 Pests and diseases
Other fungi may compete for available nutrients in the wood; as the wood ages, more competitors will show up. However, they do not provide a serious problem. Snails are more difficult to control; see the section in chapter 26 on what to do about them.
24 The collection and 'cultivation' of mycorrhizal fungi

24.1 Introduction

Some of the most appreciated mushrooms cannot be cultivated in growing rooms: mycorrhizal mushrooms like *Tricholoma matsutake*, *Cantharellus* spp., *Boletus edulis*, *Hydnum repandum*, and *Tuber* spp. only occur near their host trees. The term mycorrhizal derives from *mucor* (fungus) and *rhiza* (root). Mycorrhizal fungi (at least the ones which form edible fruit bodies) grow around and inside the root tips of the tree. Here the important chemical interaction occurs. As explained in the chapter Biology, these fungi tap carbohydrate sources from the roots of the trees. In this sense they are similar to parasitic fungi. Up to 30% of the sugar production of a tree may be used by mycorrhizal fungi! Still, it can be efficient for trees to trade the sugar against water and minerals. The tree doesn’t have to invest in a vast network of roots, as the fine threads of the fungal mycelium disclose the needed minerals. Another result of the mycorrhizal relationship is that the roots are less susceptible to infections and pathogenic fungi. The sugar content is kept low by the mycorrhiza, making the roots less attractive to pathogens. Other advantages include higher stress tolerance, drought resistance and protection against heavy metals.
### 24.1.1 Description of different species and aspects

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Popular name(s)</th>
<th>Typical aspects</th>
<th>Host trees</th>
<th>Price * indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boletus edulis</td>
<td>King Bolete, Cepe (F), Porcini (I)</td>
<td>This mushroom has a thick bulbous stem, which is partly covered by a netlike pattern. The pores do not bruise blue in contrast to other (mostly edible) boletes.</td>
<td>Broad range of both broadleaved and conifer trees</td>
<td>30 US$/kg</td>
</tr>
<tr>
<td>Cantharellus cibarius, C. subalbidus &amp; C. densifolius</td>
<td>Golden chanterelle, White chanterelle (subalbidus)</td>
<td>This yellow orange mushroom is one of the most popular mushrooms in temperate regions (USA, Europe, Asia). A tropical species with a similar taste (C. densifolius) is harvested from the African Miombo vegetation.</td>
<td>Quercus sp, Pinus sp, African Miombo, Uapaca sp, Gilbertiodendron</td>
<td>6-10 US$/kg</td>
</tr>
<tr>
<td>Hydnum repandum</td>
<td>Hedgehog, Pied de mouton (F)</td>
<td>This yellow mushroom is easily distinguished by brittle spines on the underside of the cap. It occurs in temperate regions.</td>
<td>Supposedly mycorrhizal</td>
<td>7-15 US$/kg</td>
</tr>
<tr>
<td>Tricholoma magnivelare</td>
<td>American matsutake</td>
<td>Very similar to the Japanese matsutake, this species appears in the Pacific Northwest. A typical aspect is the spicy odour, similar to sweet cinnamon.</td>
<td>Fir, pines, Douglas fir, hemlock, Lithocarpus sp, Especially Pinus contorta</td>
<td>16-200 US$/kg</td>
</tr>
<tr>
<td>Tuber gibbosum</td>
<td>Oregon white truffle</td>
<td>A pungent garlic-like smell distinguishes this truffle from many others. Found from northern California to British Columbia.</td>
<td>Douglas fir</td>
<td>200 - 300 US$/kg</td>
</tr>
<tr>
<td>Tuber magnatum</td>
<td>Italian white truffle, Truffle blanche de piemont (F), Tartufo bianco (I)</td>
<td>The most expensive mushroom in the world. Italian research indicates that this truffle is a late stage mycorrhizal mushroom. This mushroom is limited to some parts of Italy and will be difficult to grow elsewhere.</td>
<td>Quercus pubescens, Quercus pedunculata, Tilia sp, Populus sp, Corylus avellana, Salix sp</td>
<td>6000 US$/kg retail</td>
</tr>
<tr>
<td>Tuber melanosporum</td>
<td>Black truffle, Perigord, Truffle, Truffle noire de Périgord (F)</td>
<td>The best known Truffle with an outstanding taste grows in the southern part of France, and some parts of Italy and Spain. Successful fruiting outside the original habitat has been documented from North Carolina, California and New Zealand.</td>
<td>Quercus sp, Corylus sp, Carpinus sp, Pinus sp, Cedrus sp, Tilia sp</td>
<td>1000 - 1500 US$/kg retail</td>
</tr>
<tr>
<td>T. uncinatum</td>
<td>Summer truffle, Truffe grise de Bourgogne (F)</td>
<td>A lower valued Truffle from France, which typically grows in shaded forests, in contrast to the Black truffle. The taste is less outstanding, the price thus much lower.</td>
<td>Same as Perigord black truffle</td>
<td>200 US$/kg retail</td>
</tr>
</tbody>
</table>

* paid to pickers/producers unless otherwise stated; prices are very volatile, following supply and demand
Commercial mushroom picking in the Pacific. More than twenty different mushroom rooms are harvested commercially from the forests in Oregon and Washington: mushroom hunting has developed into a multi million dollar industry. In the Pacific Northwest of the USA, the government tries to minimise the negative impact caused by pickers. Littering, vandalism, campground crowding, road repairs, trespassing and reduced traffic safety are only some of the negative aspects associated with large-scale mushroom hunting. At some forests, the pickers have to buy a permission to hunt mushrooms; the collected fees are used to set up facilities for the pickers. Though the income level of the pickers is low, mushroom picking does offer employment to thousands of low educated and often poor English speaking citizens. For some higher educated pickers, the freedom and the time spent in places with great natural beauty are important reasons to pick mushrooms commercially. A relatively new development is tourism: organised trips for the Japanese who pick mushrooms which are then prepared by the chefs de cuisine of local hotels.

24.2 Benefiting from mycorrhizal mushrooms: harvesting from the wild

Two strategies can be chosen to benefit from edible mycorrhizal mushrooms: to collect them from their natural habitat or to plant inoculated seedlings and harvest the mushrooms from a plantation. Only for the most expensive mushrooms (the Truffles and Matsutake) plantations can become profitable. The high price of the Périgord black truffle can make it worthwhile to invest in a truffle plantation. The more expensive Italian white truffle is thought to be more a late stage mycorrhiza; only after 12 years the first mushrooms will appear, making research on this species a long-term venture. For the less precious mushrooms, it is in general not feasible to plant inoculated seedlings. Harvesting and processing the lower priced (but still expensive) mushrooms certainly contributes to extra income from a well-managed sustainable forest. It can thus contribute to the preservation of nature in general and forests in particular. Picking the mushrooms does not affect the ecosystem, as the mushrooms are only the fruit bodies of the underground mycelium. In this respect mushrooms differ from the harvest of medicinal plants. On the other hand, the invasion of large numbers of pickers in previously undisturbed areas will have a negative impact on the quality of the environment. In Poland erosion of hillsides has been reported, due to Chanterelle pickers. In the Pacific, pickers hunting for the most valuable button stage Matsutake sometimes disturb the mycelium when they rake the litter layer. This will affect the species negatively.

Mushrooms by accident: the Ecuador experience. Some thirty years ago, many exotic pines were planted in the mountains in Ecuador to prevent erosion and supply the people with wood. However, after twenty years more and more edible mushrooms began to grow under the trees. The mushrooms proved to be far more profitable than the wood: the mushrooms were dried and shipped to Quito, a travel of about one day. Now the focus is on preserving the forests in the area and harvesting non-wood forest products rather than just felling trees.
24.3 Certified organic mushroom collection in Zambia

Information kindly provided by Mrs. C. de Boer, Organic Producers and Processors Association of Zambia

The African woodland known as Miombo, covers a large part of this continent: it is particularly common in Tanzania, Burundi, Malawi and Zambia. The most common trees are *Brachystegia*, *Julbernardia*, *Isoperlinia*, *Marquesia* and *Uapaca*. The soil is rather poor and the trees thus have much advantage of a close collaboration with mycorrhizal mushrooms. There is a clear distinction between the rainy season in winter and a dry period in summer. Many mycorrhizal mushrooms abound here; most notably commercially attractive species like *Cantharellus*, *Russula* and *Amanita*. This case study focuses on a successful project in an area of 185,000 ha of Miombo vegetation in Zambia. The mushrooms grow commonly under forest trees (local names Mutombo, Musamba, Mutonda, Misase, and Misuku). They appear soon after the first soaking rains of the season. Mushrooms are picked and prepared during off-season for the coffee farmers. The harvested quantities depend on the amount of rain in the season.

<table>
<thead>
<tr>
<th>Latin name</th>
<th>Collection time</th>
<th>Estimated quantity / year</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amanita caesarea</em></td>
<td>Rainy season Dec-Jan</td>
<td>7.5 tons</td>
</tr>
<tr>
<td><em>Cantharellus</em> sp., marketed as</td>
<td>Rainy season Dec-April</td>
<td>22.5 tons</td>
</tr>
<tr>
<td><em>C. cibarius</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

24.3.1 History

In the raining season of 1996/7 wild mushroom drying trials were started by the Miombo Project (a forestry project founded by the European Union) at Mpongwe Farm Training Centre. The Mpongwe Development Company (MDC) took over in 1997/8 to test the commercial viability. The results were very successful and the project was consolidated as a commercial community venture. The first organic certification took place by Ecoceert in 1999. That year MDC transferred the management of the mushroom project to the Mpongwe Coffee and Organic Smallholders Cooperative, which is comprised of MDC employees and other farmers in the area who have smallholdings on which they produce organic crops like groundnuts, sunflower and sesame. They are technically assisted by the Organic Producers & Processors Association of Zambia (OPPAZ) with administrative support from MDC. Kantolo, Malembeka, Chibuli & Chisanga are some of the mushroom market/collection points.

24.3.2 Organisation of picking and collection

The organisation of the project is defined as follows: each marketing/collection point has an elected leader (Chairperson) with two assistants (Vice-chair and secretary) to organise the population in their area. There are 14 collection points registered within 4 zones. There are up to 300 pickers per collection point and 90% are women. The collection points are located roughly 50 km around the MDC plantation. The collectors
are the inhabitants of the zones. The leaders have to control the grading operation of the mushrooms on site and identify the variety delivered by the pickers. The buyers, who are members of the co-operative, may give recommendations to the leaders of the market point concerning the quality of the mushrooms as required.

24.3.3 Training
Specific training was given to the staff of MDC by the consultants of Miombo Project before the beginning of the project. Their trained staff is also part of the Mpongwe Coffee and Organic Smallholders Cooperative. All people involved in the project, including the local pickers, leaders, buyers, factory foremen, have all been trained according to their functions and are aware of the importance of the quality of the product. This wild harvest project and its associated market provide motivation to the population for preserving the environment. As in most regions in Zambia slash-and-burn agriculture has a significant negative effect on the woodlands.

24.3.4 Quality control during picking and collection
A schedule for the purchase at each market point is set by MDC before the beginning of the season. Pickers have to harvest early in the morning according to the planning of the purchase communicated to them in order to be in time for the delivery. Mushrooms covered with sand are rejected. Therefore the harvesters are taught not to pick them in this state. A shelter has been built at each marketing point for produce grading and scaling. Grading is done both by pickers and by the buyers at the market point by sorting into 1st and 2nd grade. The buyers reject some of the mushrooms and this ensures that pickers deliver high quality mushrooms. The accepted grades are weighed and recorded for each picker. The buyers bring crates of fresh mushrooms to the MDC factory for processing and drying.
Mpongwe Coffee and Organic Smallholders Cooperative buys the wet mushrooms (3000 kg/week in the 2001/2002 season), with technical assistance of MDC to ensure the processing and export operation of the products. A buyer passes by the marketing points twice a week for collecting the products. All the operations beginning from picking to exportation involved in this project are controlled.

24.3.5 Factory processing
On arrival at the coffee factory, the mushrooms are processed as follows:
Weighed, washed with water or cleaned with sponge, graded, sliced by hand or by slicing machine, dried by hot air on racks in a drying machine, sorted, packed, labelled and stored in 5kg weight boxes. The equipment is steam-cleaned with water with no addition of chemical products. The dryers are run for 3 days before use, to prevent coffee aroma to spoil the mushroom taste. This system ensures that there is no risk of mixing with other products.
The finished products are stored in a dedicated room in the coffee factory unit. The storage area is marked with the indicated “Organic mushrooms controlled by Ecocert F-32600”.
The mushrooms are only in contact with metals for the preparation and plastic is used for transportation and packaging thus preventing risk of contamination. The lot number system ensures good traceability. Each box has a serial number, which indicates the batch number, bag number, dryer number, drying day and year.

24.3.6 Documentation
All the records and methods of processing have been set as recommended by OPPAZ and Ecocert. The quality is considered at each level from the picking to the storage of the products.
A sample for each lot is kept in order to trace back and explain the origin of an eventual quality damage of the product in case of queries of the importer. The following documentation is available:
- Attendance register per day
- Weight of incoming mushrooms per day
- Mushroom daily form
- Temperature and dryer monitoring per day
- Packaging monitoring
- Sample of labels.

24.3.7 Export
MDC exported the product in 1999 to the USA and UK. In 2000/2001 mushrooms were also marketed to Switzerland via Tulimara in Zim—
babwe. In the following season England was added to the list of importing countries.

24.4 Starting a Truffle plantation

This section will focus on plantations of the Périgord Black truffles, although in Oregon, some successes with the Oregon White Truffles have been achieved. Due to the limited space available in this book, it is only an introduction; those who consider to start a Truffle plantation seriously, should obtain the cited literature (for details see the Appendix Bibliography).

With Périgord Black truffles, some successes have occurred in the last decades. It seems simple: plant inoculated seedlings and harvest truffles. However, the risks are still high as much is unknown and the first results can only be viewed after at least five years. Only if all of the following factors are met, success can be achieved:

- The right type of soil
- The right climate
- High quality seedlings ‘infected’ with the Truffle mycelium
- Good maintenance of the plantation.

24.4.1 Soil and site requirements

The Périgord Black truffle naturally occurs in France, Spain and Italy on calcareous soils, 100 to 1000 metres above sea level. The pH of the soil should be higher than 7.5 with an optimum of ca. 7.9. The topsoil is typically about 40 cm deep, overlying fissured limestone. If the soil is less deep, the subsoil should be very fractured to allow the roots to enter. Another important aspect of the subsoil is its drainage capacity. The subsoil is a factor which was often underestimated; if it does not drain well, the circumstances in the soil above become unsuitable for the Périgord Black truffle mycelium. The subsoil can change within a few metres; if calcium-poor, impermeable clay is present, the trees above that spot will yield less or no truffles at all.

The site should be protected from both cold winds or too dry winds. For that reason, the truffle-producing sites in France near the Mediterranean coast typically occur on the cooler northwestern slopes, which are protected from the dry winds. More inland, in the Haute Provence, the truffières can be found on the southwest slopes, which are protected from cold north winds.

In general the slope of a truffle plantation is less than 5°. Some successful truffières have been set up on hills with slopes of 20°; then steps were made to prevent erosion. If erosion occurs, the mycelium of the mycorrhizal mushroom will be exposed to drought and this will have a detrimental effect on truffle production.

Truffières should be well away (100 metres) from plants which host competing mycorrhiza. Even if trees are cut 5 years prior to the planting of inoculated seedlings, the soil may still contain the ‘wrong’ mycorrhiza. Inoculated seedlings should not be planted downhill from other trees; unwanted mycorrhizal fungi may then compete with the Truffle.
24.4.2 Climatic conditions
The natural distribution of the Périgord Black truffle is limited to Mediterranean regions between latitude 40° and 47°N; however, in New Zealand the first truffles were found just outside that range at 39°S. The following table summarises the approximate climatic conditions of truffle-producing areas in France and Italy.

<table>
<thead>
<tr>
<th></th>
<th>Range in France and Italy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual rainfall</td>
<td>600–1500 mm</td>
</tr>
<tr>
<td>Mean daily temperature in summer (July)</td>
<td>17–22 °C</td>
</tr>
<tr>
<td>Mean daily temperature in winter (January)</td>
<td>1–8 °C</td>
</tr>
<tr>
<td>Annual sunshine hours</td>
<td>1900–2800</td>
</tr>
<tr>
<td>Summer sunshine hours</td>
<td>1200–1800</td>
</tr>
<tr>
<td>Approximate degree days*</td>
<td>900–1900</td>
</tr>
<tr>
<td>*Mean daily temperature above 10 °C accumulated over the growing season</td>
<td></td>
</tr>
</tbody>
</table>

Source: Ian Hall e.a.: The Black Truffle.

24.4.3 Planting
The soil should be tested first before any other investments are done; specialised laboratories can assist in that field (in New Zealand the Institute for Crop and Food Research provides this kind of services). Planting is typically carried out in late autumn through early spring. The choice for hazels or oak depends on the investor’s plan: hazels produce truffles quicker (in France after four years) but have to be removed when they are 25 years old. The oaks take somewhat longer to produce (typically 7–10 years, occasionally faster as was the case in New Zealand) but last much longer.

If a simple grid is used, and assuming that the fungal mycelium develops radially, space is used inefficiently. A hexagonally close packed planting scheme is more efficient. Typical planting densities range from 100 – 500 trees per hectare. Lower planting densities are more appropriate for climatic zones and fertile soils which support rapid tree growth. On the other hand, high density plantations produce earlier at the expense of higher investments. It should be noted that the Périgord Black truffle disappears with the closing of the canopy. As the trees grow older, it may be necessary to cut down some trees to keep up the productivity of the truffière. In France truffières of over 80 years old have been established.

In the truffle-producing regions, inoculated oak (Quercus) or hazel (Corylus avellana) are available. There used to be quite some genetic variability between both plants and mycelium; this depends much on the used production technique for both plants and mycelium. Preferably a homogeneous product should be delivered. The plants may be grown either in plastic or cellulose Melfert bags. In both cases the plants should be watered very well before planting; in the case of plastic bags, these of course have to be removed before planting. The holes for the plants should be deep enough to allow the potting soil to become even with the ground. To avoid ponding around the trees, the surrounding soil in a circle of about half a metre should be slightly elevated. Compac-
tion of the soil should be avoided: it seems that one of the causes of the declined production in France is due to the use of heavy machinery, which compacts the ground and limits aeration.

24.4.4 Protecting the plants
The plants should be protected from deer, rabbits and any other animals that may cause considerable damage. Some owners use electric outriggers to control unwanted visitors. Another possibility is a tree shelter. Windbreaks can be beneficial as they prevent the soil from drying out; storms may also cause physical damage to the young trees. If strong winds often occur, a windbreak will prove necessary for at least the first years of a truffle plantation — an important consideration in parts of New Zealand.

![Claremont truffière in New Zealand; note the windbreaks (courtesy Ian Hall).](image)

24.4.5 Maintaining the truffière
A lightly cultivated soil seems better for truffles; in spring the top layer is cultivated once. Later cultivation might damage the roots or even the emerging truffles. Later in the season, weeds can be controlled by mowing. The truffle mycelium will form so-called brûlés, bare soil where the truffle mycelium has killed most of the vegetation. There is some discussion whether to cultivate the inside of a brûlé or not. There is also some debate whether the trees should be pruned or not. Local climatic conditions probably determine whether pruning is beneficial or not: pruning allows more sun to warm the soil. In relatively cool areas this will increase the soil temperature.

Harvesting. On oaks, it takes at least five years and in France and Italy usually ca. 10 years before the first truffles appear. Hazels are somewhat faster (four years). How can you tell whether you have ripe truffles? Actually it may seem unpractical for a fungus to form its fruit bodies under the ground. It does have some advantages, as the fruit bodies below ground will not be detected by above ground mammals and insects. In the natural habitat, the ripe truffles would start to produce such an aromatic odour, that pigs dig for them in a frenzied state. They will contribute to spore dispersal as they bring the truffle above the ground and consume them. In the past people used pigs for the collection of truffles, but they have mostly been replaced by dogs as these are easier to handle.
Brûlés at Oakland Truffière: the mycelium of the Périgord Black truffle kills most weeds around the stem of the trees; Right: Black (Perigord) Truffles (courtesy Ian Hall).

Yields. Secrecy still surrounds much of the truffle plantations in France. Lefevre & Hall (2001) quote yields in French truffières of up to 150 kg per hectare within 15 years after planting.
Of the twelve truffières established in New Zealand prior to 1990 six were producing Périgord Black truffles by 2002. All these plantations are situated north of Christchurch (43°S). The highest yields have been from a truffière established at 38°S on a volcanic ash soil with a natural pH of around 6.0 that had been limed to increase the pH to 7.8. Halfway through the 2002 season this truffière had produced 11 kg from 30 trees of which 2 kg were first grade. The reported yield range from European truffières is very broad: from only a few kg per hectare to 150.

Further reading on truffles:
Hall, I. et al.: The Périgord black truffle
Sourzet, P.: Guide pratique de trufficulture (French)
Callot, G.: La vie, la terre, la truffe (French)

Traditionally, pigs would hunt for truffles in France. Dogs however are easier to handle and have by now replaced pigs almost entirely. Colin Bailey with his Truffle sniffing dog Tipper (courtesy Ian Hall).
25 Post-harvest handling

The edible mushroom is a highly esteemed product with a short shelf life. Wilting, ripening, liquefaction, and change of flavour, texture and constitution decrease its value significantly. Cooling and modified atmosphere packaging can be used to delay senescence in mushrooms for the fresh market. Special conservation methods have been developed, most of which are described in this chapter. The first part pays attention to:

- quality grades and harvest,
- how mushrooms can be packed for the fresh market,
- how they can be conserved for future consumption.

The second part treats the different conservation methods:

- canning,
- brining,
- drying,
- freezing,
- lactic acid fermentation,
- pickling in either oil or vinegar.

25.1 Quality grades and harvest

Mushrooms should be picked at the stage at which they have the highest profitability. Mushrooms which have been picked at a young stage are generally more expensive. It depends on the quality requirements of the particular customer or middleman what the grower should deliver.

The mushrooms should be dry when they are picked; spraying (or rain) a few hours before picking reduces the shelf life of most cultivated mushrooms.

25.1.1 Picking

The pickers should gently break the mushrooms from the substrate or casing soil; tearing chunks of mycelium out of the substrate or casing soil should be prevented. The mushrooms are then cut to the desired stem length. Mushrooms can easily be damaged; it is therefore best if handling is minimised. Immediate grading at picking and packing them in the same packages in which they will be sold ensures that they are touched only once: at the moment of picking. Instruct pickers thoroughly to stick to the following rules:

- always pick mushrooms from newest beds/rooms first,
- do not touch sick fruit bodies (collect them at the end of picking in a separate bag, disinfect hands and clothes of the involved worker afterwards).
25.1.2 Quality grades in Shiitake

The quality of Shiitake depends on their maturation stage and cap thickness. Outside East-Asia, there is little grading of Shiitake. Deformed and broken mushrooms are sold as second quality, whole mushrooms as first quality. The grower has to reach agreement with the wholesaler about desired characteristics like stipe length and grading. Growers of wood log-grown Shiitake try to promote their mushrooms as being superior to those produced by substrate-cultivators. In fact it is the environment and the exact strain that determine which quality of mushrooms can be harvested.

In Japan, Shiitake are strictly graded by many different classes, cap opening and thickness of the cap being the prime determinants. 60 to 70% open caps for ‘donko’, 80% open for ‘koko’, and 80 to 90% open for ‘koshin’. The ‘donko’ grade with cracks on the surface of the caps is the most expensive grade. The Chinese have a similar grading system, in fact the Japanese ‘donko’ and the Chinese word ‘Donggu’ are written and mean exactly the same: Winter mushroom.

It should be noted that quality is not objective: in Taiwan Shiitake in supermarkets are packed tightly and the mushrooms smell a little fermented. If the public is accustomed to this smell, there is no need to change this way of packing. In Europe and the USA, however, evaporation in Shiitake should be allowed and weight loss has to be taken for granted.

In the USA, there is no official standard, although the following is commonly used:

- Extra large : 11,5 cm (4,5") or more cap diameter
- Large : 9–11,5 cm (3,5-4,5")
- Medium : 6,5–9 cm (2,5-3,5")
- Small : 5–6,6 cm (2-2,5")

Caps must be inrolled, otherwise the mushrooms are considered second grade. Any deformity or imperfection can cause the mushrooms to be regarded as second grade, depending on the local market.

25.1.3 Oyster mushrooms

Oyster mushrooms can either be harvested in bundles or as single fruit bodies. Some concepts of Oyster mushroom cultivation rely on harvesting whole bundles (e.g. Oyster mushroom cultivation in Japan on bottles). Especially Pleurotus ostreatus and P. cornucopiae can be harvested that way. Harvesting and marketing young bundles of Oyster mushrooms has the following advantages: many mushrooms can be picked in a brief period, the mushrooms look nice and stay fresh longer, and the buyers also pay for the stems.

Most Oyster mushrooms, however, are sold as individually picked mushrooms. They
should be picked when the outer margin of the fruit bodies is only just rolled inwards, on the verge of becoming horizontal. Storage time will increase when they are picked at a stage just before maturation. Stipe length should be discussed with the buyer.

25.1.4 *Agaricus bisporus* and *A. bisporus*

White button mushrooms are graded in three grades:
I. young button stage mushrooms
II. veiled mushrooms
III. open caps, mature mushrooms

The first two grades can be sold fresh, the third grade is usually canned. Within grade I and II, the price further depends on the size of the mushrooms. The smallest grade I are the most expensive, but they cost the pickers a lot of time to harvest. Extra large ‘giant’ White button mushrooms have less picking costs.

In some countries open caps are sold as ‘flats’ on the fresh market. A disadvantage of open caps is that they distribute a large number of chocolate brown spores. When the mushrooms are prepared, the dish looks dark, too. Apart from the grade and the size of the mushrooms, the mode of packing determines the price. The grade I mushrooms in small consumer-packages are more expensive than loosely packed mushrooms of exactly the same quality.

25.1.5 Modified atmosphere packaging

Reduced oxygen and elevated carbon dioxide levels delay maturation of harvested mushrooms. The modified atmosphere can be obtained by wrapping the product with plastic films. Care should be taken to avoid anaerobic conditions inside the package, which may occur if the plastic film has a too low gas permeability. Oxygen deprivation will result in the production of off-flavours and anaerobic spoilage. The necessary gas permeability of the plastic depends on the type of mushroom and the upper temperature at which it is kept. Plastic films should be available in a range of different gas permeabilities. The permeability can be reached by microperforations or by using a special kind of plastic with high permeability.

25.1.6 Fresh market

Under ideal conditions, mushrooms for the fresh market are packed with a plastic film and cooled rapidly after harvesting. The plastic film provides good protection from
water loss, as long as the storage temperature is more or less constant. In an ideal situation, the mushrooms are transported in a cooled container and delivered to the wholesaler, who stores them in refrigerated rooms. They are prepared for distribution, brought to shops, and sold to individual consumers in a refrigerated state. Repeated exposure to fluctuating temperatures, however, should be avoided. When the temperature goes up, the mushrooms will lose water. If the temperature drops, water will condense inside the package and on the surface of the mushrooms. This will lead to fast withering.

*Agaricus* spp.: Mushrooms kept at 4 °C will stay fresh for about one week. Optimal CO₂ concentration (3-6%) can be achieved by a plastic film around the packaging. Quick cooling with moistened circulating air directly after picking is best. *Agaricus bitorquis* is more robust than *A. bisporus*, which makes it a better candidate for the fresh market.

*Pleurotus* spp.: Experiments in the tropics showed that keeping the mushrooms at 8-10 °C in pre-packs wrapped in perforated polyethylene films is a good way to keep the mushrooms fresh. They can be kept for four days. In Western countries, Oyster mushrooms are cooled down to 2 °C directly after picking.

*Volvariella volvacea*: Only mushrooms harvested in the egg-state can be marketed on the fresh market. Open mushrooms deteriorate too fast, they should be eaten within two days. The ideal storage temperature is 15 °C. At low temperatures (4 to 6 °C) the mushrooms liquefy rapidly. Mushrooms packed in completely closed bags can be kept for only two to three days. In perforated bags at 10 °C the mushrooms can be kept for four days. The weight loss is then about 10%. Storing at 15 °C gives a better quality, however, and the mushrooms can be kept for four days, too.

### 25.1.7 Transportation and packing for export markets

*Volvariella volvacea*: Mainland China used to export its fresh straw mushrooms to Hong Kong by boat or train. The containers consist of three compartments, of which the middle one contains the mushrooms, and the outer ones ice. The air-freight shipments from Taiwan and Thailand are sent in bamboo baskets with an aeration channel in the middle and dry ice wrapped on top of the mushrooms.

*Lentinula edodes*: The experiences of Shiitake importers indicate that keeping the right temperature is less important than good aeration. It was frequently observed that Shiitake in completely isolated containers with dry ice arrived in good condition at a low temperature, but in a few days they would become spongy and could no longer be sold. Loosely packed Shiitake – 2 kg per crate (crates providing aeration from all sides) with dimensions of approximately 40 cm long, 30 cm wide, 11 cm high). 500 kg per air-freight container with aeration holes – lost more weight, but the quality was kept. Because the mass is great (500 kg), the container will only slowly warm up. As soon as it gets out of the airplane the container has to be cooled. Weight loss during the first three days may be as high as 6-7% and after one week even 10%.
25.2 Conservation methods

The aim of conservation is to keep the nutritional value of a crop for a longer period. The taste and nutritional value of fresh mushrooms is usually better than that of conserved mushrooms. In some cases however, the taste may become stronger because of the conservation treatments. Oyster mushrooms and Shiitake, for example, obtain a specific fragrance after drying. Mushrooms which have been conserved by lactic acid fermentation obviously taste sour.

The choice for a specific conservation method depends on the market requirements and the resources of growers and marketeers. If a canning plant is too expensive, growers can decide to market the product in brine. This process is much easier to perform. Conservation methods are also necessary when only part of the harvest can be sold fresh.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Principle</th>
<th>Requirements</th>
<th>Typical aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>canning</td>
<td>the mushrooms are sterilised and sealed from possible contaminants</td>
<td>canning lines with either autoclaves or gas burners under the conveyor belt</td>
<td>usually performed by larger companies, who have access to worldwide markets</td>
</tr>
<tr>
<td>brining</td>
<td>the salt concentration inhibits the growth of contaminants because of its high osmotic value</td>
<td>only containers and salt</td>
<td>suitable for developing countries as investments are limited and it is easier to perform than canning</td>
</tr>
<tr>
<td>freezing</td>
<td>the low temperature reduces the growth rate of contaminants</td>
<td>tunnels which can be cooled by liquid nitrogen</td>
<td>the cooling chain has to stay intact; high investments</td>
</tr>
<tr>
<td>freeze-drying</td>
<td>no water available for contaminants</td>
<td>freeze-drying equipment: compressors, freezers</td>
<td>high energy cost, but no need for cooling during transport. The product looks like fresh, but is much lighter</td>
</tr>
<tr>
<td>drying</td>
<td>no water available for contaminants</td>
<td>heat or sunshine, or simple plastic tunnel with ventilator</td>
<td>simple method, very suitable for developing countries</td>
</tr>
<tr>
<td>conservation in oil</td>
<td>seal from air, which is necessary for the development of contaminants</td>
<td>only containers and oil</td>
<td>simple method, very suitable for developing countries</td>
</tr>
<tr>
<td>vinegar pickling</td>
<td>the environment is unsuitable for most contaminants because of low pH value</td>
<td>only (acid-resistant) containers and vinegar</td>
<td>simple method, very suitable for developing countries and small-scale farmers</td>
</tr>
<tr>
<td>lactic acid fermentation</td>
<td>the environment is unsuitable for most contaminants because of low pH value</td>
<td>only containers and starter cultures of lactic acid bacteria</td>
<td>simple method, but a limited market for the product because of its typical (sour) taste</td>
</tr>
</tbody>
</table>
In some cases mushrooms are fried in oil and consequently canned, e.g. Shiitake mushrooms (marketed as Po Ku). The following table shows which conservation methods are commonly applied. These are marked with an x. Canning, brining and drying are the most used techniques. Not all conservation methods are equally suitable for all the different mushrooms. Canned Oyster mushrooms taste just horrible (except for Pleurotus cystidiosus and P. abalonus).

<table>
<thead>
<tr>
<th></th>
<th>Brining</th>
<th>Canning</th>
<th>Drying</th>
<th>Freezedrying</th>
<th>Freezing</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Auricularia</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Coprinus</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Dictyophora</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Flammulina</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Ganoderma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Hericium</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Pleurotus</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Volvariella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

* except for Pleurotus cystidiosus and P. abalonus

Vinegar pickling and covering with oil are usually domestic techniques, trial and error should determine whether these techniques are suited for a specific situation.

25.2.1 Canning

Canning is the most important conservation technique for Agaricus, Volvariella, Pleurotus cystidiosus and P. abalonus. Agaricus bitorquis is less suited for canning in whole pieces, because the skin turns grey after the canning process. Therefore it is usually sliced before canning; the grey colour of the skin is less apparent because the white of the flesh dominates. The taste of mushrooms suffers from canning, but the product lasts very long and can be shipped cheaply in standard sea containers.

Cleaning: The mushrooms are graded and sorted. Spots and blemishes are cut off. The casing soil in Agaricus cultivation should be washed away completely. The washing water may contain 0.1% citric acid or 0.3% sodium metabisulphite. These prevent the mushrooms from turning brown.

Blanching: The mushrooms are cooked in water at 95-100 °C containing 1% sodium chloride and sometimes citric acid.

Canning: Blanched mushrooms are canned with a brine solution or the blanching broth with a tablet of salt. The cans are then sealed.

Sterilisation: Two methods can be employed:
- a continuous process with gas burners under the conveyer belt with the cans,
- a batch process in an autoclave.

Monitoring the correct sterilisation time is important in order to prevent the growth of the anaerobic bacterium Clostridium botulinum. Exact canning times have to be determined for each situation, since they differ significantly, depending on factors such as:
the germination capability of the harvested product, the hygienics in the canning factory, the heat dissipation in the retorts, etc.

**Cooling:** Prevents boiling over.

**Labeling and packing:** When the temperature has dropped to 35-40 °C, labels can be put on the cans or glasses. Typical canning line capacities are: 1000/1500/2500/5000 kg fresh mushrooms per hour.

Smaller amounts can be canned using domestic techniques. About 40% of the fresh weight of the mushrooms is lost in canning. Some pre-treatments may increase the canning yield, like ‘evacuating’ the mushrooms. This process substitutes the gases in the mushrooms by evacuating the air in the mushrooms, immersed in water. About 5% higher canning yields have been reported for *Agaricus bisporus* grade I with a five minute evacuation period.

Metal cans are most used for packing. Glass is heavy and its use is thus limited to local consumption. Many customers feel the product looks nicer when put in glass. The cheapest grades are usually canned and not packed in glass. It is important to follow the regulations of the country the product is destined for. Exact specifications on packing, cap diameter, wet weight, drained weight, allowed preservatives and volume can be obtained from the FDA (Food and Drug Administration) in the USA and from the EC for export to the European market.
25.2.2 Problems in canning
In both Agaricus and Volvariella canning, some problems have occurred. Cans were reported to be spoiled by a sour, flat, sulphur smell. The following causes can be recognised:
- The initial bacterial spore count of Volvariella (before the canning process) was 10⁴ or even 10⁵ per gram. The high spore count may be caused by insufficient trimming or leaving straw, soil and cotton waste attached to the straw mushrooms. Agaricus usually has only 7 to 8 per gram.
- Non-uniform thickness of the slices. Different slice thicknesses require different times for heat penetration while sterilising.
- The sterilisation temperature may be too low or the sterilisation time too short. Before the spoilage reports appeared, one hour sterilisation at 121 °C was common. Later some factories increased the sterilisation temperature to 130 °C. The quality of the product decreases, however.
- Cooling the cans with water may introduce contaminations if the cans are not properly sealed.
- If the cans are not sterilised as soon as possible after blanching, the microbial count will increase considerably. This makes sterilisation more difficult.

25.2.3 Brining
Brining is a conservation method based on the principle of limiting free water. The high concentration of salt in the brine solution increases osmotic tension to a level which prevents the growth of micro-organisms. Spores cannot germinate either, because no water is available to them, although there is water all around them. The same principle is used in making syrups.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>The method is easy to perform.</td>
<td>Mushrooms in brine bring lower prices than canned ones.</td>
</tr>
<tr>
<td>The original shape and texture of the mushrooms are reasonably preserved.</td>
<td>A long period for desalting is required.</td>
</tr>
<tr>
<td>The method can be used for many different species of mushrooms.</td>
<td></td>
</tr>
</tbody>
</table>

The salt solution should be 18-25%. Per litre of water 180 to 250 g salt must be added. The water is brought to a boil, then stirred until the salt is dissolved. The brine has to cool down before use. The required quantity of this brine solution equals about half the volume of the containers.

The mushrooms have to be cleaned as described for canning. They should be blanched in a 5% salt solution for five minutes. Drain and let the mushrooms cool down. Arrange the mushrooms layer by layer and cover each layer with the cooled brine. Cover with a clean cheesecloth or close the container. All the mushrooms should be completely covered with brine.

The mushrooms need to be desalted before use. In some cases mushrooms in brine are
sent from developing countries to canning factories in Europe and the USA. There the mushrooms are desalted and put in glass or cans. Mushrooms in brine are less well suited for the consumer market because desalting may take considerable time: from two hours, if the water is changed frequently, to two days.

*Brining the Monkey head mushroom:* Another method to brine mushrooms is the following, which is in use in the countryside in China. The case study mentions Monkey head mushrooms, but other mushrooms can also be brined in this way. Remove the stalks of the Monkey head mushrooms since they have a bitter taste. Wash the mushrooms in clean water. Boil them in a 0.1% citric acid solution, then place them in cold water to cool. 25% salt (compared to the weight of the mushrooms) is used to brine the mushrooms. A layer of mushrooms is put in a container, a layer of salt is added, then the next layer follows, etc., until the container is full. The mushrooms are pressed down to make sure they are covered by the salt. The mushrooms have to be rinsed several times before they can be used.

25.2.4 Drying

Drying is based on the principle of limited free water availability, too. Drying has several advantages: it is easy, quick and safe and well-dried mushrooms can be stored for a long time. However, drying cannot be used for all species; species from the genus *Coprinus* for instance, liquefy when they mature. Other species have little marketing potential when dried.

Apart from the mushrooms mentioned in the table above, there are many wild mushrooms that are commonly sold dried. Among these are different members of the *Boletus* family, all kinds of morels (*Morchella* species) and several species of the genus *Cantharellus*.

In cultivated mushrooms, this conservation technique is mostly used for *Lentinula*. The Shiitake mushrooms get tastier after the drying process. Oyster mushrooms also become tastier. Nevertheless, the market for dried *Pleurotus* is small compared to the market for dried Shiitake.

Watch the following points during drying:

- the mushrooms should not touch each other,
- air circulation is very important: put the mushrooms on a grill rack or a metal grid,
- the area around the drying oven should be well ventilated in order to provide fresh dry air, while the moist air can flow out.

Mushrooms do not have to be crisp to the touch after drying, they should still be slightly flexible. Longer drying at low temperatures is safer than faster drying on high
heat, as the mushrooms could become toasted at high temperatures. If the fresh mushrooms are very wet, the starting temperature should not be low, otherwise they might start to rot. This is especially important for large unsliced mushrooms.

**Artificial drying:** Revolving driers are suitable for mass production. The temperature for Shiitake should start at 30 °C and increase every hour by 1 or 2 °C. In 12 to 13 hours the temperature should be 50 °C. Finally, the mushrooms are heated up to 60 °C for one hour to increase the lustre of the cap. Fluctuations in drying temperature will cause the cap to wrinkle, according to Chinese growers.

If artificial heating is used for drying *Tremella*, the temperature should start at 30 °C. When the surface of the mushroom is dry, the temperature is gradually raised to 48 to 60 °C. In 24 hours the fruit bodies will be dry.

**Drying by sun:** The quality of sun-dried Shiitake is generally less than that of artificially dried ones. Their moisture content will be higher. The sun-dried mushrooms can thus be kept for a shorter period than the artificially dried ones. *Volvariella* fruit bodies can be sun-dried by cutting them longitudinally in halves and drying them on a concrete floor in the sun.

A low energy input method of drying is by constructing a simple plastic tunnel and blow in cold air from one side. The freshest mushrooms should be added downstream, as they evaporate much water.

*Auricularia* can be sun-dried in a similar way as *Volvariella*. It does not need to be cut into smaller pieces. A disadvantage of drying mushrooms on the ground is that sand and dust decrease the quality and value of the product. It is better to dry on wire mesh above the ground.

Insects can be attracted by the smell of the drying mushrooms and deposit eggs on them. The larvae from the hatching eggs can spoil the dried mushrooms. Ideally the mushrooms should be covered during the sun-drying process with a wire mesh on both sides to prevent insects from spoiling the product.

25.2.5 **Freezing**

Freezing is a good way to preserve taste, flavour and consistency of the mushrooms. It requires, however, good transport lines with cooled containers. The technique is unlikely to be used in developing countries because it requires a non-interrupted cooling chain from factory to consumer. The mushrooms can be kept frozen for at least three months. They are easy to prepare for bulk consumers, like hotels and restaurants. The quick freezing method gives a whiter product, thus improving the appearance of the mushrooms. After blanching, the mushrooms are transported through a tunnel where
they are cooled with nitrogen vapour to -25 °C. Little energy is required if liquid nitrogen is employed. Only energy for ventilators, conveyor belts, etc. is needed, once liquid nitrogen is available. Typical capacities of equipment are 500 to 1000 kg per hour. Disadvantages of this conservation method are:

- it requires a high investment,
- a water layer is formed around the blanched mushrooms, which hinders loose freezing,
- the process of freezing and transportation from farm to market requires special equipment.

The market for frozen fungi is expected to grow, as both wild and cultivated frozen mushrooms are on sale in Italy and France in supermarkets. In other countries the distribution of the frozen product is mainly limited to bulk consumers.

25.2.6 Freeze-drying

The freeze-drying process consumes large amounts of energy, but there is no need for cooling the mushrooms during transport. Taste and flavour are well preserved, but a high investment is needed for the equipment. Energy costs will determine whether freeze-drying is profitable or not.

The mushrooms have to be cleaned and frozen in a closed container at -20 °C. The container is then evacuated and kept at a pressure of about 50 Pa (about 0.5 mbar). By subsequently increasing the temperature slowly to room temperature (over a time span of 10-12 hours), the internal water is transformed from the frozen state directly into the gas phase (sublimation).

The high cost of energy to keep the vacuum restricts wider use of this method. The mushrooms lose almost all their water, but it is recommended not to lower the moisture content below 7%. If the moisture content gets lower, the fats will start to oxidise and smell rancid. The freeze-dried product looks very similar to the fresh product, but its density is more than 10 times lower. Freeze-dried products are brittle and have to be packed in sturdy boxes. For long-term conservation, they are kept under nitrogen gas. In water, the freeze-dried product will quickly recover 80% of its initial moisture content. Freeze-drying will increase the flavour. Most freeze-dried mushrooms are sold in 200 litre packages to the food industry (the product can be used in instant soups). The best storage conditions are a temperature of 15 °C and a dry atmosphere.

25.2.7 Lactic acid fermentation

The lactic acid fermentation technique is a cheap and simple method to conserve food for several months. The technique is very suitable for developing countries, especially
on the countryside with limited resources. The main consideration is whether the market will accept mushrooms which have been conserved in this way.

**Method:** The mushrooms are cleaned and blanched by boiling them for a few minutes in water. The water should be boiling when the mushrooms are immersed. The mushrooms are filled in containers, e.g. glass ‘twist-off’ jars. They have to be covered by a solution of 50% sauerkraut juice and 50% water (or blanching liquid). For 1 kilo of blanched mushrooms, about 750 ml solution has to be added. In only a few days the lactic acid bacteria will have decreased the pH to below 4.1. This is considered the critical value, below which a harmful microflora will not develop. Once the pH is below 3.8 the product can be stored for 12 months at 25 °C.

25.2.8  Conservation in oil

This technique is also well suited for developing countries. The mushrooms should be cleaned thoroughly before blanching. It is best to bring the blanching solution to a boil first. Then add the mushrooms and cook for five to fifteen minutes. Take the mushrooms out of the blanching broth and put them in acid-resistant containers, like glass, or glazed pottery. Then add the blanching broth to the rim. No airpockets should be allowed between the mushrooms under the oil. The blanching solution may consist of a 2:1 oil to vinegar mixture, salt and herbs can be added as desired. Different types of oil can be used, it depends on local resources and taste which type of oil is chosen. Alternatively, mushrooms can be blanched in water with spices and other vegetables, and subsequently filled in glass containers. The blanching broth is filled to cover the mushrooms, after which a layer of oil is added to ensure that the blanching liquid is sealed from the air.

More value can be added to mushrooms when processed with herbs as a ready-to-use meal (courtesy D. Martinez-Carrera).
26 Pests and diseases

This chapter discusses the pests and diseases which may occur in mushroom cultivation and hygienic measures, disinfectants and pesticides to combat them. Recently, the use of pesticides is legislated more strictly; this trend will certainly continue in the near future. The chemical multinational Dupont considers taking a product like Benomyl from the USA market, as the costs of keeping it on the market increase significantly. Hygienic measures and biological control will therefore become more and more important. Please check whether the chemicals which are mentioned in this chapter, are permitted in the local situation before acquiring them.

All mushroom farmers will sooner or later encounter pests and diseases in their crop. They have to identify them and try to find out at which stage they have entered. Only if the cause is known, can appropriate measures be taken. Some pests indicate improper preparation of the substrate, others wrong management during fruiting. In all cases strict hygienic measures are most important. If pests are recognised at an early stage, then the damage can usually be restricted. If contaminants are allowed to spread, they will greatly increase the infection pressure on the farm.

A holiday break of several weeks or seasonal cultivation of mushrooms have the advantage that the infection pressure is not continuous. The infection pressure decreases when there is no substrate available for a number of weeks. Pests and diseases can affect the crop in several ways:

- by keeping the spawn from growing into the substrate (e.g. mice consuming grain spawn before the mycelium colonised the substrate),
- by colonising the substrate faster than the mycelium of the spawned mushroom (thus competing for nutrients),
- by damaging the mycelium of the spawned mushroom (nematodes or insect larvae feeding on the mycelium; parasitic fungi which produce hydrolytic enzymes),
- by damaging the mushroom itself (e.g. bacteria causing brown spots on the cap or insect larvae tunnelling the mushrooms).

Other factors, not treated in this chapter, which will adversely affect yields are:

- poor (degenerated) strains,
- unsuitable substrates,
- unsuitable climatic conditions.

The organisms responsible for the reported pests and diseases in mushroom cultivation are:

- insects,
- termites,
- mites,
- eelworms (nematodes),
- snails and rodents,
- parasitic fungi,
- saprophytic fungi (competitors),
- bacteria,
- viruses.
The best way to fight pests and diseases is to prevent them. Strict hygienic measures and physical barriers are most important. These are therefore discussed first.

26.1 Hygiene

The following hygiene should be routine:

- Start picking mushrooms from the uninfected (young) substrate and end with picking from the older (possibly contaminated) substrate.
- Inspect the farm in the same order: from uninfected young substrates to older, possibly infected substrates.
- Sterilise multiple-use picking baskets, mushroom containers or substrate containers before re-using. 1% Aldecol, 2% Deosan Super, 1% hypochlorite, or 2% formaldehyde solutions can be used. A more environmentally friendly disinfectant is H2O2, which has been reported to work well in concentrations above 5% on spores; it is less effective against mycelial fragments.
- Keep doors and windows closed or use a wire mesh to prevent insects from entering the growing rooms and spreading diseases.
- Always apply the spent compost at considerable distance from the farm. Heavily infected substrate should be burned.
- Remove undeveloped primordia from the substrate.
- Keep the floor of the growing house as clean as possible. Immediately sweep the floor after picking.
- Do not leave the cut stalks in the growing room. Feed them to pigs before they deteriorate, or dispose of them at some distance from the farm.
- Do not touch sick fruit bodies during picking. Collect them at the end of the picking and dispose of them immediately. Some farmers use a tissue, saturated with alcohol, to take sick spots out of the beds.
- Dip shoes in a disinfected solution before entering the growing rooms.
- Remove contaminated substrates as soon as the contamination occurs.

These hygienic measures are the first to be considered closely if an infestation occurs. In general this can be achieved by constantly maintaining a strict hygienic regime. Mushroom growing is a constant fight to keep parasites from spoiling the crop, and to keep competitor fungi from consuming the available substrates. If all else fails, the use of pesticides can be considered.

26.1.1 On disinfectants

The objective of the use of disinfectants is to kill spores of harmful organisms on equipment, walls and floors of rooms, shoes, picking baskets etc. Before the application of disinfectants, the rooms, equipment etc. should be thoroughly cleaned to remove organic debris. The presence of organic material reduces the effect of disinfectants considerably.

A much used disinfectant is a 2% formaldehyde solution (formalin is often sold in 40% solutions; use 100 ml of the 40% solution in 5 litres of water). Do not use a higher percentage: the formaldehyde creates a biological vacuum then, which is quickly filled by (possibly harmful) green moulds, such as *Trichoderma*. Be careful with the applica-
tion of formaldehyde as it causes irritation of skin and eyes and is possibly carcinogenic. At times, steam can be used to sterilise instead of formalin. In ecological mushroom growing no formaldehyde is allowed. The growers sterilise the casing soil with steam. \( \text{H}_2\text{O}_2 \) has been shown to be unable to kill *Trichoderma* mycelium. If green moulds cause problems at the farm, Deosan Super or Aldecol can be used. A 1% solution of Aldecol is sufficient, for Deosan 2% is recommended. A cheaper alternative for use under more natural conditions is quick lime. This is also used against some insects.

<table>
<thead>
<tr>
<th>Ingredients (gr/litre)</th>
<th>Aldecol</th>
<th>Deosan Super</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>144</td>
<td>40</td>
</tr>
<tr>
<td>Glutahyde</td>
<td>158</td>
<td>72</td>
</tr>
<tr>
<td>Didiciarnoliumchloride</td>
<td>113</td>
<td>103</td>
</tr>
<tr>
<td>Isoproponol</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

26.2 *Insects*

Flies and mosquitoes will be attracted by the smell of the substrate, the mycelium or the mushroom. An infestation at an early stage (during the spawn run) can be very harmful, as the larvae will feed on the mycelium and a second, third or maybe even fourth generation will attack the rest of the crop. At later stages they may even tunnel the mushrooms. Once the tissue is attacked by flies, contamination by pathogenic fungi can easily occur. Flies also act as the transport medium for mites and spores of contaminating fungi. Flies are most likely to occur in composted and pasteurised substrates. They are less likely to contaminate substrate in sterilised plastic bags during spawn run because the substrate is sealed from the air.
<table>
<thead>
<tr>
<th>Insects</th>
<th>Affected mushrooms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lycoriella</em> spp. sciarid flies</td>
<td>all cultivated mushrooms</td>
</tr>
<tr>
<td><em>Megaselia</em> spp. phorid flies</td>
<td>all cultivated mushrooms</td>
</tr>
<tr>
<td><em>Lepidocyrtus</em> spp. springtails</td>
<td>outdoor grown mushrooms</td>
</tr>
<tr>
<td><em>Achorutes</em> spp. springtails</td>
<td>outdoor grown mushrooms</td>
</tr>
</tbody>
</table>

Left: Puppet stage of phorid fly; right: adult *Megaselia halterata* (courtesy PPO).

*Lycoriella auripulla*, the phorid fly; right larvae with black head (courtesy PPO).

26.2.1 Monitoring
To see whether insects are present, use a yellow piece of plastic with glue. In this way it is possible to monitor which and how many insects are present in which stages. If the
threshold is passed, then control measures have to be taken (the threshold has to be examined by determining the minimum number of flies, at which an unacceptably low yield results). Depending on the circumstances, control measures can be taken like removing the infested substrate, or by using an insecticide.

26.2.2 Prevention
It is best if insects cannot get access to the mycelium. Wire meshes and filters in the ventilation openings of the growing room can help in keeping the insects from getting into contact with the mycelium. Special fly traps are available from specialised companies nowadays, special lights which attract insects are used in indoor mushroom houses. The insects which come near the light are electrocuted. This kind of light can only be used indoors. The lights are often hung above the doors of each growing room.

26.2.3 Treatment
Some pesticides can be used to prevent an outbreak of insect contamination, like Dimilin. This may affect the yields negatively, however.
For sciarid flies, a biological solution is to use the parasitic nematode *Steinerma feltiae*. It is capable of infesting and killing this particular kind of insect. The nematodes are distributed commercially by at least two companies, Koppert Biological Control (The Netherlands) and Beckerunderwood from the UK. These nematodes are not effective against phorid flies however.
Try to use as few chemicals as possible. Pesticides are relatively expensive and routine use of them could lead to resistant insects. Maintain strict hygienic procedures to prevent rather than to combat pests and diseases with pesticides. Be sure to obtain pesticides in a proper packaging, stating clearly when to use them and how to act in case of poisoning. The following pesticides can be used against insects, if local legislation permits. Some highly toxic pesticides like endosulfan have been banned in some countries.

**Diflubenzuron**: Dimilin (by Duphar) is non-toxic and will stop the development of the larvae. It has a long-lasting effect and can be applied in the substrate or in the casing soil as a preventive measure.

**Diazinon**: brands: Luxan, Basudine, Diazinon.

**Dichlorvos**: brands: Nogos, Nuvan, Vapona, Dedevap, Benfos, Bibisol, Canogard, Coopervap, Devipan, Dyvos, Erasekt, Mafu, Marvex, Mutox, Nutrax, Phosvit, Roxo. The following insecticides all belong to the group of Pyrethroids.

**Malathion**: Trade names: Carbofos, Cythion, Maldison, Malathon, Mercaptation. This is a non-systemic insecticide, which is less toxic than parathion. Its half-life is one month. It is widely used in tropical countries because of its low cost.

**Cypermethrin**: Dutch trade names include: Cymbush, Luxan Cypermethrin, and Polythrin.

**Deltamethrin**: Trade names: Decis Flow 25, Deltamethrin Flow 25, Parimco Delta-methrin.
26.3 Termites

Termites feed on plant debris and mushroom mycelium. Do a survey on the presence of termites before the start of the cultivation. If termite hills are near the beds, then the following pesticides can be used to control them, but a more environmentally sound practice would be a construction to keep the termites from entering the beds.

Fenvalerate: Pyrethroid from Sumitomo Japan (‘Goldrest Tribute’).

Cypermethrin: Pyrethroid from ICI, UK (‘Cyperator’), Dutch trade names: see above.

Permethrin: Pyrethroid from ICI, UK, also developed by Shell, Mitchell Cots, Ciba Geigy.

Brand: (‘Imperator’)

Chloopyrifos: Phosphorus ester from Dow Chemical Benelux, Antwerpen, Belgium.

Chloopyrifos is a pesticide used worldwide.

A cheaper alternative for outdoor cultivation is to sprinkle quick lime around the growing site.

26.4 Mites

Mites are tiny spiders (eight-legged creatures), that may feed on the mycelium or on the mushrooms themselves. Mites are especially active on weed fungi in a pasteurised substrate. In that case the mites show that the substrate was not properly prepared, and competing fungi are present. Mites can carry spores of unwanted fungi into the substrate; in this way they can spoil sterilised substrate in small bags, too. Once mites have entered, they are difficult to control. Try to prevent the other crops from being infected by disposing of the contaminated substrate as quickly as possible.

Sometimes mites are so numerous they will cause discomfort and irritation to pickers. In some countries sold mushrooms have to be free of insects or mites. Mites are difficult to remove from harvested mushrooms. Quick lime
sprinkling can control them, but do not spray it directly on the mushrooms or on the substrate. The following pesticides can effectively control mites:

**Diofopen**: trade names: Kelthane, Duphar Kelthane.

**Malathton**: (see brand names above). Apply during spawn run or in between flushes, do not spray on the mushrooms.

### 26.5 Eelworms (nematodes)

Millions of these very small worms are present in only one m³ of soil. In substrate, parasitic eelworms will feed on the mushroom mycelium and can thus affect the yield considerably. Saprophytic eelworms are only problematic if they are present in large numbers. In compost e.g. if the pasteurisation process hasn’t been performed properly, or if too much water is sprayed through the casing soil.

Check for the presence of eelworms by shining with a torch on the substrate in a dark growing room. The eelworms will waive in the light. On the other hand, specific eelworms can be helpful to mushroom growers as they feed on the larvae of sciarid flies.

It is interesting to notice that the mycelium of many Oyster mushrooms catches and consumes nematodes. Eelworms have not been reported to cause problems in Oyster mushroom cultivation.

**Treatment**: heavily infested cultures should be cooked out. The rooms have to be disinfected thoroughly before new substrate is placed in them. Maintaining a strict hygienic regime is most important to prevent eelworms from becoming a plague. Dipping shoes in a disinfectant before entering the growing room is an important measure to prevent eelworms.

### 26.6 Snails and slugs

Slugs and snails form a major problem when cultivating outdoors. Especially large slugs (either those with black and white striping or brown ones with an orange ridge) can bring substantial damage to the mushrooms. The smaller slugs eat less, but also degrade the appearance of the mushrooms. Don’t spill any beer to catch the snails; a physical barrier works best. Special slug edges, produced from metal, can keep the slugs out of your beds. Alternatively, you can order a load of broken shells (e.g. oysters) and distribute it around your beds. The slugs and snails hate the sharp edges and keep away from your mushrooms.
The *Stropharia* on the left is damaged by both snails (still present on the stipe) and slugs; the Shiitake on the right are attacked by a brown-orange coloured slug.

A biological treatment is to order nematodes, which infect the snails. The nematodes are sold in the form of clay, which contains millions of them. The clay and nematodes are dissolved in water and sprayed on the ground where the snails occur.

A chemical treatment with limited ecological hazard can be performed with special snail/slug pellets, which are not harmful for rodents, cats and hedgehogs. One of the chemical substances is ferri phosphate 1%; it works for a number of weeks if the pellets have been placed around the beds.

Recently, an article in Nature (2002) on the use of caffeine to kill slugs received much media interest. Some preliminary results indicate that coffee (which contains ca. 1-2% caffeine) sprayed on wood logs with Shiitake may actually help in keeping slugs away.

### 26.7 Parasitic and saprophytic fungi

Many fungi are known to be parasites of different kinds of cultivated mushrooms. They can be recognised by the shape and colour of their sporophores and/or their mycelium, or by the symptoms they cause. Some parasitic fungi cause problems during the spawn run phase, where they may feed on the mycelium of the inoculated mushroom. Others grow on the mushrooms themselves. A serious fungal attack results in complete loss of the crop. Saprophytic fungi compete with the spawned mycelium for nutrients. Some of them will grow faster into the substrate, thus preventing the spawned mycelium from colonising all of the substrate. The main causes of fungal infections are: improper preparation of the substrate and/or contamination at the time of spawning.

The following table shows which mushrooms can be affected by which unwanted fungi.
Only the major weed- and parasitic fungi are included; numerous others exist.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Vernacular name</th>
<th>Affected mushroom species</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Chaetomium spp.</td>
<td>Olive green mould</td>
<td>Mushrooms on pasteurised substrates</td>
<td>Slows down growth rate of inoculated mushrooms.</td>
</tr>
<tr>
<td>2 Cladothryum cepliculum</td>
<td></td>
<td>Pleurotus spp.</td>
<td>Causes soft rot of fruiting bodies.</td>
</tr>
<tr>
<td>3 Cladothryum varioporum</td>
<td>Cobweb moulds</td>
<td>Flammulina velutipes, Agaricus, Pleurotus, and others</td>
<td>Parasitises on surface mycelium or mushrooms. Causes quick spoilage of mushrooms.</td>
</tr>
<tr>
<td>(C. cladothryum), C. dendroides spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Coprinus spp.</td>
<td>Inkeaps</td>
<td>Mushrooms on pasteurised substrates</td>
<td>Substrate competitor.</td>
</tr>
<tr>
<td>5 Gliocladium deliquescens, G. virgins</td>
<td></td>
<td>Pleurotus spp., Lentinula edodes, Pholiota nameko</td>
<td>Causes brown rot of fruit bodies. Antagonist (competitor) during spawn run.</td>
</tr>
<tr>
<td>6 Neurospora spp.</td>
<td>Agent orange</td>
<td>Sterilised substrates</td>
<td>Substrate competitor.</td>
</tr>
<tr>
<td>7 Mucor spp.</td>
<td>Black pin mould</td>
<td>Contaminant in pasteurised and sterilised substrates</td>
<td>Substrate competitor.</td>
</tr>
<tr>
<td>8 Mycogone perniciosa</td>
<td>Wet bubble disease</td>
<td>Agaricus spp.</td>
<td>Irregular bulbs of wet mycelium occur.</td>
</tr>
<tr>
<td>9 Paecilomyces varioti</td>
<td>Green mould</td>
<td>Wood-inhabiting species</td>
<td>Temporary inhibitor of mycelial growth.</td>
</tr>
<tr>
<td>10 Penicillium spp.</td>
<td></td>
<td>Agaricus spp., Pleurotus spp. and others</td>
<td>Substrate competitor in both pasteurised and sterilised substrates.</td>
</tr>
<tr>
<td>11 Penicillium chermatinum</td>
<td>Smoky mould</td>
<td>Agaricus compost</td>
<td>Heavy infections reduce the yield considerably.</td>
</tr>
<tr>
<td>12 Sibirina fungicola</td>
<td>Sibirina rot</td>
<td>Pleurotus spp.</td>
<td>Causes parts of the fruit bodies to rot.</td>
</tr>
<tr>
<td>13 Trichoderma spp.</td>
<td>Green moulds</td>
<td>Agaricus, Pleurotus</td>
<td>Heavy infections with parasitic and other strains reduce the yield considerably</td>
</tr>
<tr>
<td>14 Verticillium fungicola</td>
<td>Dry bubble disease</td>
<td>Agaricus</td>
<td>Malformed fruit bodies: stipe thicker than cap if infected in a young stage. Mushrooms don't open normally.</td>
</tr>
</tbody>
</table>
1  *Chaetomium spp.* (olive green mould)
The presence of the olive green mould indicates anaerobic conditions during pasteurisation or spawn run, and/or too high pasteurisation temperature. *Chaetomium* excretes toxic metabolites.

**Symptoms.** The olive green mould often grows at black spots in the compost, where *Agaricus* mycelium doesn’t grow. The mycelium of *Chaetomium* is greyish white at first, when the ascospores are formed they can be recognised by olive green fluffy structures on the straw.

**Treatment.** Once *Chaetomium* has established itself, it is impossible to control. To prevent it from occurring, maintain proper conditions during substrate preparation. An oxygen concentration below 16% in combination with relatively high pasteurisation temperatures (> 62 °C) favours *Chaetomium* infections. A too dense substrate or a rather high moisture content also favour *Chaetomium*.

2  *Cladobotryum apiculatum*
The spores of this species are commonly present in the soil. Take hygienic measures to prevent them from getting into the substrate, like dipping shoes in disinfectant before entering growing rooms.

3  *Cladobotryum spp.* (cobweb moulds)
At times cobweb moulds are an indication of dead air in the growing room, with too
little air movement, high relative humidity and relatively high temperatures. It can spread
very fast, and grows as a parasite on the mushroom mycelium.

**Symptoms.** The name is quite appropriate: cobweb-like structures grow radially from
dead stumps or dead primordia, forming a veil which spreads rapidly. Cobweb moulds
spread through aerial hyphae, pickers and insects. With maturation, the colour can
change to yellowish-pink.

**Treatment**
- Remove stumps and dead mushrooms regularly from the beds/bags at the end of
  picking,
- Spray a 0.5% formaldehyde-solution on the spots where the cobweb occurs,
- Use a fungicide like benomyl, carbendazim or thiofanate-methyl in severe cases in
  between flushes.

4 **Coprinus spp.** (Inkcaps)
The large genus *Coprinus* contains mushrooms which vary in size from about 0.2 mm to the 40
cm high *Coprinus comatus*, the Shaggy mane. Most species share the characteristic feature of
dissolving the rim of the cap by autolysis. After the basidia have released the spores, they dissolve
and form an ink-like fluid, hence their vernacular name. Their appearance indicates: a too high
ammonia content in *Agaricus*- or *Volvariella* substrate, and improper heat treatment in case of
pasteurised *Pleurotus* cultivation.

**Prevention.** When the mushrooms appear it is too late to do anything about it. Prevent the next crops
from being colonised by Inkcaps by proper substrate preparation:
- increase conditioning period for *Agaricus,*
- decrease ammonium-content in the substrate-ingredients for *Volvariella,*
- ensure proper pasteurisation for *Pleurotus.*

5 **Gliocladium deliquescens,** **G. virens**
Species from the genus *Gliocladium* look similar to *Penicillium.* Both have compact
phialides at the end of the branches, but *Gliocladium* phialides bear slimy rather than
dry structures with the conidiospores inside. *G. virens* has been reported to grow even
on 0.2% formaldehyde!

**Treatment**
- Take hygienic precautions to prevent contamination during spawning,
- Use other disinfectants than formalin (run a test on petri dishes to check sensitivity
  for the applied disinfectant),
- Remove contaminated substrate as quickly as possible from the farm.
6 Neurospora spp.
The presence of Neurospora (‘Agent orange’) in plastic bag cultivation is an indication that the plugs became moist during sterilisation. Neurospora can enter the substrate through the wet plug. It is usually not very aggressive.

**Symptoms.** An orange powder-forming mass on top of the cotton plugs is typical for Neurospora.

**Treatment.** Check the airflow in the sterilisation chamber/autoclave and try to prevent the steam from wetting the plugs. Cover the plugs with a plastic sheet, or fold them downwards covered with plastic from the bags. Alternatively, dry heat can be used to sterilise, in which case the sterilisation time has to be adapted.

7 *Mucor* spp. (Black pin mould)
The presence of *Mucor* indicates poor hygienic conditions during spawning.

**Symptoms.** The vernacular name describes the macroscopical features quite well. The black pins can be seen on the surface of contaminated substrate.

**Treatment.** Check the source/vector of contamination: is it the air, the grower, or insufficiently sterilised substrate? If the heat treatment is the cause, then a larger number of bags should show signs of a mix of contaminants. Especially bags which have been packed too densely in the autoclave will be affected then. Sometimes individual bags show signs of contamination. A closer examination will reveal whether these are due to holes or cracks in the bags or not.

8 *Mycogone perniciosa* (Wet bubble disease)
Spores of this mould can be spread by mushroom flies and other insects, as well as by people and spraying water. They can infect the mycelium of the Button mushrooms and cause the formation of wet bubbles. In a later stage, the bubbles excrete reddish-brown drops. The mycelium of the Dry bubble mould doesn’t grow in the compost.

**Symptoms.** The Wet bubble disease distinguishes itself from the Dry bubble disease by the formation of moist bubbles, which produce a foul stench when they mature.

**Treatment.** Carefully analyse how the infection could have occurred and take appropriate measures. Fungicides like benomyl, carbendazim or thiofanate-methyl can be used in severe cases in between flushes.

9 *Paecilomyces variotii*
A relatively heat-resistant disease. It survives heat treatments below 80 °C.
10 *Penicillium* spp.
This is a well-known genus, containing about 100 species, of which *P. chrysogenum*, the producer of penicillin, is the most famous. Apart from *P. chermesinum*, few *Penicillium* species cause trouble in mushroom growing. If they do occur, it is usually a sign of improper pasteurisation or unsterile conditions during the spawning of sterilised substrate.

11 *Penicillium chermesinum*
Typical for this fungus is the large number of conidiospores which look like smoke when the contaminated compost is touched. The mycelium is white at first, only later it slowly turns brown. Infections can reduce yields by up to 80%. It has very small conidiospores (smaller than 2 μm), its optimal temperature for mycelial growth is 28 °C.

**Treatment.** Most important are hygienic conditions during spawning and the immediate removal of infected substrate.

12 *Sibirina fungicola* (Sibirina rot)
Can be spread by organic debris. Take hygienic measures.

13 *Trichoderma* spp. (green moulds)

A group of very common green moulds is often encountered in mushroom cultivation. Different species of this genus (*T. reesei, T. viride, T. harzianum, T. aeroviride, T. pseudokoningii, T. hamatum*) are hard to distinguish from one another macroscopically and can be identified with certainty only microscopically. *T. viride* has a somewhat darker green colour than the others. Most *Trichoderma* species turn green when they start to form conidiospores; before that time their mycelium is whitish and difficult to distinguish from mushroom mycelium. The spores are sticky and can easily be carried by flies, mites and pickers’ hands to previously uninfected areas. *Trichoderma* can be found as green spots on dead mushrooms, left stumps, on the casing soil, in compost and both pasteurised and sterilised substrate, as well as in freshly
cut wood logs. As long as there are only occasional spots of saprophytic Trichoderma, there will be no or little damage.

**Parasitic behaviour.** If parasitic Trichoderma species get the chance of contaminating substrate at the time of spawning, they can wreak havoc upon the complete farm. Millions of dollars have been lost in the past decades because of parasitic infections with Trichoderma harzianum and T. viride in Shiitake, Agaricus and Pleurotus production. The mushroom mycelium is killed by toxic substances and hydrolytic enzymes, which are produced by the Trichoderma mycelium. Trichoderma is also capable of growing around he hyphae of the mushroom mycelium and eventually invading them. Strains from the T. harzianum-group are currently investigated for use as bio-fungicides. If Trichoderma is inoculated in compost without Agaricus, it can hardly colonise the substrate. The growth of Trichoderma is hindered by antagonistic bacteria in the compost. Agaricus bisporus however is capable of inactivating this antagonistic microflora. Only in combination with Agaricus (or Pleurotus as a case in The Netherlands showed) the green mould can completely take over colonisation. Trichoderma viride is a feared parasite in Shiitake wood log cultivation in the Far East.

**Causes.** The main problems arise when the substrate is infected with conidiospores of parasitic Trichoderma strains within a week after spawning. Once an infection has established itself, it will produce billions of spores which will reinfect the just inoculated substrate on the farm.

A case in Ireland showed that spawning substrate and filling bags near to the place where raw materials were composted and delivered caused the problem. Another cause was that spawn was repacked in rooms where conidiospores were present. If only one grain kernel per bag was infected with Trichoderma, the yield was reduced by 25%; five contaminated grains would reduce it by 75%!

Agaricus cultivation in Ireland is performed in sheds with plastic bags on the ground. The problem became even bigger when mice ate the grain spawn and spread the contamination all over the sheds. Mites increased the infection pressure even more. Because contaminated substrate was left near the farm, reinfection occurred easily. New plastic bags were static and attracted the conidiospores.

**Treatment.** Once the substrate is severely infected, it cannot be saved. Remove contaminated substrate as quickly as possible from the farm and dispose of it at a considerable distance. Spawning should occur in a disinfected room. Bags with substrate can also be infected if they are left to incubate in a room with high concentrations of spores. If heavy infections occur, use Deosan Super or Aldecol for disinfecting. Trichoderma is relatively insensitive to low concentrations of formaldehyde. Even if higher concentrations were used, a biological vacuum would be created which would soon be filled by Trichoderma. Check and clean everything which comes into contact with the spawned substrate. The fungicide benomyl has been overused and is found to be ineffective in the substrate nowadays. At least some strains of Trichoderma have become resistant against it.
14 **Dry bubble disease:** *Verticillum fungicola* var. *fungicola*

*Origin:* The cultivation of mushrooms, 1988, used with permission from copyright holder PPO, with some modifications.

Dry bubble (old name *V. malthousei*) had not yet been found in The Netherlands in 1938, but it is very common now. In 2001, the damage caused by this fungus was estimated at ca. 0.5% of the total Dutch mushroom sector of ca. 300 million Euro. *V. fungicola* var. *fungicola* forms only one type of spore and can therefore also easily be distinguished microscopically from *Mycogone perniciosa*. Dry bubble varies in size from a pea to a large grape. It does not turn brown, nor does it become falty like wet bubble. A film of greyish-white spores hangs over the bubble. Van Zaayen & Gams (1982) carried out research into the pathogenicity of different species of *Verticillium*. One thing that emerged was that *V. fungicola* var. *aleophilum* can cause brown blotches on the caps of *A. bitorquis*, or even burst caps.

Dry bubble (*V. fungicola* var. *fungicola*) does not occur in *A. bitorquis* crops, but only in *A. bisporus*, and can cause considerable damage and loss of yield. Although *V. fungicola* var. *aleophilum* can also cause brown blotches in *A. bisporus*, the growing temperature of *A. bisporus* is too low for this heat-loving species of *Verticillium*. The same story applies to *V. psalliota*, which can cause rather more merging brown blotches in *A. bitorquis*.

An artificial infection of *A. bisporus* with *V. psalliota* proved difficult or impossible (Van Zaayen & Gams, 1982). In circumstances in which it has not been possible to keep the compost temperature below 20 °C in the harvesting phase (with *A. bisporus*) an infection with *Aphanocladium album*, also involving brown circular blotches on the cap, can also occur. With high air humidity an air mycelium is formed on the affected patches. *Hormiactis fimicola* can also cause brown blotches on the cap (Fermor, 1979). The correspondence with the mycelium of *V. fungicola* is only macroscopic. This patho-
gen occurs only sporadically. The symptoms involved in a dry bubble infection can also vary widely depending on the variety of mushroom. Due to the development of resistance to benzimidazoles, which have been permitted for use in mushroom growing since 1973, research had to be carried out into substitute fungicides (Van Zaayen & Adrichem, 1982). Since then, prochloraz and chlorothalonil had been given a permit and worked very effectively. However, prochloraz accumulates in the tissue of plants easily and is toxic for algae. For this reason, hygienic measures have to be taken first.

**Symptoms.** If mushrooms are affected in an early (pinhead) stage by *V. fungicola* var. *fungicola*, the typical onion form is produced; the stem is thicker than the cap, which is often no longer distinguishable from the stem. At a later stage of infection, you often find a very crooked stem, because the infected cells stop growing and strips of surface tissue have peeled away from the curved side and curled back. If part of the cap is affected, we have the so-called hare lip. *V. fungicola* does not grow in the compost, but comes onto the farm with the casing soil. The disease can spread very rapidly in a crop, particularly if the growing room temperature is on the high side during growing (above 20 °C). Owing to the rapid spread, the damage caused by *V. fungicola* can be very great. The affected mushrooms are greyish in colour and are unsaleable. If the infection occurs at a later stage, you can see a grey mouldy fuzz on the cap of the mushrooms. Sometimes little pustules or lumps appear on the cap. The colour of the cap can also often be light brown, or light brown blotches appear on the cap (*Verticillium* spot). This picture could be confused with bacterial blotch. The brown blotches in bacterial blotch are often much darker in colour and are shiny, and they are more on the surface of the cap. In the case of *Verticillium* blotches are a duller, more chestnut brown, and penetrate more deeply into the mushroom tissue (necrotic lesions). The mushrooms remain leathery, and there are no rotting bacteria present, so that no unpleasant smell occurs. With *A. bitorquis* the dark brown blotches (lesions) caused by *V. fungicola* var. *aleophilum* are sometimes covered with a layer of grey-coloured mycelium, particularly in the centre. Cracks often occur in the lesions, or cracks can occur over the whole width of the cap if the infection is serious. The blotches have ragged edges.

**Spread.** *Verticillium* is carried onto the farm by the infected casing soil, although the possibility of *Verticillium* spores also being carried in by dirty packing containers cannot be ruled out. Once *Verticillium* infection has occurred in a growing room, it can be spread to other growing rooms by spores in ventilation air, by phorid and sciarid flies, dirty equipment, hands, clothing and the like. The spread of dry bubble is quicker than that of wet bubble, particularly through the fact that the small conidia are easily spread by phorid and sciarid flies, but also through picking work. The reason for this is the sticky mucigel with which the clusters of *Verticillium* conidia are surrounded. Even washing the hands with soap and hot water is not enough (Fletcher et al., 1986). During watering, spores can fall on the ground and can be carried by the air flow after drying up.

**Control**

- Pay the greatest possible attention to hygiene: see the first paragraph of this chapter.
- Control of phorid flies and sciarid flies.
- Spore filters in air supply and venting ducts.
- Careful treatment of the casing soil (see paragraph 1.4.), even if a container is used.
- Control local infections by spraying the affected patch with 2% commercial formalin. Remove diseased specimens and take away in plastic bag.
- In case of a serious infection, or if it occurs often, a systemic fungicide (benomyl, carbendazim or thiophanate-methyl) must be used throughout the growing period.
- Chlorothalonil can be used immediately after casing and one week before harvesting.
- If a formalin treatment is carried out immediately after casing, prochloraz can be sprayed (9 days after casing) if the infection is serious.
- Ensure that infected mushrooms are not picked with the others during harvesting.
- Work in the correct sequence, i.e. from new to older crops.
- Cook out properly: compost temperature (not room!) 12 hours at 70 °C.

26.8 Bacteria

Bacteria generally cause less problems than insects or fungi, and at times they are even indispensable for mushroom growers.

Bacteria play an important role in the fermentation and pasteurisation processes. One of the results of the pasteurisation is the establishment of a specific microflora, which encourages the growth of the desired mushroom and is often antagonistic towards pathogenic fungi, like *Trichoderma*.

Bacteria are also important in *Agaricus* cultivation, because *Agaricus* only forms fruit bodies on a casing soil if *Pseudomonas putida* bacteria are present. However, in the table on the opposite page you will find in which cases bacteria cause damage to mushroom growers.
<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Vernacular name</th>
<th>Affected mushrooms</th>
<th>Damage</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many genera</td>
<td>Bacteria</td>
<td>All wood-inhabiting mushrooms cultivated on either pasteurised or sterilised substrates</td>
<td>Bacteria may reduce mycelial growth. Bacteria are often active if the substrate is too wet (high free water activity), if sterilisation has not been performed properly. Some bacteria form spores, that survive a heat treatment up to 100 °C</td>
<td>Proper substrate preparation, check sterilisation room for heat-leak, reduce easily available carbohydrates, in pasteurised substrate (e.g. by conditioning or by changing the substrate formula)</td>
</tr>
<tr>
<td><em>Pseudomonas tolaasi</em></td>
<td>Bacterial blotch</td>
<td>Mainly <em>Agaricus</em>, sometimes also <em>Pleurotus</em> and <em>Lentinula</em></td>
<td>The bacteria cause yellow to brown blotches on the caps and in severe cases also on the stipes of the mushroom</td>
<td>Control humidity: let the mushrooms dry with enforced ventilation within 2 hours after spraying. Check whether air movement is sufficient everywhere in the growing room. Reduce compost temperature. Isolate the contaminated spot by digging a slit at 1.5 metres and disinfect with 2% formalin. Decrease RH. Cook out more thoroughly.</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>Mummy disease</td>
<td><em>Agaricus</em></td>
<td>Deformed mushrooms have weblike mycelium around the (extra thick) base of the stipe. Casing soil and mycelium are easily torn loose upon picking. Spreads only by sick mycelium (thus not from bed to bed, but within the infected bed).</td>
<td></td>
</tr>
</tbody>
</table>

*PESTS AND DISEASES*
26.9 Virus disease

Viruses multiply by changing the genetic system of their host and 'ordering' the host to produce new viruses. Many mushrooms are sensitive to virus infections. In the past the notorious die-back disease has led to large reductions in yield in Agaricus bisporus and many reports have been published on this subject. Several virus-like particles have been detected in Lentinula and Pleurotus too. The die-back disease is well-known in Agaricus cultivation, the abnormalities found in Pleurotus ostreatus and P. pulmonarius also can be attributed to viral infections, but these have received much less attention in the literature.

26.9.1 Indications

Agaricus bisporus: There may be spots in the casing soil where no mycelial growth occurs. Around these spots, mushrooms of low quality appear, with long stems and dirty caps. Sometimes the only indication of a virus infection is a lower yield. In severe cases, complete beds may produce only a small number of deformed fruit bodies. In the cultivation of other mushrooms, the indications of viral infections are also lower yields and deformed fruit bodies.

Viruses caused abnormalities in P. pulmonarius and P. ostreatus var. colombinus, causing the fruit bodies to look like mini-cauliflowers. These viruses are spherical in shape. In Lentinula edodes, a swollen stipe and cap torsion can indicate viruses. Lentinula-associated viruses detected by prof. Poppe were rod-shaped.

26.9.2 Measures

Virus diseases can easily spread if contaminated (sick) fruit bodies sporulate. Therefore, infected mushrooms should be picked before the caps open (in Agaricus bisporus), or as soon as it is clear that the mushrooms are infected. It is actually even safer to cook out a room when viruses appear. Contaminated mycelium in wooden panels can contaminate fresh substrate/mycelium even after a heat treatment like cooking out. Shield the wood with plastic from the mycelium.

Ecological factors that will put stress on the mycelium, like high temperatures or a highly compressed and wet substrate, have to be avoided during spawn run. Check whether trucks which supply spawn or substrate (-materials) to different farms have been thoroughly cleaned after leaving each farm. The viruses can be spread easily by vehicles moving from contaminated farms to clean farms. Viruses can be present in spawn, even if it looks completely normal. Spawn should only be made from healthy
mushrooms, or a reliable culture. In practice, it is very difficult to guarantee virus-free spawn. Even sophisticated spawn laboratories in Europe sometimes encounter problems.

When cultivation of a mushroom is intensified, viruses are likely to occur in due time unless strict hygienic measures are taken. The presence of high spore loads in closed growing rooms offers opportunities for viruses to express their presence. The presence of viruses can be detected by electrophoresis in *Agaricus bisporus* cultures and probably also in other species, but this can only be performed in well-equipped laboratories. *Agaricus bitorquis* has been reported to be virus-resistant.
Appendix A Safe use of pesticides

Pesticides vary widely in their toxicity. Some pesticides can cause poisoning already in very low quantities, while others are relatively harmless. Chemicals should be obtained in their original package. An adequate guideline on how to use them should be printed on the package in the local language. Handle the chemicals according to the guidelines. Furthermore, take the following general precautions:

A.1 Storing pesticides

- Store the pesticides in a special room or locker which can be properly closed and locked.
- Do not store any other goods in the same area, especially food, fodder, clothing and tools.
- Do not leave pesticides unguarded outside the storage area.
- Dispose of empty packages properly. Do not store drinking water in metal containers which previously contained pesticides.
- Store pesticides in their original packaging.

A.2 Use

- Read the label thoroughly before using the pesticide. Do not use improperly labeled pesticides. The labels should describe first aid treatment in case of poisoning and give recommendations on how to apply the pesticide safely.
- Do not mix different pesticides. A chemical reaction may occur, giving unexpected results.
- Be careful when diluting the pesticides. Use proper pouring equipment and protective clothing (at least impermeable gloves). Prevent powders from being blown by the wind.
- Check spraying equipment for leakages before use. Never use leaky equipment.
- Water, soap and towels should be available during the application of the pesticides, so the skin can be washed immediately in the event of contamination.
- Do not eat, drink or smoke when preparing or applying the spraying solution. Wash hands thoroughly after application.
- Be aware of the possibility of poisoning fish, birds and other animals.
- Avoid overlong working days when spraying.
- Never rinse spraying equipment in surface water.
- Keep away from recently sprayed crops.
A.3 Protective clothing

The best way to avoid poisoning oneself is to prevent physical contact with the pesticide. In warm weather protective clothing is uncomfortable, but it must be worn if the label states so. It can be very difficult to persuade farmers to act according to the guidelines. A large number of casualties in pesticide poisoning occurred because the protective measures are uncomfortable and farmers thus preferred not to wear protective clothing.

- Impermeable gloves are necessary because the hands come in frequent contact with the spraying solution. Talcum powder or a lining in the gloves can make the gloves more comfortable to wear.
- Rubber boots should be worn with trousers over the boots to prevent the spraying solution from dripping in.
- Protective glasses or a face-shield prevent the eyes from being hit by splashes.
- Jacket and trousers: an impermeable apron should be worn if the user can become wet during usage.
- A hat will protect hair and face from dusty pesticides.

A.4 In case of poisoning

Check which substance has caused the poisoning (look at the label of the package used) and immediately call a doctor.

Take the following actions in case the patient is unconscious:

- bring the victim in a room with fresh air,
- take personal safety precautions,
- put the victim on his/her side, mouth downwards, head bent backwards,
- do not induce vomiting,
- do not give anything to drink,
- if the victim breathes insufficiently, provide artificial respiration.

If the victim is conscious, the following actions can be taken, depending on where the poison has made contact with the victim.

- poison on skin: take wet clothes off, wash the skin thoroughly. First with cold water, later with warm water and soap.
- poison in eyes: immediately rinse with tap water. Highly irritating substances should be washed out for at least 15 minutes.
- poison in stomach: if the victim can hold a glass, let him/her drink two glasses of water. Induce vomiting by pressing two fingers as deep down in the throat as possible. Act swiftly. With highly aggressive pesticides, do not induce vomiting later than 30 minutes after swallowing the pesticides. Use Norit (active coal) to absorb the chemicals.
- poison in lungs: bring the victim into an environment with clean air.
Appendix B  Conservation of strains and culture collections

B.1 Degeneration

If a strain is cultivated for some time it may eventually lose some of the genetic characteristics that made it desirable to grow in the first place. Fresh starter cultures may be obtained from a young and healthy fruit body. A tissue culture of a mushroom of a degenerated strain, however, will still yield a degenerated culture. Therefore other techniques have been developed to keep the genetic characteristics. Cells in fungi can degenerate due to lack of nutrients or oxygen, infections (viruses, for example), a change in substrate pH and the accumulation of unfavourable metabolites. The objective of strain conservation is to keep the vigour and genetic stability of the pure mycelium.

B.2 Techniques to preserve cultures

Several techniques have been developed to preserve the cultures, all with specific advantages and disadvantages.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar-to-agar transfer</td>
<td>• Simple, can be performed in any spawn laboratory</td>
<td>• Risk of glass tube breakage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Some degeneration can still occur because the mycelium keeps growing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Laborious if large numbers of cultures have to be maintained (strains have to be transferred every two to six months)</td>
</tr>
<tr>
<td>Mineral oil on agar slant</td>
<td>• Sealing with oil prevents infestations by mites</td>
<td>• Laborious if large numbers of strains have to be maintained</td>
</tr>
<tr>
<td></td>
<td>• Relatively cheap</td>
<td>• Cultures can be kept for 1-3 years, but degenerate easily</td>
</tr>
<tr>
<td>On agar in demineralised water in refrigerator</td>
<td>• No contamination with mites possible</td>
<td>• Laborious for large collections</td>
</tr>
<tr>
<td></td>
<td>• If cooling breaks down, the water will still cool the agar for some time</td>
<td>• Mycelial growth not completely halted, degeneration will still occur</td>
</tr>
<tr>
<td></td>
<td>• Cheap method</td>
<td></td>
</tr>
<tr>
<td>Cryogenic freezing</td>
<td>• Best method as mycelial growth is halted completely, thus delivering stable mother cultures</td>
<td>• High cost of specialised staff and equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Constant surveillance and careful maintenance required</td>
</tr>
</tbody>
</table>
### Appendix B: Conservation of Strains and Culture Collections

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilisation</td>
<td>• Good technique for storing spores</td>
<td>• Rarely used for mycelial cultures</td>
</tr>
<tr>
<td>(freeze-drying)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### B.2.1 Agar-to-agar transfer

The agar-to-agar transfer technique is identical to sub-culturing (refer to the chapter *Spawn manufacturing*). Most cultivated mushrooms are incubated for 10 to 14 days at a temperature of 25 °C. *Volvariella volvacea* is incubated at a temperature of 32 °C for seven to ten days. *Pleurotus tuberregium* can also be incubated at 30 °C. If the cultures are grown on the same medium each time, degeneration will more rapidly occur compared to switching between a nutrient-rich and nutrient-poor medium. Cultures may be protected from drying out by sealing the tubes with paraffin wax. On the other hand, a too hermetrical sealing will result in even faster degeneration due to oxygen deprivation.

The time between transfer is six months for most of the mentioned mushrooms at a temperature of 5 °C. *Volvariella volvacea*, however, should be kept at a temperature of 15 °C and has to be transferred every two months. *Volvariella* is rather unstable, but switching the medium from rice straw to agar and back will help in retaining its characteristics.

**Beware of mites:** If mites start eating their way through the culture collection, then the strains are in great danger. Mites carry all kinds of contaminants and bits of mycelium with them, thus spoiling the pure culture character of the collection. Cover the cotton plugs of the cultures with cigarette paper. The small pores in the paper allow aeration, but are too small for mites. Sticky mats can also control mites to some extent. These sticky mats are normally used for trapping flies and monitoring insect populations.

#### B.2.2 Case study: conservation of strains on compost

At the Mushroom Experimenting Station in The Netherlands *Agaricus* species are usually kept on a compost medium. This medium can also be used for other species which prefer fermented substrates, like *Lepista nuda*, *Coprinus comatus* and others. The compost medium is probably the best medium for mushrooms thriving on compost, because it contains all required nutrients for their growth. The strains are transferred every two years, thus reducing the risk of breakage, mislabelling and degeneration. Media preparation is as follows: use 300 gram dried compost (pasteurised and conditioned) and grind it in 5 mm pieces. Moisten with 1 litre of tap water. Then wash the compost three times with hot water. This treatment will remove the gases that otherwise would blow the plugs from the test tubes during sterilisation. The test tubes are filled for only one-quarter for the same reason. Sterilisation temperature is 121 °C at 100 kPa (1 bar) for two hours. The next day the heat treatment is repeated. Bacteria that form heat-resistant spores will be killed by this treatment.
B.2.3 Mineral oil on agar slants
Cultures stored under mineral oil can be kept at either room temperature or in the refrigerator. The oil prevents mite infestation and the agar from drying out. The oil should be sterilised for half an hour first and (when cooled) poured aseptically on top of the culture. Use oil with a specific gravity of 0.865-0.890 and test tubes with screw caps, or bottles. When the culture is to be used, the oil has to be drained first. Transfer sections of the full-grown agar to new slants. The strains will often show some signs of degeneration after being kept under oil for several years. They will stay viable longer if kept at a low temperature.

B.2.4 Cultures on agar in refrigerated, demineralised water
A simple technique is to grow the culture on an agar medium and to keep small pieces of colonised agar floating in demineralised water. If 100 ml bottles are used, then these should be filled with 75 ml demineralised water. Sterilise the bottles for two hours, let them cool, then transfer aseptically small pieces from the agar culture. Put about three to four pieces of 0.5 x 0.5 cm² in each bottle. Always inoculate at least three bottles per strain, so if some contamination occurs then there is still a back-up. Strains can easily be recovered by taking a piece of the agar out of the water and transferring it to a new slant. This operation is not as messy as the mineral oil technique. Strains can be kept for at least one year without losing vigour (except for Volvariella volvacea, which cannot stand prolonged storage at temperatures below 12 °C).

B.2.5 Cryogenic freezing
Cryogenic freezing is the best preservation technique, but also the most expensive. Only large research institutes and spawn companies can afford the necessary equipment. In former years, nitrogen containers were most used. Nowadays electric cooling units are available, which can reach temperatures of -135 to -150 °C. Specialised biologists and laboratory assistants have to maintain the cultures. This technique is therefore beyond the scope of this book, but it is important to know the advantages of cryogenic freezing. Ampoules with mycelium and a cryogenic preservative are kept at temperatures of -150 to -180 °C (nitrogen gas) or -196 °C (nitrogen liquid). At these temperatures, no mycelial growth is possible. The mycelium, however, can be revived with unaltered characteristics. If cultures hang in nitrogen gas, the temperature of the gas has to be checked regularly, as it can rise above the desired temperature range.

B.2.6 Lyophilisation or freeze-drying
Lyophilisation or freeze-drying results in rapid removal of water out of the tissue. This method is most used for spores, which stay viable for at least 20 years. At present there are no reliable techniques to lyophilise mushroom mycelium. Spores are preferably collected from a young mushroom. The outside is washed and the mushroom is allowed to discharge spores under sterile conditions. The spore print is freeze-dried. It should be remembered that a culture derived from spores may differ from its parent
mycelium. If a multitude of spores is used to obtain a new culture, then its characteristics will probably be very similar to those of the parent. Freeze-drying requires special equipment, but the technique itself is relatively simple. The spores have to be frozen in a closed container at -20 °C. The water is transformed from the frozen state into the vapour phase without passing through the liquid state. This can be achieved by raising the temperature slowly under a low pressure (50 Pa, about 0.5 mbar). Once the spores have been freeze-dried, they can be kept at room temperature or preferably in a refrigerator or cool room (15 °C).

B.3 Culture collections

Each research station and spawn manufacturer should have its own culture collection. Some preserve many thousands of strains, while others only preserve the cultures they use regularly.

It is of course very important to label each culture properly and accurately. It is impossible to see from the outside which strain one is dealing with. In large culture collections the tubes are labelled with scientific names and collection numbers. Cultures must be retrieved quickly, therefore their place in the store is linked to the culture number as well. The preparation and maintenance of culture collections requires expert knowledge in different fields, such as taxonomy and microbiological techniques. Small-scale mushroom farms cannot keep the cultures in a good condition. Farmers should therefore obtain their cultures or spawn from reliable scientific institutions or spawn producers. If you happen to isolate a mutant with favourable characteristics (such as higher fruiting temperature than most other strains of the species, higher yield on a certain substrate, faster spawn run, etc.), then you may send it to one of the larger type culture collections in the world. If you want the strain to be stored solely for your own use, then you will have to pay for this service. It is also possible to store the culture for general use, for which no fee is charged. It can then be ordered by scientists and spawn manufacturers all over the world. It is important for future development to have many different strains of the same species.

B.3.1 Addresses of culture collections

Many universities have a culture collection of some kind. The large culture collections charge a considerable fee for their services, so it is rather costly to experiment with different strains. The type collections form the gene resources of the world and preservation of strains is expensive, so it is understandable that a fee is charged for their services. Another way to obtain cultures is to write to scientists who published on specific strains and ask them for help.

CBS (Centraal Bureau Schimmecultures)
Upssalaan 8, 3584 CT Utrecht, The Netherlands. P.O. Box 85167, 3508 AD Utrecht, The Netherlands
Telephone +31 (0)30 2122600 – Telefax +31 (0)30 2512097 www.cbs.knaw.nl
E-mail: info@cbs.knaw.nl This largest culture collection in the world has over 30,000 strains in stock. Prices per strain differ for commercial enterprises, or scientific and
nonprofit organisations. A number of commercially cultivated strains of many different mushrooms from Asia has been deposited here by the author of this manual.

**ATCC (American Type Culture Collection)**

12301 Parklawn Drive, Rockville, Maryland 20852, USA. ATCC has more than 21,000 strains of filamentous fungi available. It has a wide range of commercially cultivated mushroom strains, too.

**CCRC Taiwan (Culture Collection and Research Centre)** P.O. Box 246, Hinchu, 30039 Taiwan. The cultures of CCRC are relatively inexpensive and their small collection has many commercial high-temperature strains of cultivated mushrooms. It is interested in exchanging strains to enlarge the collection.

**Mycelia (manufacturer of exotic mushroom spawn)**

Jean de Bethunestraat 7-11, B-9040 Gent, St. Amandsberg, Belgium, tel: +32 (0)91 287090, fax: +32 (0)91 288028. This spawn manufacturer can also mail agar cultures of many commercially cultivated species at a reasonable price.

**MUCL (Mycothèque de l’Université Catholique de Louvain)**

Faculté de Sciences Agronomiques, UCL, Place Croix du Sud 3, B-1348 Louvain-la-Neuve, Belgium, tel: +32 (0)10 473742, fax: +32 (0)10 451501 Apart from many other species, *Dictyophora indusiata* and *Pleurotus tuberregium* have been deposited here.
Appendix C Spore allergies

C.1 Diseases caused by sporulating mushrooms

Mushrooms produce millions of spores. Many people are sensitive to these spores and respond with allergic symptoms when exposed to extreme numbers of them. Up to now, Oyster mushrooms, Auricularia and Shiitake have been reported to cause allergic alveolitis, a lung disease. Other mushroom species are likely to give the same problems if they are cultivated on a large scale in closed growing rooms. Spore allergies can also be caused by Actinomycetes during the fermentation process for Agaricus compost (see paragraph C.2). Especially in closed rooms the spore load of the air can be extremely high. Sometimes clouds of spores actually create a fog. It is not advisable to enter the growing room then. The allergies have mainly been reported from Western countries, where large volumes of mushrooms are cultivated in closed growing rooms and labour inspection is stricter than in developing countries. The growing rooms in developing countries are generally not as closed, so the spores will disperse quickly. Still, attention should be paid to possible allergies. The problem does not occur in outdoor cultivation of mushrooms, because the spores are quickly dispersed by wind. It is also unknown in the cropping phase of Agaricus cultivation, as the mushrooms are picked before they start to release spores.

Symptoms: Fever up to 40 °C, coughing, dyspnoea (shortage of breath), decrease of lung vitality. Often the symptoms misleadingly appear six to eight hours after exposure to the spores and not at the time of the exposure itself. If the patient gets sick and temporarily stops working, he will recover in a few weeks, but become ill again after renewed exposure to the spores.

C.1.1 Treatment

The patient will recover completely within weeks if contact with spores is avoided. A repeated exposure, however, can even lead to scars in the lungs. It is therefore important to diagnose the allergy and not to mistake it for some kind of influenza.

Prevention: Blow fresh air in or open the windows before picking mature mushrooms. Picking them at a less mature stage will also decrease the spore load in the growing room. Some strains of Oyster mushroom and Shiitake are known to produce much less spores than others, but farmers claim they get less yield from them, also. Simple caps before the mouth can decrease the number of spores by up to 90%. However, 10% of several millions of spores still yields numerous spores. The best masks provide filtered air, but these are rather expensive.
C.2 Diseases caused by compost dust

The second paragraph of this appendix stems from Van Griensven, 1987, with permission by the copyright holder Applied Plant Research

Compost must be prepared in the correct way to function as an optimum substrate for mushroom cultivation (see Chapter 17). Following peak heating, where the compost loses its ammonia, it is conditioned. During phase II, the temperature of the compost is very important. One of the countless biological processes that go on in this phase of composting is the wholesale growth of Actinomycetes, a type of bacteria that live on organic refuse. They are characterised by the formation of thread-like spores. The thermophilic Actinomycetes considered here have an optimum growth temperature of 40 to 60 °C. When the phase II compost is spawned, its temperature has been reduced substantially (to 25 °C), but the Actinomycetes are still present in enormous quantities. These Actinomycetes are very important for the health of the workers, since they can cause the condition known as mushroom grower’s lung. How does it happen? Well, during spawning, the compost mass is mixed out to permit even distribution of the spawn throughout the compost. In opening up the compost, a lot of dust is released, dust laden with Actinomycetes. The larger dust particles are trapped by the nose, throat and upper wind-pipe, while the finer particles, 1 to 5 micron in diameter, are inhaled and work their way right into the lung tissue, right down to the air sacs or alveoli. The tiny spores of the Actinomycetes can now cause inflammation. Lung inflammation does not always occur, and not everyone suffers, but certain sensitive people overreact or have an allergic disease. The official name for mushroom grower’s lung is allergic alveolitis (Bringhurst, 1959; Van Haaren, 1985).

The symptoms of the disease are characteristic. They are typically seen some six to eight hours after the patient has done a job that has released a lot of compost dust. Compost dust is released not only during spawning, but also during emptying of the rooms, although to a lesser extent. Typically, spawning in the morning is followed by symptoms in the late afternoon and evening. The symptoms are tightness of the chest, a dry cough, a “fluey” feeling with a slight fever, loss of appetite or nausea and vomiting. If there is no subsequent exposure to compost dust on the immediately following days, then the symptoms diminish and disappear in one or two days. Repeated exposure to compost dust at frequent intervals can make the condition chronic, with the additional symptoms of listlessness, shortness of breath and severe weight loss.

The changes that occur in the lung following chronic exposure, are loss of lung tissue and its replacement by scar and fibrous tissue. The lung function deteriorates and it loses its capacity to take up oxygen. The patient becomes an invalid. Mushroom grower’s lung is thus a serious illness if not discovered in time or if left untreated. Not only does the health of the sufferer deteriorate, so too does his capacity for work, to the point of total unfitness for work. Several questions relating to this serious occupational disease have yet to be answered: who is susceptible? who is not? how long must someone be exposed before contracting the disease? which bacteria, or which bacterial components precisely are involved? how can the disease be diagnosed with certainty? Much research remains to be done in order to answer these questions. It is not such a common illness, that is to say it is only seen in one or two percent of mush-
room workers. However, given its seriousness, each case is one too many.

C.2.1 Prevention

It is obvious that prevention is necessary and must be given every attention, even though we do not know the precise mechanism of the disease. If the hazardous substance is present in the compost after conditioning then dust formation must be prevented. The best solution would be a technical modification of the working practices that reduced dust formation or excluded it altogether. This is too difficult and expensive for most farms. Thus there is no alternative at the moment to the use of personal protection against the inhalation of dust by using dust masks. This is not a perfect answer either, but experience does suggest that the half-face fine-particulate dust mask offers quite good protection. This is a mask that covers mouth and nose, traps particles larger than 1 micron and can be replaced daily. A dust helmet naturally gives better protection. It is important that everyone who is engaged in spawning or emptying rooms should be protected in this way. It may not be assumed that those who have never suffered from the disease have built up sufficient resistance to it. Sensitisation to the disease can be triggered by each new contact. If detected in an early stage, recovery can be complete. If discovered in a more advanced state, recovery can be difficult if not impossible. It is therefore of the utmost importance that mushroom growers should recognise the symptoms, so that they can identify them themselves and consult a doctor at the earliest opportunity.
Appendix D Maintenance chart

Information provided by Cpoint

Good maintenance obviously extends the life span of the equipment of a farm and ensures the right climatic conditions; it also keeps energy costs down. Cpoint developed the following maintenance chart to monitor maintenance.

D.1 Description of the chart

The chart is divided into months and week numbers. Items where a check box appears weekly should be replaced or inspected weekly. The room number should be noted in the check boxes. If, for example, in week number 5, growing room 3 is filled, note “clean water reservoir and replace water and wick” by filling in a 3 at the check box for week 5. Items marked by an asterisk indicate where a growing room number is filled in. (*). Note periodic maintenance by crossing the check box concerned or noting the date when the activity was carried out. Each check box is only filled in once.

The various items are listed below, with an explanation:

D.1.1 RH measuring box inside

Clean water reservoir and replace water and wick: During cultivation the reservoir can become soiled. After cultivation the water is no longer free of impurities. For good measurements, clean the reservoir and replace the water. The wick loses quality due to the constant evaporation and cook out in the growing room. For reliable measurements, place a new wick after each growing cycle.

Check fan working: Check if the measuring box fan is still working. This fan is needed to guide air past the sensor at a certain speed. This gives a more accurate measurement than measurement without air speed.

Check measurement: As measurements can deviate over a certain period it is important to check measurement reliability after each growing cycle. Check the measurements by removing the wick and the wet bulb thermometer. Both thermometers should then indicate the same temperature.

D.1.2 RH measuring box outside

Check meters: The outside meters should also be regularly checked. Check if there is enough water in the container, if the measurements are still accurate and if the wick needs replacing.

D.1.3 CO₂-meter

Calibrating the CO₂-meter with a zero cartridge: Calibrate the meter regularly for accurate and reliable measurement. This is done using gas with a known concentration.
Calibration using outside air is not advisable as the concentration of this air can show large local, temporary variations.

**Check filter and suction tube:** Regularly check the suction tubes for bends and leakage. Also check the measuring tube connections for suction of false air, using cigarette smoke, for example. Check the filters for obstructions. Obstructed filters can give false measurement readings.

**Replace filters:** Replace internal dust filters to prevent obstructions and false measurements.

**Drain condensation container:** Regularly drain the condensation container to prevent it from overflowing which could lead to (permanent) damage.

### D.1.4 Heating installation

**Check water pressure:** Check the water pressure to ensure the boiler has enough water to work properly.

**Maintenance boiler/burner:** An authorised installer should periodically check the heating installation. Make sure this is done before the winter begins – when a reliable heating installation is essential! Figures show that a badly maintained boiler performs 5 to even 10% less than a well maintained one.

### Example: save energy

You grow mushrooms on a total surface area of 2000 m². This requires a heating capacity of 170kW. The boiler performance is 88%. Insufficient maintenance means however that it only provides 82%. The calorific value of natural gas is 32 MJ/m³. This corresponds to 8.9 kW. 8.9 kW can be obtained from one cubic metre of natural gas. A performance of 88% gives: \[\frac{170}{(8.9 \times 0.88)} = 21.7\] cubic metres required to achieve 170 kJ capacity. A performance of 82% gives: \[\frac{170}{(8.9 \times 0.82)} = 23.3\] cubic metres. A difference of 1.6 cubic metres per hour at full capacity. With 2000 running hours annually this can save you 3200 m³ gas. At a price of US$ 0.15 per m³ this will save US$ 480 per year.

**Check main pump:** The main pump re-circulates water. It is essential that the main pump is in good working order.

**Check and test pump/mixing valves:** Check and test correct functioning of pumps and mixing valves. If they do not work correctly the result can be unnecessary and/or unwanted energy loss.

**Check (pipe) insulation:** Inspect pipe insulation. Bad insulation leads to unnecessary heat loss.

### D.1.5 Steam boiler

**Blow out:** When steam is produced sediment forms in the boiler. This sediment can cause irregular boiling and impurities in the steam. If the boiler is blown out after each use, the sediment will be removed. This keeps the boiler in good working order.

**Clean and refill water softener:** Soft water means less lime deposits in the boiler. A clean boiler gives more effective heat conversion and more efficient energy consumption. Regularly clean and fill the water softener to ensure proper functioning.
Maintenance boiler/burner: Service the boiler annually to ensure correct functioning. Only allow an authorised installer to perform maintenance activities.

Check boiler water level: There should be enough water in the boiler to allow correct functioning.

Check (pipe) insulation: Insulate pipes correctly to prevent steam condensing on them.

Check steam cut-off valves: Check the steam cut-off valves for leakage.

D. 1.6 Climate units

Replace coarse inlet/outlet filters: Replace the coarse filter after each growing cycle. Resistance is raised as the filters become soiled, leading to higher energy consumption. Old filters are also unhygienic.

Replace fine filters: The fine filter wears less than the coarse filter which catches most of the dust particles. However, it will not last forever. A yearly check/replacement should be sufficient. In general, the higher the filter's resistance, the lower the amount of air. With insufficient capacity this can be detrimental to mushroom quality. When replacing filters, switch off the fan and observe all hygiene regulations to prevent any pathogens entering the growing room.

Calculation on effect of filter replacement

The resistance over a soiled filter can often become very high without it being noticeable. Raised pressure values of up to 250 Pa are no exception.

A farm with a growing surface of 2000 m² requires an air capacity of: required air volume per m² growing surface x total growing surface x simultaneity factor = 22.5 x 2000 x 0.7 = 31500 m³ air per hour.

The following example compares the energy consumption of a filter replaced after one year and a filter replaced after two years. Assume that the average pressure increase due to soiling is 50 Pa for a 1-year-old filter and 150 Pa for a 2-year-old filter (realistic values). The difference between the two is 100 Pa.

The higher resistance of 2-year old filter causes higher fan energy consumption to achieve an equal volume of air. This additional consumption can be expressed as:

(air volume per second* pressure loss) / (fan performance and (ave. 70%) x electromotor performance (ave. 95%). With a 100 Pa difference, the extra energy consumption is:

(31500/3600) x 100) / (0.70 x 0.95) = 1316 W = 1.32 kW. If electricity costs US$ 0.10 / kW this represents US$ 0.14 per hour extra for energy. A year has 8760 hours, which means extra costs of 8760 x 0.30 = US$ 1226.40.- This makes it worth considering the most economic option: the cost of extra energy or the costs of buying new filters or using a certain type of filter.

Check and test inlet and mixing valve: Check if the valves are working as indicated by the computer. If the computer indicates that an inlet is fully closed, it must actually be closed!

Clean internally: Remove all dirt from the unit itself. Cleaning the unit helps keep resistance minimum and aids optimal hygiene.
D.1.7 Cooling unit

Check condensation drainage: The cooling unit often cools to such low temperatures that condensation occurs. This is removed to prevent moisture collecting in the unit and being introduced with room air, which would mean extra dehumidification. Ensure the condensation drainage hole is always unobstructed.

Clean internally and externally: A cooling unit will function more efficiently if it is clean on the inside and outside. Clean the exterior of the cooling unit with compressed air in the opposite direction to the airflow. A soiled block has a higher air resistance, which leads to bad cooling transfer properties and can quickly result in a 5% energy loss. If spring water is used for cooling, the block must also be cleaned on the inside to ensure the most efficient cooling transfer and to prevent blockages.

D.1.8 Heater unit

Clean heater unit: To ensure heater unit resistance is a low as possible, clean the unit regularly. Clean the exterior of the heating unit with compressed air in the opposite direction to the airflow. A soiled block has a higher air resistance, which leads to bad heating transfer and can quickly result in a 5% energy loss.

D.1.9 Fan

Lubricate bearings in fan housing: Lubricate the fan bearings regularly for smooth fan operation.

Adjust V-belt tension: Regularly checking the V-belt tension will ensure the fan works efficiently. If the belt is too loose it will slip. This uses more energy and increases wear and tear on the V-belt. If the belt is too tight there will be higher resistance, which also uses more energy and will wear the belt out sooner.

Clean fan blades: The fan blades will become soiled in time by dirt, flies etc. This increases resistance and reduces efficiency, resulting in higher energy consumption.

D.1.10 Humidifier

Check valve: Inspect the magnetic valves to check they are not letting water through, or are obstructed. Clean as necessary.

Clean misting nozzles: Clean the misting nozzles to prevent obstructions.

Clean/replace water filters: Regularly clean or replace the water filter to prevent obstructions.

D.1.11 Cooling installation

Maintenance to the cooling equipment: Service the cooler annually to ensure correct functioning. Only allow an authorised installer to perform maintenance activities. If a cooling installation in The Netherlands uses CFC’s, HCFC’s or HFC’s to cool, it will be covered by the ‘Resolution concerning substances that damage the ozone layer’. This means that the cooling installation must be regularly inspected for leakage. How often these inspections take place depends on the amount of cooling medium used.

Check the condenser: The functioning of the condenser greatly influences the cooling capacity. It should be kept clean to ensure correct heat transfer. Clean with compressed air in the opposite direction to the airflow.
Remove rust and dirt: Remove rust and dirt particles to prevent obstructions.
Set temperature cooling medium: Set the temperature of the cooling medium so the
cooling capacity is used as efficiently as possible. For example, if it is possible to
achieve a temperature difference of 4 °C instead of 3 °C between the pumped up and
drained spring water, huge energy savings can be made.

D.2 Buildings

Check walls and ceiling for leaks: Check if there are any cracks and gaps which could
cause energy losses or allow diseases and pests to enter the building. Localise cracks
by switching off all the lights inside the building and seeing where daylight appears.
When checking the walls and ceilings also inspect the insulation. It can be difficult to
see if the insulation is effective. If any problems are discovered replace or repair the
insulation material. If in doubt contact an energy company to check the insulation.

D.2.1 Doors

Check and re-hang hinges and locks: Hinges and door locks are often not correctly
adjusted, so doors do not close properly. Air and energy can escape from the room.
From a hygienic point of view it is important that doors close firmly.

Check and/or replace rubber seals: Worn or damaged rubbers will also prevent doors
closing properly, resulting in energy loss. From a hygienic point of view it is important
that doors close firmly.

D.3 Climate computer

Check settings: By regularly checking the settings the optimal values can be entered.
This can save a great deal of energy, 5 to 10% is a realistic figure. For example, if the
outside air has the right temperature to cool down a growing room at night, it is better
to use outside air. Otherwise the daytime air must be cooled down first before it is used.

D.3.1 Cold storage

Maintenance: Only allow an authorised installer to perform maintenance activities.
The cold storage unit is covered by the “Resolution concerning substances that damage
the ozone layer” in The Netherlands.

Check insulation: Check the insulation material for breaks and damage. If there are
gaps in the insulation material, fill them with polyurethane spray. Leaking insulation
causes high energy losses.

Clean condensers: A soiled condenser influences the cooling capacity. For good heat
transfer, the condenser must be kept clean. Use compressed air to clean, blown through
the fins in the opposite direction to the outlet direction. Check the fan for soiling.

D.4 Conclusion

Our conclusion is that correct maintenance in all its facets has a positive effect. Firstly,
more efficient energy use is better for the environment. Secondly, correct maintenance
will reduce energy consumption and save money. Even though the individual savings
are slight, cumulatively they add up to a considerable amount each year. And last but not least, the quality of your produce will improve due to better regulated and more accurate climate control and the reduced risk of infection and diseases by firmly closing gaps and cracks.

### D.5 Items on the maintenance chart

<table>
<thead>
<tr>
<th>RH Measuring box inside</th>
<th>Cooling unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean water reservoir and replace water per growing cycle</td>
<td>Check condensation water drainage 4 x year</td>
</tr>
<tr>
<td>Replace wick per growing cycle</td>
<td>Clean cooling unit 1 x year</td>
</tr>
<tr>
<td>Check fan per growing cycle</td>
<td></td>
</tr>
<tr>
<td>Check measurements per growing cycle</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RH Measuring box outside</th>
<th>Heater unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check meters 1 x month</td>
<td>Clean heater unit 1 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CO₂ meter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrate CO₂ meter with zero cartridge 4 x year</td>
</tr>
<tr>
<td>Adjust V-belt tension 4 x year</td>
</tr>
</tbody>
</table>

| Check suction hose + filter 4 x year |
| Check condenser working 4 x year |
| Remove rust and dirt 4 x year |
| Set cooling medium temperature 4 x year |

| Drain condensation pot 2 x month |
| Check steam cut-off 1 x year |

| Replace filters 1 x year |
| Clean misters 4 x year |
| Replace/clean water filters 4 x year |

<table>
<thead>
<tr>
<th>Humidifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check valve 4 x year</td>
</tr>
<tr>
<td>Clean misters 4 x year</td>
</tr>
<tr>
<td>Replace/clean water filters 4 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubricate bearings 4 x year</td>
</tr>
<tr>
<td>Adjust V-belt tension 4 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Steam boiler</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance boiler/burner 1 x year</td>
</tr>
<tr>
<td>Check boiler water level 4 x year</td>
</tr>
<tr>
<td>Blow through per growing cycle 1 x year</td>
</tr>
<tr>
<td>Check insulation (piping) 1 x year</td>
</tr>
<tr>
<td>Check steam cut-off 1 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Check/refill softener</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance 1 x year</td>
</tr>
<tr>
<td>Check insulation 1 x year</td>
</tr>
<tr>
<td>Clean condensers 2 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Climate units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check &amp; test inlet + mixing valve 2 x year</td>
</tr>
<tr>
<td>Replace coarse filters 1 x year</td>
</tr>
<tr>
<td>Replace fine filters per growing cycle 1 x year</td>
</tr>
<tr>
<td>Clean inside 1 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cooling installation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance cooling installation 2 x year</td>
</tr>
<tr>
<td>Check condenser working 4 x year</td>
</tr>
<tr>
<td>Remove rust and dirt 4 x year</td>
</tr>
<tr>
<td>Set cooling medium temperature 4 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cold storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance 1 x year</td>
</tr>
<tr>
<td>Check insulation 1 x year</td>
</tr>
<tr>
<td>Clean condensers 2 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Buildings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check walls/ceilings for leakage 1 x year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Doors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check and re-hang locks/hinges 1 x year</td>
</tr>
<tr>
<td>Check/replace sealing rubbers 1 x year</td>
</tr>
</tbody>
</table>
### Maintenance Chart (Essential Part)

<table>
<thead>
<tr>
<th>Month</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>RH measuring box inside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>clean water reservoir and replace water and wick*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check fan working*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check measurements*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH measuring box outside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check meters</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CO₂-METER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calibrate CO₂ meter (zero cartridge)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check suction hose plus filter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>replace filters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>drain condensation jar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEATING INSTALLATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check water pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>maintenance boiler/burner</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check main pump</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check/test pumps/mixing valves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check insulation (piping)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEAM BOILER</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>blow through</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>clean/refill softener</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>maintenance boiler/burner</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>check boiler water level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check insulation (piping)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check steam cutoffs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLIMATE UNITS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>replace coarse filter plus outlet*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>replace fine filters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>check/test inlet plus mixing valve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>clean inside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOING UNIT</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Appendix E Termitomyces: a common genus in Africa and South-East Asia

E.1 Introduction

The genus Termitomyces is highlighted in this manual because extension officers in developing countries often receive inquiries about it. At present, species of this genus cannot be cultivated. The mushrooms are much sought after, so it is certainly worthwhile to start cultivation experiments with members of this genus. It may well prove to be difficult to cultivate, however, because of the very specific conditions under which it grows in nature.

The indigenous population in many tropical countries (from Zambia, Tanzania and Burundi to Thailand and Taiwan) is familiar with this genus of mushrooms. The following tale reveals how much Termitomyces is loved by people in Malawi: *When the first rains fall, the older women go out in the fields to check the spots where they have harvested Termitomyces before. When small cracks in the soil indicate the emerging mushrooms, they jealously cover these spots with leaves and litter. They will declare the right to harvest the mushrooms at that particular spot.*

Termitomyces is equally liked by animals. Not only insect larvae and termites from the adjacent termite hill, but also boars have been reported to consume it. The nutritional value of most Termitomyces species is better than that of the cultivated White button mushroom. They generally contain more proteins than Agaricus.

E.2 Taxonomy

There are about twenty different species of Termitomyces. Quite a few are depicted on stamps from countries where they abound, which shows their popularity. They have to be differentiated macroscopically, as their microscopical characters are remarkably simi-
lar. The spores are hyaline (colourless), cystidia are always present and often abundant, the basidia are tetrasporal.

The following macroscopical factors are important in distinguishing different Termitomyces-species:
- cap diameter,
- colour and structure of the cap (sclary, striate),
- annulus (ring) present on the stipe or not,
- colour of pseudorhiza (the ‘root’ below the stipe, leading to the chamber where the mushroom emerged from; see below).

The identity of the termites also indicates which kind of Termitomyces can be found and vice versa.

Two species of Termitomyces are easy to identify:
1. *Termitomyces titanicus*, with a cap diameter of up to 1 meter (!), grey scales or grains on the cap and a ring on the stipe.
2. *Termitomyces microcarpus*, which has very small fruit bodies (smaller than 2 cm), growing in large numbers on the soil or on termite hills.

The second species is different from all the others, in that the termites which grow the mycelium of this mushroom, carry numerous parts of fungus combs outside the nest. They only act in this way during the rainy season. So many mushrooms may emerge all at once that the surface of the mound is completely covered with them. Because of their small size, they are time-consuming to collect.

The following species have been reported from Thailand, Burundi and Tanzania:

<table>
<thead>
<tr>
<th>Species</th>
<th>Thailand</th>
<th>Burundi</th>
<th>Tanzania</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Termitomyces aurantiacus</em></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><em>Termitomyces clypeatus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Termitomyces eurhizus</em></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Termitomyces fuliginosus</em></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Termitomyces globulus</em></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><em>Termitomyces lelestui</em></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Termitomyces mammiformis</em></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Termitomyces microcarpus</em></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><em>Termitomyces robustus</em></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><em>Termitomyces singidensis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Termitomyces striatus</em></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><em>Termitomyces tilanicus</em></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

*T. robustus* and *T. fuliginosus* possibly belong to the same species.
E.3 Termite behaviour

About 100 of the 1800 known termite species are mushroom growers (*Macrotermintinae*). They live like ants with females, males, workers and soldiers in colonies. They differ so much from ants, however, that they have been placed in a different insect order, the Isoptera. The name white ants is therefore misleading. They are active year-round; food should therefore also be available year-round. It consists of plant materials, either live, dead or partly decomposed. Termites belong to the most successful degraders in tropical countries, and play a very significant role in the degradation of millions of tonnes of plant debris each year. Why are they more successful than other degraders, like saprophytic fungi, bacteria and other insects? The answer can be found in the ‘termite cathedrals’, the nests of the termites. These nests host numerous fungus chambers, where fungi degrade the lignin components of the solid excrements of the termites. The chambers are coated with fluid excrements. Galleries connect the chambers and ventilation ducts provide aeration. The termites construct gardens, fungus combs, from plant material. These combs look like coral, sponges or brains. The *Termityوميَس* mycelium grows on these structures, which provide sufficient aeration because of their specific form. The termites nibble at the mycelium, which induces the mycelium to form nodules. The termites constantly add organic material on top of the fungus combs. The mycelium is constantly in an atmosphere with the right humidity and temperature. Other degraders of plant debris suffer from periodical drought and fluctuating temperatures. This is the reason why the *Macrotermintinae* in cooperation with fungi belong to the most common degraders in tropical countries.

E.4 Fungal development in the fungus chambers

(The following case study has been reported from Thailand by P.J. Bels and S. Pataretgitt, in Tropical Mushrooms, see Annotated bibliography for details)

In the development of *Termityوميَس* species three distinct phases can be distinguished. **First phase.** During the first phase the termites are actively maintaining the mycelial culture. The termites bring in plant debris (after having ingested it first) and put it on top of the combs. They will nibble at the bottom of the combs, where the mycelium has
partly degraded lignin, thus easing digestion by the termites. These actions of the termites probably stimulate the development of nodules and also suppress the growth of \textit{Xylaria}, another fungus which is present in the chambers. The mycelium is present in the form of small round colonies, which have not yet grown together.

**Second phase.** The second phase is entered when the termites start to neglect a number of chambers and hardly consume any \textit{Termitomyces} mycelium from them anymore. The number of nodules decreases and both the white tufts of \textit{Termitomyces} mycelium as well as the olive green ones of the \textit{Xylaria} spp. can grow together. The combs become covered with a velvety layer of \textit{Termitomyces} mycelium, and some of them may eventually give rise to pseudorhiza. During the rainy season some pseudorhiza can actually turn into fruit bodies, which will push themselves through the soil. Some species are equipped with a so-called \textit{perforatorium} on the cap to break through the soil. It has been shown that the temperature in the chambers decreases slightly when the termites abandon the chambers. CO$_2$ concentrations will therefore also decrease, as it is produced by the termites, too. Many basidiomycetes respond to lower temperatures and CO$_2$ levels with the formation of fruit bodies. This could well be the case with \textit{Termitomyces}, too.

**Third phase.** The third phase is entered when all termites have moved to another part of the hill. By now, the entire comb has turned greenish-black by the \textit{Xylaria}-mycelium, fruit bodies of \textit{Xylaria} are all over the chamber and no more nodules can be seen. Unlike \textit{Termitomyces}, the \textit{Xylaria}-fruit bodies lack the potential to break through the (often hard) soil.
Appendix F Suppliers

The following companies have participated in the production of this Mushroom Cultivation book. They are listed in an alphabetical order; the table below shows which type of products and services are offered by these companies.

<table>
<thead>
<tr>
<th>Spawn</th>
<th>Mycelia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micelios Fungisem</td>
</tr>
<tr>
<td></td>
<td>Sylvan America Inc.</td>
</tr>
<tr>
<td>Substrates</td>
<td>Micelios Fungisem</td>
</tr>
<tr>
<td>Substrate preparation equipment</td>
<td>Thilot Holland BV</td>
</tr>
<tr>
<td>Climate control, building growing rooms and tunnels</td>
<td>Unicorn</td>
</tr>
<tr>
<td>Consultancy and research</td>
<td>Limbraco bv</td>
</tr>
<tr>
<td></td>
<td>Gicom</td>
</tr>
<tr>
<td></td>
<td>Peeten bv</td>
</tr>
<tr>
<td></td>
<td>Hato bv (lighting only)</td>
</tr>
<tr>
<td>Plastic bags for spawn and substrate</td>
<td>Applied Plant Research</td>
</tr>
<tr>
<td></td>
<td>Blaak Specialty Mushrooms Consultancy</td>
</tr>
<tr>
<td></td>
<td>Cpoint</td>
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<td></td>
<td>Spore/ECO Consult Foundation</td>
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<tr>
<td></td>
<td>SacO₂</td>
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<tr>
<td></td>
<td>Unicorn</td>
</tr>
</tbody>
</table>

Applied Plant Research (Mushroom Research Unit)

The Mushroom Research Unit at Applied Plant Research carries out contract research for both industry and public sector organisations. We develop expertise and products related to cultivated mushrooms, including cultivation techniques, new varieties and bioactive compounds. We also have in-house expertise relating to mycorrhizal fungi and wild mushrooms.

Postal address:
P.O. Box 6042, 5960 AA Horst
The Netherlands
Tel: 31 (0)77 4647575  Fax: 31 (0)77 4641567
Website: www.ppo.dlo.nl  E-mail: infopaddestoelen@ppo.dlo.nl
Blaak’s specialism is the cultivation of lignivorous mushrooms in all their aspects. Substrate production according to 4 principles: sterilisation, pasteurisation at 95 °C, pasteurisation at 70 °C, pre-heating method. Blaak advises you in the preparation of a realistic business plan for substrate production and mushroom cultivation. Precise instruction will be provided by way of workshops or “coaching on the job” to obtain optimal results from your production unit. Whatever your requirements in business advice, workshops or practical training, it will always be tailor-made services.
Blaak Specialty Mushrooms Consultancy
Scheemderzwaag 4, 9679 TM Scheemda, The Netherlands
Tel: +31 597 593314    Fax: +31 597 591226
Website: www.eblaak.com  E-mail: info@eblaak.com

GICOM Composting Systems laid its foundations by growing mushrooms. By seeing the mushrooms grow, at GICOM’s own mushroom farm, the first computer control systems were developed in 1984. One innovation led to another. Nowadays GICOM, based in the heart of The Netherlands, is specialised in designing, manufacturing and commissioning composting installations for mushroom substrate and organic waste treatment and mushroom growing rooms worldwide.

GICOM’s factory, based in the heart of The Netherlands

Gicom BV, Oogstweg 9,
8256 SB Biddinghuizen, The Netherlands
Tel: 31 (0)321 22 26 82    Fax: 31 (0)321 22 27 84
E-mail: info@gicom.nl    Website : www.gicom.nl
Cpoint, Training and Consultancy for mushroom growing, offers training and advice in order to provide employers and their staff with the necessary expertise. Cpoint is an organisation oriented towards the mushroom sector. Cpoint has resulted in a unique organisation which is able to tailor to the particular needs of mushroom growers and supply companies regarding expertise. We are in close contact with the mushroom sector, the Mushroom Experimental Station and supply and delivery companies. The knowledge and experience of an enthusiastic team of teachers and advisers guarantee high results of your investments in advice, training or courses!

Advice and consultancy at Cpoint
Cpoint is specialised in giving expert advice in many areas to mushroom growers and composting farms. The advice is aimed at improving and optimising the production. A good example is cultivation and business consultancy. A company is visited an agreed number of times a year. Each visit results in practicable advice which will improve the production. Apart from cultivation and business consultancy, Cpoint can give you tailored advice with regard to equipment and mechanisation, results pertaining to farm economics, personnel management, construction and quality systems.

Training and courses at Cpoint
Cpoint is also specialised in offering training and courses to employers and their staff. Training can be offered on location, i.e. on the employer’s farm. The acquired knowledge can be field-tested directly, which is a great advantage. This also means that the participants are very familiar with the situation.
Training can also be offered on another location to staff members of one company or several companies jointly. The extra advantage is that people are detached from their own company and see the acquired knowledge being applied on another farm first.
There are also courses with open enrolments. Of course the advantage of this is the unique opportunity to exchange experience with other participants.
Because of the unique combination of teachers and advisers you can expect optimal advice from us regarding your need for expertise.

Short Course and Theme-days
The objective of the Short Course is to gather and/or extend your knowledge about mushroom growing and to expand the background information of a mushroom farm. This course is meant for workers of a mushroom farm, (starting) self-employed persons and farm managers. This course will be organised every year in springtime. One week is focussed on compost, the other on growing. It is possible to subscribe for one or two weeks.
The Theme-days are meant for workers of a mushroom farm or compost yard, self-employed persons and farm managers. Experience in mushroom growing is desirable. The agenda of the course will be as follows: Monday & Tuesday: composting and tunnel management; Wednesday: farm visits; Thursday & Friday: growing and harvesting. This course will be organised every year in fall.
P.O. Box 6035, 5960 AA Horst, The Netherlands
tel.: +31 77 398 45 55 fax: +31 77 398 41 60
E-mail: info@cpoint.nl Website: www.cpoint.nl
Hato BV Lighting is specialised in permanent lighting systems for mushroom houses. Hato lights can withstand high temperatures and are resistant against ammonia, formaldehydes and the most used chemicals in mushroom cultivation. If you need permanent lighting without problems, please contact us.

Hato BV, Handelsstraat 31
6135 KK Sittard, The Netherlands
T: +31 (0)46 4585050  F: +31 (0)46 4585090
Website: www.hatobv.com  E-mail: info@hatobv.com

Limbraco BV manufactures and installs complete climate control units, steam, heating and electro equipment, as well as computer controls and machinery for the mushroom industry all over the world already for more then 30 years. Your address to go to.

Bremweg 2, 5961 NE Horst, The Netherlands
Tel: +31 77 3983359  Fax: +31 77 3983543
Website: www.limbraco.nl  E-mail: info@limbraco.nl

Micelios Fungisem, S.A.
Producer of high quality ready to fruit substrate of Shiitake, Pleurotus, Lepista nuda, Pholiota aegerita, Pleurotus eryngii etc. Also produces spawn of above mushrooms. Research and technology on all phases of mushroom cultivation.

Carretera de Calahorra, Km 2, 26560 Autol, La Rioja, SPAIN  (34) 941 390001
**MYCElia**

**Mycelia** is a production lab for axenic mycelium of cultivatable mushrooms. We dispose of a large strain collection of species and strains (see), of which we sell:
- mother cultures, in large test tube, 1 p. per Microsac: for further multiplication
- mother spawn, 500 g per bottle, 1 p. per Microsac: for further multiplication
- ready spawn, 5 or 10 litre per Microsac

Mycelia also offers individualised training sessions on axenic mycelium production, either in their own lab or at the customer’s premises. They assist in the lay-out and organisation of mycelium production labs all over the world. Ask for our tariffs!

Jean Bethunestraat 9, B-9040, Gent (Belgium)
Tel. +32-(0)9/228.70.90  Fax. +32-(0)9/228.80.28
info@mycelia.be  www.mycelia.be

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**Peeten bv**

More than 50 years experience in mushroom installations. We help you to set up the most modern mushroom farm according to the latest technology:
- composting systems phase 1,2,3: bunkers, tunnels
- growing rooms
- machinery.

Delivery according to your demand:
- consultancy, advice, engineering, training
- climate installations, computers, machinery
- turnkey projects.

Molenstraat 40, 5995 BJ Kessel, The Netherlands
Tel: 31 (0)77 462 1441  Fax: 31 (0)77 462 2485
E-mail: info@peeten.nl  Website: www.peeten.nl

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**Sylvan America Inc.**

Sylvan offers reliable and stable mushroom spawn, supplements and bio control agents throughout the world. It is currently the world’s largest producer and distributor of mushroom spawn.

West Hills Industrial Park, Kittanning, PA 16201, USA
(724) 543 2242
Saco2 produces autoclavable “breathing” bags for the multiplication of mycelium. The working principle is regularly distributed gas exchange through a number of parallel filter strips. Each strip consists of an endless series of filter plugs, the latter ensuring a regular gas exchange without risk of infection or drying out (see www.saco2.com).

The same gas exchange principle is used for the in-vitro multiplication of plants, axenic fermentation products and various applications where gas exchange is required while avoiding the passage of particles.

Rozenstraat 1a, 9810 NAZARETH (Eke), België
Tel: 32 (0)9/280 09 80, 0496/217 617 Fax: 32 (0)9/280 09 16
E-mail: info@saco2.com Website: http://www.saco2.com

Spore / ECO Consult Foundation

ECO Consult promotes mushroom cultivation with training and lectures, mushroom articles and books. The trade name Spore is used for the distribution of spawn and mushrooms to the general public in Europe. The main author of this book can be contacted through Spore.

Eco Consult Fax: 31 (0)344 630 225
Website: www.spore.nl E-mail: spore@antenna.nl

Thilot Holland BV is the leading manufacturer of composting and mushroom farm equipment. Experience and knowledge we have gained through the sale and service of hundreds of machines worldwide to more than 42 countries. Over the years, Thilot has earned a reputation for rugged machines that set standards for performance, durability, quality and value.

Thilot Holland BV
Hoofdstraat 11-17, 5973 ND Lottum The Netherlands
Tel: +31 (0)77 463 1774 Fax: +31 (0)77 463 2648
E-mail: thilot@thilot.nl Website: www.thilot.nl
UNICORNBAGS

We create the perfect environment for mushroom cultivation.

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Appendix G Chemical analyses for mushroom cultivation

Information provided by Cpoint

A number of measurements must be made to determine if the finished compost satisfies the requirements to successfully produce mushrooms.

G.1 pH-measurement (acidity)

Pure water has slight electric conductive properties. This means that water (an extremely small amount) is split into ions:

\[ \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^- \]

If per time unit, no concentration change is observed in the start or end product, the state of the product is in chemical balance. A state of balance means that per time unit the same amount of \( \text{H}_2\text{O} \)-molecules are divided as are formed. This can also be expressed as:

\[ \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^- \]

The chemical equation above means that the concentration (amount per litre) of \( \text{H}^+ \)-ions exactly equals the concentration of \( \text{OH}^- \)-ions. The concentration of both ions is very small – just \( 10^{-7} \) mol/l. Pure water has no acid or basic properties. We refer to pure water as a neutral substance.

If the chemical balance is disturbed (e.g. by a change in the concentration in the begin-and/or end product), the resulting reaction will be to try and reverse the disturbance as far as possible. If this rule is applied to the equation above, the following is obtained:

If acidity, i.e. \( \text{H}^+ \)-ions, is added to water, a portion of these (surplus) \( \text{H}^+ \)-ions will react with the \( \text{OH}^- \)-ions present:

\[ \text{H}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O} \]

Because the \( \text{H}^+ \)- and \( \text{OH}^- \)-ion concentration is often extremely small, the term \( \text{pH} \) or degree of acidity has been introduced. The \( \text{pH} \) level indicates the concentration of \( \text{H}^+ \)-ions.

The principle is:
pH + pOH = 14

Expressed differently: concentration H⁺-ions x concentration OH⁻-ions = 10⁻¹⁴
The pH may vary between 0 and 14.

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If the pH drops from e.g. 8.5 to 7.5 during conditioning, this means the concentration of OH⁻-ions will decrease tenfold and the concentration of H⁺-ions will increase tenfold. A low pH means: a high concentration of H⁺-ions (and a low concentration OH⁻-ions). A high pH means: a low concentration H⁺-ions.

G.2 NH₄⁺-determination

If the ammonia content is mentioned in relation to mushroom cultivation, the term covers various aspects. The NH₄⁺-content, measured at the moment of filling, includes the concentration of NH₃ (ammonia) in the compost air and the concentration of NH₄⁺-ions (ammonia) in the compost moisture. If the ammonia content is referred to just before spawning, this means only the NH₄⁺-concentration in the compost air! The reason behind this confusion is the fact that NH₃ and NH₄⁺ concentrations have a clear link. This relationship is shown in the equation below:

\[ \text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^- \]

G.3 Nitrogen (N) determination

Nitrogen is not just present in the form of ammonia. Approximately 2/3 of the total amount of nitrogen found in compost is present as proteins and amino acids.
In mushroom cultivation, two methods of nitrogen determination are used. When referring to the N-content of e.g. compost, the method used for N-determination must be stated.
1. the normal Kjeldahl method;
2. the altered Kjeldahl method.

1. In this method, an amount of compost is first dried for a period of 10 hours (oven temperature 105 °C). When the compost has cooled, a sample is ground and the nitrogen content determined. With this Kjeldahl-determination the total amount of amino-nitrogen is determined (i.e. protein-nitrogen). Details relating to N-determination are not handled here. An important fact is, however, that all the nitrogen present in NH₃ or NH₄⁺-form disappears during the drying process. This form of nitrogen is erroneously not included in the N-determination.

2. With the altered Kjeldahl-method an undried compost sample of a few grams is used. This method of N-determination takes the ammoniac nitrogen (NH₃⁻ N) into account.

Based on observations carried out over a period of years, Overstijns (1982) concludes that there is an obvious relationship between N-content determined by method a) and b):
total N-content = \( \text{NH}_4^+ - N + \text{N-content} \)  
(method b)  
(method a)

As the N-content of a compost sample is always expressed as a % in relation to the d.m.-content, a dry matter determination must always be made at the same time as an N-determination. Dry matter determination requires a minimum of 8 hours drying at 105 °C.

### G.4 Ash determination

The ash content is determined by burning dried compost. A dried sample is heated for 4.5 hours at 500 to 600 °C. The remaining unburned matter is ash (inorganic matter). The ash content is expressed as a % of the dry matter. E.g.: a dry sample of 5 gram still weighs 1 gram after incineration; the ash content is 20% of the dry matter. The ash content has little value in relation to compost quality; the ash content is however needed to calculate the C/N-ratio.

### G.5 Organic matter (o.m.)

When compost is heated to 500 °C the organic matter is burnt. If the ash content can be determined, so can the organic matter content:

\[
\text{dry matter} = \text{ash} + \text{organic matter}
\]

The organic matter content is always expressed as a % of the dry matter. In the previous example the o.m. content is 80% of the dry matter.

### G.6 Carbon (C)

Pure carbon makes up half of organic matter. If the o.m. content is known, the C-content can be calculated. C is always expressed as a % of the dry matter. In the previous example the C-content is 40% of the dry matter.

### G.7 C/N-ratio

The C/N ratio is a figure that shows the weight ratio between the amount of carbon and nitrogen. The nitrogen amount can be expressed including (N\text{total} = N + \text{NH}_4^+ - N) or excluding (N) the ammonium-nitrogen, e.g.:

\[
N = 2,0\% + \text{NH}_4^+ - N = 0,4\% = \text{Ntot.} \ 2,4\%
\]

\[
C = 40\%
\]

\[
\frac{C}{\text{N}} = 40/2 = 20
\]

\[
\frac{C}{\text{Ntot.}} = 40/2,4 = 17
\]
Appendix H Annotated bibliography

A large number of books, scientific articles and journals have been consulted for the compilation of this manual. The following list of books and journals is a selection of what the author feels are the most valuable books for mushroom growers, extension officers and researchers. Researchers should of course also consult more specific literature for in-depth studies. Two co-authors added references to their contribution (consult the relevant chapters 9 on organisation and 24 on mycorrhiza).

H.1 Literature on mushroom cultivation

Growing gourmet and medicinal mushrooms, 1993/2000, Paul Stamets, Published and distributed by Ten Speed Press, P.O. Box 7123, Berkeley-CA 94707, USA. This third edition is definitely a must for everybody involved in growing speciality mushrooms (anything except White button mushrooms). 393 photographs and diagrams (of which 59 in colour) on 574 pages clearly describe precise growth parameters for 25 gourmet mushrooms, including species like *Polyergus umbellatus*, *Grifola frondosa*, *Agaricus blazei*, all edible Oyster mushrooms etc. Commercial techniques, liquid inoculation techniques, growing room and spawn laboratory design, and contamination vectors are all described extensively in this volume.


Five Module Technology Guides to Mushroom cultivation, 1991, by T.H. Quimio, National Institutes of Biotechnology and Applied Microbiology, University of Los Banos, Laguna, Philippines. This set of five nicely illustrated brochures treats the following subjects: 1 Mushrooms and their benefits, 2 Pure culture techniques, 3 Spawn production, 4 Cultivation of *Volvariella* 5 Cultivation of *Pleurotus*. It is a good example of how to communicate on a simple level. Unfortunately it is out of print. Most of the information it contains can be found in the Manual you are reading right now.

Tropical Mushrooms, Biological Nature and Cultivation Methods, 1981, edited by S.T. Chang and T.H. Quimio, Chinese University Press, Hong Kong. Price approximately US$ 30 (about HK$ 200). Contains chapters by different authors on genetics and breeding, spawn production, storage, chemical analysis methods, morphology and physiology of *Volvariella*, *Pleurotus* and *Auricularia*, cultivation
techniques in different countries and an ecological study of Termitomyces. Some of the chapters are rather specialised and there is some overlap, but the book is a must in every experimenting station or extension service in tropical countries.


The scientific background of the three authors is clearly reflected in the book. It contains practical information on spawn production, outdoor cultivation of Volvariella, cultivation of Shitake on wood logs and problems encountered in cultivating various fungi. Most of the information in the FAO manual can be found in the present manual also.


This much cited collection of 33 papers from international experts covers aspects of most kinds of cultivated mushrooms. Relatively new developments like the Shitake cultivation on small plastic bags and Agaricus blazei are not treated, social aspects and case studies of implementation of mushroom projects are also not included. Chapters include strain preservation techniques, nutritional value, medical effects, cultivation practices of Agaricus bisporus. Agaricus bitorquis. Coprinus fimetarius. Lentimla edodes (on wood logs), Pholiota nameko, Pleurotus. Stropharia rugoso-annulata, Volvariella volvacea, Auricularia spp. and Tremella fuciformis.


A good example of a mushroom manual adapted to local circumstances. Contains easy-to-understand chapters on spawn production. Auricularia cultivation on both sawdust substrate and wood logs. Rice straw and Oyster mushroom cultivation and an English/Thai word list. The used terms (for example spore instead of mycelium) are sometimes incorrect, however.


Good descriptions of sterile techniques, spawn production, compost preparation, and a key to common contaminants in agar cultures. Specific information on tropical mushrooms is limited, but the book provides a good overall view of many aspects of mushroom production, including that of hallucinogenic Psilocybe and Agaricus.


All the aspects of Agaricus production around 1987 in The Netherlands are treated extensively. The situation in The Netherlands has changed since then, but the book remains valuable for its in-depth treatment of all aspects of mushroom growing. The chapters on breeding, spawn production, compost preparation, organisation, and climate control are of interest to Agaricus-growers all over the world.

Guide to Low Cost Mushroom Cultivation in the Tropics, 1986, T.H. Quimio, Department of Plant Pathology, University of Los Banos, Laguna, Philippines.

Cultivation practices in the Philippines are described in detail. The book is, however, out of print. Most information is included in this manual, too.

H.2 Journals

Mushroom Journal for the Tropics, published by the International Mushroom Society for the Tropics, Chinese University of Hong Kong, Department of Biology. This interesting journal has ceased publication. It was a quarterly journal with both practical and highly theoretical articles, and announcements. Contact Hong Kong University for back-issues. Annual subscription used to be US$ 15, add another US$ 5 for airmail delivery.

Micologia Neotropical Aplicada, yearly journal. Subscriptions should be addressed to: Micologia Neotropical Aplicada, Apartado Postal 490, Xalapa, Vera Cruz 91000, Mexico. Subscription rate: US$ 15, add US$ 5 for airmail delivery. The journal promotes the exchange of information between scientists. Some of the articles are in Spanish. All articles have summaries in both English and Spanish. The journal is of particular interest to extension workers and mycologists in Latin America.

Mushroom News, American Mushroom Institute, 907 E. Baltimore Pike, Kenneth Square-PA 19348, USA. Covers aspects of Agaricus cultivation in the USA, with occasional reports on other mushrooms.

Mushroom Journal, published by the British Mushroom Growers Association, Agricultural House, Knightsbridge, London SW1X 7NJ, United Kingdom. This journal mainly focuses on Agaricus production in the United Kingdom, with occasional reports on other mushrooms.

ISMS Newsletter, published by the International Society for Mushroom Science, 50 St. Flora’s Road, Littlehampton, Sussex BN17 6BB, United Kingdom. Tel: +44 (0)1903 716469, fax: +44 (0)1903726318. The newsletter is sent to all members of ISMS. Membership costs £ 30 per year. The quarterly published newsletter contains a useful overview and summaries of all recently published articles on mushroom cultivation and related aspects.

Mushroom Science I-XV
The ISMS organises international mushroom congresses every four years. The articles presented at each congress are bundled and published. They contain very heterogeneous articles, from very specialised research reports to straightforward descriptions of cultivation practices. The most recent publication from the Maastricht congress in 2000 was Mushroom Science XV. The Oxford papers can be found in Mushroom Science 14. Science and cultivation of edible fungi. 1995. The Mushroom Congress in Ireland in 1991 yielded Mushroom Science 13: Science and cultivation of edible fungi, part 1 & 2. Braunschweig, 1987, published its papers in 1989: Mushroom Science 12. A copy of these papers can be consulted at many research institutes. Mushroom Science 13. 14 and 15 can still be ordered from the publisher, A.A. Balkema. at: Swets Publishing Services, PO Box 825, 2160 SZ Lisse, The Netherlands. Price: approximately US$ 150.

H.3 Literature on taxonomy and identification of wild mushrooms
The following books are just a selection of available literature. Many more books exist
in the USA and Europe. Many mushroom species in developing countries, however, have not yet been described. Few developing countries have good field guides, but lately more and more books on this subject are being published. Some of the full colour picture books are certainly nice to look at but only if they compare this with determination keys they can be used to identify mushrooms. In the end taxonomical monographs with keys to the different species are necessary.

A full colour book with many fantastic pictures of the most astonishing mushrooms (Termitomyces titanicus with a cap diameter of almost 100 cm!, brightly coloured Cantharellus) and good descriptions of edible mushrooms from this part of Burundi. It also contains chapters on how to collect and preserve mushrooms, as well as a description of the Miombo-vegetation which yields numerous edible and tasty mushrooms. It can be acquired from: Administration générale de la coopération au développement. Rue du Trone, 4, B – 1050 Brussels, Belgium.


Authoritative reference work on the taxonomy of all Agaricales, with references to monographic keys.

Edible mushrooms of Tanzania. 1995, by M. Härkönen, T. Saarimäki, and L. Mwasumbi. This work is a combination of ethno-mycological knowledge from Tanzania and modern natural sciences. The book (with 93 pages and colour pictures) covers the most important edible and poisonous mushroom species. It is the result of four field trips and hundreds of interviews with Tanzanians. Vernacular names, modes of preparation and an introduction on how to identify mushrooms are all discussed. KARSTENIA Vol. 35 suppl. 1995, Helsinki. ISBN 951-45-6962-8.

A guide to the most common edible and poisonous mushroom species in Malawi, Zambia and Zimbabwe. 200 pages, colour pictures. Contains more information than Edible mushrooms of Tanzania.


More than 900 species are depicted in this volume, which is one of the least expensive quality picture books available. Many species in this publication are cosmopolitan. Local species in tropical countries (like the genus Termitomyces) are not treated in this volume, however. There is a separate edition for North America, but not for Africa, Latin America or Asia.
H.4 Literature on medicinal aspects and dyeing

Icons of Chinese Medicinal Fungi, 1987, Mao et al., Science Press. Beijing. Price in China: RMB 100 in Chinese, around FEC 300 (foreign exchange currency) in English. Fungi Perfecti mails it at USS 100. 272 beautiful colour drawings of a large number of mushrooms with descriptions of the species and their medicinal effects. For people doing a survey on possible usage of extracts from mushrooms the book can be useful.

Reishi mushroom: herb of spiritual potency and medical wonder. by T. Willard. The history and medical use of Reishi (Ganoderma) full of anecdotal reports. Willard is a devoted (and therefore possibly not always objective) Ganoderma lover, who writes entertainingly about the subject.

Mushrooms for colour, Miriam Rice. 154 pages, US$ 16. Mushrooms can be used for differently coloured dyes. The book describes how to extract the dye components and apply them to silk, cotton and wool with different types of mordants.


H.5 Literature on mycorrhiza, including ‘cultivation’ of truffles

Managing Forest Ecosystems to Conserve Fungus Diversity and Sustain Wild Mushroom Harvest, 1996. D. Pilz and R. Molina, editors. United States Department of Agriculture, PNW GTR 371. This publication describes on roughly 100 pages the experiments which the USDA has carried out in relation to wild mushrooms and (controlled) harvesting.


Appendix I Management and motivation

By Marc Maas, Cpoint

The cost price of mushrooms depends much on the cost of labour; especially picking takes considerable time. For this reason it is important to get as high a “yield” as possible in return for the cost of labour. Clearly, a well motivated team of employees will ensure high productivity.

One needs to take a closer look at a company’s management before being able to motivate its staff. Try and list its strengths and weaknesses, for the person in charge will set an example to the rest of the staff.

A good manager should have the following qualities:
- Personal qualities
- Social qualities
- Skills

E.1 Personal qualities
- The company’s policy should be open and honest. If staff fail to do their job properly they must be informed of this fact. Without notification they will not change their attitude towards work and as a result productivity will be low and the atmosphere at work will suffer.
- A good manager must be able to sense things. One should feel it if the atmosphere at work is negative. Sensing is intuition.
- The management should have the guts to change things rather than hold onto them simply because that is how they have always been. Clearly, these changes must be improvements.
- One must make a reliable impression. If steps are declared these should be taken.
- The management must believe in the company’s products. This will be noticed by members of staff and also serve as advertising.
- A true leader has the mind of a winner and will always try to produce a good product, together with staff members.

E.2 Social qualities

The management must be able to communicate and inform. This means the management is able to have a good talk and explain things to all those concerned. Diplomacy is desirable. Calling someone to order in the company of customers and other members of staff often has the wrong effect, not only on the person concerned but also on those present.
The management must be sensitive to changing values and developments in society, for
example those regarding part-time work and women who would like to return to work.
This requires careful planning and organisation.
If staff members are having domestic problems these might harm performance and
atmosphere. It may have a favourable effect if the management is able to sense when
this is the case.

E.3 Skills

It is frustrating for staff if decisions take a long time to make.

- Decisive action creates clarity, so that employees know where they stand.
- The management must be able to show and encourage the importance of teamwork.
- The management must be able to delegate. This means passing on responsibilities
to members of staff. It certainly does not mean shirking problems as the manage-
ment will remain responsible.
- Organisation and planning are an important part of managing. Careful planning
creates clarity and a just division of labour within the company.
- A good manager has problem-solving skills. The more problems one can solve the
more time will be left for other things.
- The management should at all times set an example. Expecting staff to do things
you won’t do yourself will only weaken the management’s position.
Those who can meet all these criteria are the kind of managers a company is looking
for. However, it should be noted that there are four different managing styles.

- Leading style
  The management tells employees what needs to be done.

- Supervising style
  The management shows what needs to be done and gives an explanation.

- Supporting style
  The management gives an explanation and lets staff have a try themselves.

- Delegating style
  The management leaves the whole process, including planning, to the staff, but not
without offering help and support.
If you now think you know what kind of manager you are yourself you may feel there
is still room for change and improvement. In any case it is important to be critical of
yourself. The next step would be the motivation of staff by means of the skills and
styles mentioned earlier. In order to motivate people you first need to determine what
motivation exactly is. This can be described as inciting someone to action. Motivating
people requires a target which is attainable yet not too easy. Furthermore it is important
to pay attention to those who have achieved something. There are a number of things
that prove to motivate most people well. Money is often mistakenly thought to be an
important incitement. However, people quickly get used to a pay increase and soon
forget about it.
Research has shown the following incitements:

- Extending knowledge results in growing motivation as this leads to more involve-
ment in the job.
As a rule motivation will also improve by allowing people to make their own decisions regarding working hours and procedures, in other words the way in which things are done. This is often thought to make employees work more slowly and sloppy, but in practice this proves not to be the case.

- If people achieve something and get a word of appreciation their motivation will improve.
- It is a fact that people like to influence others.
- Passing on responsibilities to those who can handle them will therefore lead to more achievements and improved motivation.
- If the management and staff are able to be creative this will in general give members of staff a positive feeling. Allow employees to help solve problems and consider and discuss their solutions seriously.

If you decide to apply the above to your own company the following possibilities will improve your staff’s motivation:

**Attention:** Pay attention to your employees and be genuinely interested in their well-being.

**Praise:** Praise your employees if they have achieved something and do not allow such an opportunity to pass.

**Self-confidence:** Stimulate your employees’ self-confidence if they have tried to come up with solutions to problems that have occurred. This also makes them feel responsible for the smooth running of the company.

**Pride:** Let your employees be proud of the end product.

**Information:** Give your employees as much information as possible. Do not keep them in the dark.

**Co-operation:** Encourage your employees to co-operate as much as possible. Point out its importance. Discuss each others’ ideas, replace each other if necessary and jointly discuss the procedures.

Please note that each employee needs a different incitement and therefore a different approach. Where management and motivation are concerned, every owner, or manager will have their own ideas.

Please take a sheet of paper and briefly describe what you do to motivate yourself on a day-to-day basis. What aspects of my work give me sufficient motivation?

The next question is: How do I as a manager motivate people?

### E.4 Some practical tips to motivate employees.

- Make sure the mushrooms look good. This will result in a high picking rate because it is enjoyable to pick a good product. Try not to harvest too many qualities at the same time.
- Employees’ assessments should be discussed regularly with those concerned. These conversations must be prepared well.
- If groups are small, progress should be discussed regularly, during coffee breaks if necessary. Do not worry if these breaks take longer than usual. Everyone should be able to say what is on their mind. Putting things on paper may be helpful. In general
the last item will be remembered best, so that might be something to take advantage of. Discuss the most important item last.

- Your attitude towards your staff. Some require a direct approach whereas others need to be handled with kid gloves. Whatever the approach, it should be consistent. Staff need to know where they stand. Chopping and changing your points of view will create uncertainty amongst staff and lead to demotivation, so a consistent policy is important. For example, at one time some companies allow all mushrooms of a certain quality to be picked whilst at another moment their demands are stricter. Inform staff of the reason why quality requirements may change from one hour to the next.

- Who plans the holidays and how flexible are the rules?
- Are staff part of a permanent or variable group and do picking staff always start in exactly the same place in the growing room?
- It is best for groups to be “like-minded”. People should feel comfortable within a group. This relates to age, residence and place of origin.
- A top 10 can show your staff’s picking rate. Bringing this into the open may be both positive and negative, depending on the way it is presented and its consequences.
- Some growers always end work at the same time. For example at five o’clock, without working overtime. This will create certainty for employees rather than a feeling of “We won’t be able to finish work. This means putting in overtime.” Of course this supposes an adequate number of staff. At the same time people will be able to make their own plans.

- Many people, such as women who would like to return to work, prefer working part-time. Often this is easier to fit into the work scheme than one would expect.
- The younger the employees, the more a manager will need to supervise and direct.
- Will there be any social evenings? If so, how often? And what about the division of the costs?
- Careful planning creates clarity so that staff can plan other activities outside work, knowing these need not be cancelled because of work.
- Keep work and private life separate.

**Pay special attention to the following**

- Some measures cost money but should be regarded as an investment in the future. For example investing time in discussions on work.
- The fluctuation of the picking rate in the course of the day and week needs to be looked at.
- Not every item can be applied to all companies so there is room for discussion.
- In addition to this, certain items cannot be applied without taking other areas for special attention into account.
- Some of the things mentioned earlier, such as discussing assessments, will need to take place several times in the course of the following years. One such discussion will not be enough.
ACTINOMYCES: filamentous bacteria, sometimes classified as Deuteromycetes in older literature ('Ray Fungi').

AGAR: an extract from a seaweed used to solidify media; alternatively, (cheaper) gelatin may be used. Agar is available in bar or powder form.

ASCOMYCETES: a group of fungi that have in common that they produce their sexual spores inside specialised cells (asci), which usually contain eight spores.

ASEPTIC: sterile condition: no unwanted organisms present.

AUTOCLAVE: a container, the contents of which can be heated up to 121 °C. It must be able to withstand an overpressure of 1 bar, otherwise the temperature can not rise sufficiently.

BACTERIA: unicellular micro-organisms that may cause contamination in culture work. Grain spawn is very easily contaminated with bacteria. On the other hand there are some bacteria that are needed for the fruiting of Agaricus. These are present in the casing soil.

BASIDIOMYCETES: a group of fungi which produce their spores externally on so-called basidia. Often four spores are produced per basidium. Many basidiomycetes show clamp-connections on their hyphae, ascomycetes never do.

BREAK: see FLUSH

BUTTON STAGE: the young mushrooms are still fully closed, but are already fully differentiated.

CASING SOIL: Some mushrooms (mainly Agaricus) need a covering layer of soil with a specific microflora for fruiting.

CELLULOSE: an organic compound in wood, straw, etc. It is more easy to degrade than lignin. Cellulose is probably best known as the raw material for paper. Cotton waste contains high amounts of cellulose, sawdust contains cellulose, hemicellulose and lignin.

COMPOST: the fermented (or fermenting) substrate. The reason for composting substrate in mushroom cultivation is to make it more selective for the desired mushroom.

CONDITIONING: the stage after pasteurisation in compost preparation, during which the temperature is held between 45 °C and 50 °C. This range is optimal for the development of a thermophilic microflora.

CRYOGENIC FREEZING: is the best technique to preserve strains. The strains are kept under liquid nitrogen at a temperature of less than 180 °C below zero.

CULTURE: here used to describe the way the pure strains are kept; for example on agar slants, under mineral oil or under liquid nitrogen.

CULTURE MEDIUM: micro-organisms differ in their nutritional needs. A large number of different growth media have been developed, PDA-agar and malt agar can be used for most cultivated mushrooms.

DIKARYOTIC MYCELium: contains the nuclei of both 'sexes' and can therefore produce fruit bodies.

FERMENTATION: the process of composting. Easily accessible nutrients will be degraded by micro-organisms which makes the substrate more selective. Unwanted fermentation may oc-
cur if the compost is still very ‘active’ or if thick layers or large bags are used. In that case the temperature inside the substrate will rise too high for the desired mycelium.

FLUSH: the sudden development of many fruit bodies at the same time. Usually there is a resting period between flushes or breaks.

FREE WATER: the water actually available to the micro-organisms in the substrate. Water content is the absolute measure. Free water is related to the water film around each particle in the substrate and the concentration of salts in the water.

FRUITING: the mycelium will form mushrooms in its reproductive stage. This is called fruiting as the mushrooms are actually the fruit bodies of the mycelium.

GERMINATION: the spreading of hyphae from a spore.

GILLS: the radially arranged, vertical plates below the cap of a mushroom on which spores are formed.

HETEROTHALLIC: a species that requires a compatible thallus (sexual partner) to mate. At least two (often four) different mating types can be recognised.

HOMOTHALLIC: species like *Agaricus bisporus* need only a single spore to form a mycelium capable of fruit body formation.

INCUBATION: the period after inoculation (preferably at a temperature optimal for mycelial growth) during which the mycelium grows vegetatively.

LAMELLAE: see GILLS

LIGNIN: a difficult-to-degrade organic compound in wood, straw, etc. White-rotting basidiomycetes are well equipped to degrade lignin.

MOTHER SPAWN: spawn which is not meant for inoculating substrate, but for inoculating another batch of spawn.

MYCELIC: the network of hyphae that form the vegetative body of the fungus. Mushrooms are the fruit bodies of the mycelium.

MYCOLOGY: study of fungi.

MYCORRHIZA: a symbiotic relationship between fungi and plants.

NUCLEUS: the central part of a cell containing genetic information (DNA).

PARASITE: organism that lives at the expense of others, usually causing diseases in its hosts. Ultimately it may cause the death of its host.

PASTEURISATION: heat treatment applied to a substrate to destroy unwanted organisms but keeping favourable ones alive. The temperature range is 60-80 °C. The treatment is very different from sterilisation, which aims at destroying all organisms in the substrate.

PATHOGENIC: causing disease.

PETRI DISH: a round glass or plastic dish with a cover to observe the growth of microscopic organisms. The dishes are partly filled with sterile growth medium (or sterilised after they have been filled). Petri dishes are much used to grow the mycelium which will inoculate the mother spawn.

pH: A measure to describe the acidity of a medium. pH 7 is neutral; higher means alkaline, lower acidic. Most wood-inhabiting mushrooms prefer a slightly acidic substrate. *Agaricus* a more or less neutral substrate.

PINHEAD: a term to describe a very young mushroom when the cap has the size of a pin.

PRIMORDIUM: the initial fruit body.

PURE CULTURE: an isolated culture of a micro-organism without any other micro-organisms. Pure cultures are essential to the spawn production process.

RELATIVE HUMIDITY: the percentage of moisture in the air compared to the maximal amount that the air can hold at that temperature and pressure.

SLANT: a test tube with growth medium, which has been sterilised and slanted to increase the surface area.
SPAWN: the pure culture of mycelium on grain, sawdust, etc., used to inoculate the final substrate.

SPAWN RUN: the vegetative growth period of the mycelium after spawning the substrate.

SPECIES: fundamental unit of biological taxonomy. Generally spoken, two individuals belong to the same species if they can produce fertile offspring.

SPENT SUBSTRATE: the substrate remaining after the mushrooms have been harvested.

SPORES: the means of reproduction in fungi. In cultivated mushrooms, they are formed on the gills (or on the spines of the Monkey head mushroom) and dispersed in the air. One mushroom can produce millions and millions of spores.

STIPE: stalk of the mushroom.

STERILE conditions: see aseptic.

STERILISATION: completely destroying all micro-organisms present, by heat or chemicals.

Spawn substrate always has to be sterilised prior to inoculation.

STRAIN: the equivalent of race in plants and animals. The same species may consist of strains that vary considerably in genetic make-up, but all are sexually compatible.

STROMA: heavy mycelia on top of the casing soil, with negative effects: the casing soil will not absorb water readily. Stroma is correlated to the use of spawn with fluffy mycelium.

SUBCULTURE: a culture derived from another culture.

SUBSTRATE: the material on which the mycelium grows.

THERMOPHILES: micro-organisms with an optimal growth range of 35 to 60 °C.

TISSUE CULTURE: a culture made from the tissue of a young and healthy mushroom. The mycelium emerging from the tissue will have the same genetic properties as the mushroom the tissue derived from.