

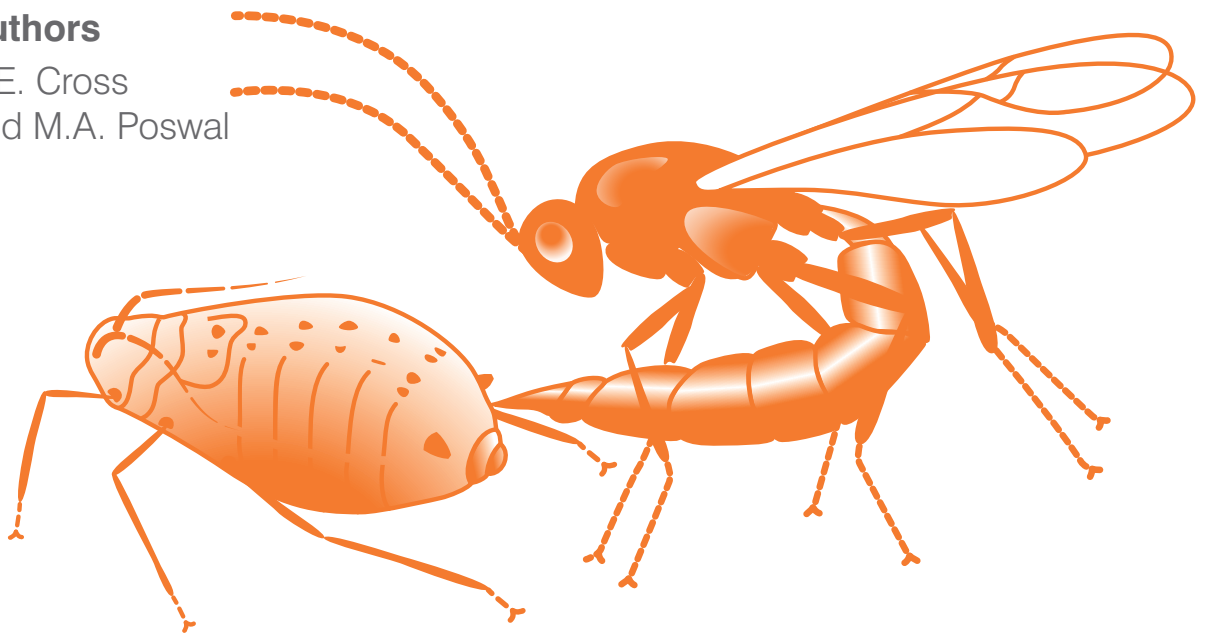
# Dossier on *Pauesia antennata*: Biological Control Agent for the Brown Peach Aphid, *Pterochloroides persicae*, in Yemen

August 2013



## Authors

A.E. Cross  
and M.A. Poswal



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A. (Tony) E. Cross (t.cross@cabi.org) was an entomologist at the International Institute of Biological Control (IIBC) UK Centre at Silwood Park in 1996; he is now CABI Facilities Manager.

M. Ashraf Poswal (a.poswal@cabi.org) was Scientist-in-charge of the PARC-IIBC Station, Pakistan, in 1996; he is now Global Director, Plant Clinic Implementation for the CABI Plantwise Programme.

## Preface

In the mid-1990s, the Yemen's stone and pome fruit sector was confronted with an outbreak of an accidentally introduced insect pest, the brown peach aphid (*Pterochloroides persicae*) causing severe losses to the sector. An emergency response project was set up with the General Department of Plant Protection (GDPP), Republic of Yemen under FAO Technical Cooperation Programme: 'TCP/YEM/4555 - Emergency Assistance for Control of Aphid', in collaboration with the Yemeni–German Plant Protection Project.

CABI (as the International Institute of Biological Control) was tasked to search for potential biological control agents, assess their suitability and provide one or more selected agents for introduction to control the brown peach aphid. A literature survey and assessment of prospects (Kairo and Poswal 1995) was followed by surveys in Pakistan where brown peach aphid is indigenous (Poswal 1996). This led to the selection of a braconid wasp, *Pauesia antennata*, for further study, which was carried out in Pakistan and in the CABI quarantine facility in the UK. The CABI inputs were initially the responsibility of Ashraf Poswal in Pakistan and Moses Kairo in the UK, although Tony Cross took over from Kairo when the latter was appointed Scientist-in-charge of CABI's Caribbean and Latin America Centre.

*Pauesia antennata* was imported to the laboratories of the GDPP in Sana'a, Yemen at the end of January 1997. A rearing colony was established, and by July the GDPP had reared more than 65,000 parasitoids, and of these more than 50,000 were released in the field. At the peak of production 1000 parasitoids a day were being collected and released. Releases were concentrated at three main sites around Sana'a and within two months the aphid populations in these areas, and beyond, had completely collapsed as a result of parasitoid attack. The parasitoid was found at farms more than 50 km from Sana'a two months after release, and 120 km away four months after. The release programme was extended to the south and south-east to increase dispersal throughout the country, and to attack the aphid on other hosts being affected such as almonds, apricots and pears (IIBC 1997). A successful country-wide control programme was achieved as the parasitoid continued to spread and establish. The GDPP shared the Edouard Saouma Award 1998–1999 which recognizes particular efficiency in implementation of a project funded by the Technical Cooperation Programme (FAO 1999).

At the time of the project, introductions of biological control agents were made following the newly endorsed first version of the International Standards for Phytosanitary Measures No. 3 'Code of conduct for the import and release of exotic biological control agents' (ISPM3, FAO 1996). Particularly in countries lacking specific legislation, this standard provided a mechanism to help countries implement classical biological control safely (Kairo *et al.* 2003). A key step in the regulatory process set out in ISPM3 is the preparation of a dossier on the proposed biological control agent, on the basis of which the risks associated with its proposed introduction can be assessed by the regulatory authorities. Here we present the dossier prepared for *P. antennata* based on the 1996 edition of ISPM3. Fuller guidance is available now regarding information requirements for the importation and release of biological control agents (e.g. Bigler *et al.* 2005), and ISPM3 has since been revised as 'Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms' (FAO 2005).

We are now using the mechanism of a CABI Working Paper to put this original dossier in the public domain, for the following reasons:

- It provides a historical document, i.e. an example of a biological control agent dossier from the first days of ISPM3.
- There is no likelihood of the research documented here being written up for journal publication, due to the changed responsibilities of the lead scientists.
- It helps document a little known, but very successful biological control programme, which deserves to be better known.

As a historical document, the dossier has been reviewed but only minor editing done, to update some species names, and adjust some references. Similarly, it has not been updated with new information, such as Rakhshani *et al.*'s (2005) very relevant publication.

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Matthew Cock  
CABI Chief Scientist  
August 2013



**Dossier on *Pauesia antennata* (Mukerji):  
Biological Control Agent for the Brown  
Peach Aphid, *Pterochloroides persicae*,  
in Yemen**

A.E. Cross<sup>1</sup> and M.A. Poswal<sup>2</sup>

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**<sup>1</sup>INTERNATIONAL INSTITUTE OF BIOLOGICAL CONTROL**  
Silwood Park  
Buckhurst Road  
Ascot, Berkshire, SL5 7TA  
UK

**<sup>2</sup>PARC-IIBC Station**  
P.O. Box 8  
Rawalpindi  
Pakistan

## CONTENTS

Executive summary	3
Introduction	4
Brown Peach Aphid	4
Taxonomy, origin	4
Distribution and pest status	5
Control options	5
Prospects for Biological control	6
Natural enemies of the Brown Peach Aphid	7
Previous biological control programmes against the brown peach aphid	7
<i>Pauesia antennata</i>	8
Characterisation	8
Biology and ecology	8
Safety and likely impact	14
Assessment of Potential Risks	16
To non-target organisms	16
To human and animal health	16
To those handling the parasitoid	17
Contaminants	17
Procedures for eliminating contaminants	18
References	19
Annex 1	
Confirmation of identifications of the pest, and the parasitoid for introduction	22

## Executive summary

1. Since 1993 an aphid pest has been reported as becoming a serious problem in the Yemen. This accidentally introduced pest, *Pterochloroides persicae*, the brown peach aphid, is a known pest of fruit trees in the genus *Prunus* and in Yemen is causing severe damage to *Pr. persica*, *Pr. amygdalus* and *Pr. domesticus*. The pest is thought to originate in China and have slowly dispersed westwards invading new areas.
2. A suggested strategy is for integrating chemical control by the application of compatible and selective chemicals on a need basis, allied with some cultural methods, with a complementary release of biological control agents to achieve a sustainable long term solution. The exotic nature of the pest, and its outbreak in the absence of its natural enemy complex, would indicate that it would be an ideal candidate for classical biological control. This would involve the introduction of one or more of the natural enemies primarily responsible for its control.
3. A number of natural enemies have been identified as attacking the brown peach aphid, however it is proposed that the host specific parasitoid *Pauesia antennata* be the first agent imported and released. This parasitoid is the most commonly recorded and predominant natural enemy of the aphid in Central Asia. It has equivalent biological attributes to other aphid parasitoids which have proved successful in classical biological control programmes.
4. The biology and behaviour of *Pauesia antennata* have co-evolved with the aphid to such an extent that it will not pose a risk to other non-target species, human or animal health or the environment. Procedures will be taken prior to importation to ensure there is no risk of introducing any harmful contaminants into the Yemen.

## Introduction

In 1993 an exotic aphid was first reported attacking fruit trees in the Yemen. It was identified as the brown peach aphid, *Pterochloroides persicae* (Cholodkovsky), which has been known as a pest of peach (*Prunus persica*) and almond (*Pr. amygdalus*=*Pr. dulcis*) in some countries since the turn of the century. In recent years it has continued to spread and in many new localities is reported to be causing widespread damage.

In Yemen it is a problem on fruit trees of the genus *Prunus*, particularly peach, almond and plum (*Pr. domesticus*) (van Harten *et al.* 1994), causing considerable damage and even death of the trees. These fruit trees are grown by over 70,000 farmers and are a vital source of income in addition to being an important constituent of their diet. In an attempt to alleviate the growing problem posed by the brown peach aphid, the Government of Yemen began a nation-wide spraying program. For the long term, however, the intention is to develop an IPM strategy with the emphasis on biological control. The exotic origin of the brown peach aphid in Yemen makes it a good candidate for classical biological control. This would involve the introduction and release of exotic natural enemies, and the suggested agent in this document is the host specific parasitoid *Pauesia antennata* (Mukerji) (Hymenoptera: Braconidae, Aphidiinae).

This dossier provides information on the parasitoid in line with the FAO Code of Conduct for the Import and Release of Exotic Biological Control Agents (FAO 1996).

## Brown Peach Aphid

### Taxonomy, origin

The brown peach aphid, also referred to as the giant bark, giant brown bark, peach trunk, peach stem and black peach aphid, was first described in 1899 as *Lachnus persicae* from material collected in the Trans-Caspian area of Central Asia (Cholodkovsky 1899). It was later placed in the genus *Pterochloroides* raised by Mordvilko (1914). Several synonyms have been used in the literature for this species, namely *Cinara persicae*, *Pterochlorus persicae*, *Tuberodryobius persicae*, *Dryobius amygdali* and *Dryaphis persicae*. It is a monotypic species belonging to the tribe Lachnini of the sub-family Lachninae (Homoptera: Aphididae). This is a large subfamily containing several economically important genera, perhaps the most notable being *Cinara*, which contains several major pest species (Ciesla 1991). Its closest relatives are probably *Lachnus* spp.

The main host of *Pt. persicae*, peach (*Pr. persica*: Rosaceae), is reported to have originated in China (Wang 1985), as are a number of its other minor hosts in the genus *Prunus* including the second most frequently attacked species *Pr. armeniaca*. Assuming that these *Prunus* species are the main natural hosts of the brown peach aphid, it has been suggested that the centre of origin of the aphid is also in China (Kairo and Poswal 1995). This has not been confirmed however as there are no records of the aphid from that country. The spread through Central Asian countries into the Middle East, Mediterranean, Southern Europe and North Africa is well documented (Kairo and Poswal 1995).



In addition to these species native to China, the brown peach aphid has also been reported from other *Prunus* species e.g. sloe (*Pr. spinosa*) and cultivated plums (*Pr. domestica*), the former being a particularly suitable host.

### **Distribution and pest status**

*Pterochloroides persicae* commonly occurs on peaches in southern Europe, the Mediterranean, North Africa, the Middle East and Central and South East Asia. Records show that the aphid has slowly increased its range westwards and southwards from a focus in Central Asia. In Europe, the most westerly records are those in Spain (Hermoso de Mendoza and Lacassa 1995), and on the North African coast the aphid also occurs in Tunisia (El-Tariki and El-Sharif 1987).

The pest status and host range of the brown peach aphid varies from country to country, depending on what fruit species are being grown. *Pterochloroides persicae* is however perhaps most important as a pest of peach and almond. Other recorded hosts, which are considered to be unusual, include *Malus* spp. (Rosaceae), *Pyrus malus* (Rosaceae), *Cydonia vulgaris* (Rosaceae) and *Citrus* spp. (Rutaceae). These other hosts are reported to be unsuitable for the brown peach aphid to complete its life cycle (Archangelsky 1917).

The brown peach aphid is an aggregative aphid, forming huge colonies, mainly on the underside of larger branches as well as stems of young peach trees. In addition to the stress the aphids place on trees through removing sap, the copious amounts of honeydew produced encourage development of sooty moulds. This reduces the photosynthetic capacity of leaves, and fruit obtained from such trees are abnormal in size, shape and colour and may suffer from premature fruit drop (Batra 1951). The extent of the damage varies with tree age. In Egypt, for example, it has been noted that the aphid does not develop on seedlings less than 3 years old (Darwish *et al.* 1989). Severe infestation on mature trees over many years will often cause the death of the trees.

### **Control options**

#### *Resistant varieties*

One potential component for the long term control strategy is the development of fruit tree cultivars with resistance to the brown peach aphid. Recent work in Turkmenistan has identified almond varieties resistant to *Pt. persicae* and other pest and disease problems (Denisov 1985). Additional studies by Monet (1985) on peach resistance to leafcurl and the aphid *Myzus persicae* (Sulzer) support the possibility of developing resistant cultivars. Scorza and Okie (1985) reported that many peach cultivars have been developed from a restricted germplasm base. This practice has thought to be the root cause of many problems including pests, diseases, tree vigour and fruit firmness. Although the development of resistant cultivars would appear to require considerable research effort and collaboration between countries, there may be many potential benefits to be gained from it in the long term.

#### *Cultural control*

Several biological attributes of the aphid would indicate possible success from cultural measures such as pruning of infested branches. However there are few records in the

literature on such cultural control measures being used. One example exists in Pakistan where the aphid colonies are traditionally brushed away or crushed with a piece of gunny sack or rags tied to a stick (Janjua and Chaudhry 1964).

### *Chemical control*

Although attempts to control the brown peach aphid with insecticides date back to the 1950s (Batra 1951), there are no recorded studies of insecticidal control as part of an IPM programme. The efficacy of different chemical insecticides has been examined by several workers (Ciampollini and Martelli 1977, Velmirovic 1977, Sadhu and Sohi 1978, Mann *et al.* 1979). Some have also investigated different spraying methods, including high volume sprays, ULV and aerial spraying (Ahmad *et al.* 1966, Sadhu and Sohi 1978). However, despite the effectiveness of many contact insecticides there are risks associated with their use including pest resurgence, and the danger of killing pollinators (Avidov and Harpaz 1969). Even systemic insecticides are likely to have detrimental effects, as they will affect predators or parasitoids attacking the aphids and insecticide residues may persist in the fruits (Kashyap and Hameed 1986).

### **Prospects for biological control**

For accidentally introduced pests, classical biological control has frequently proven to be a very efficient and cost effective pest management strategy. Success has apparently been achieved in 46% of the introductions against homopteran insect pests (Greathead 1989). At least 26 species of Aphidoidea have been the subject of over 100 biological control projects, with a success rate of 48% (Hågvar and Hofsvang 1991). Information from projects on the classical biological control of aphids indicates that 23 species of braconids have been used, and that parasitoids became established in 32 of 55 attempts (Greathead 1989).

A review of aphid biological control in field situations has shown there to have been eight substantial successes, six of these being from the use of braconids (Hughes 1989). Some recent examples of the control of lachnine aphid pests include successes with *Pauesia* species; for example, the release of *Pa. cedrobii* Starý & Leclant against *Cinara (Cedrobium) laportei* (Remaudière) in France (Fabre and Rabasse 1987) and of *Pa. bicolor* (Ashmead) against *C. cronartii* Tissot & Pepper in South Africa (Kfir and Kirsten 1991). Another project presently being implemented is the release of *Pa. juniperorum* Starý against *C. cupressi* (Buckton), a devastating pest of cypress in Africa (Murphy *et al.* 1994).

*Pterochloroides persicae* is exotic to several countries where it is now a serious pest, usually due to the absence of any effective natural enemies. The lack of any records of the aphid from its potential area of origin may indicate that control in this area, by its natural enemy complex, is extremely effective.

The successful record of parasitoids in aphid control projects, the exotic origin of *Pt. persicae* to the Yemen, and its apparent absence from its supposed area of origin would suggest that the prospects for successful classical biological control are good. One of the key biological control agents identified for this is *Pa. antennata* (Kairo and Poswal 1995).

## Natural Enemies of the Brown Peach Aphid

### *Predators*

A range of aphidophagous predators from seven families have been recorded attacking the brown peach aphid (Kairo and Poswal 1995). In areas most recently invaded the predator fauna is poor, only the predatory midge *Aphidoletes aphidomyza* Rondani, some coccinellids and syrphids being routinely found (Talhok 1977). The majority of these are generalists that have probably either moved from the surrounding habitat to exploit this new prey source or were already present in orchard systems exploiting other aphid species. These predators with a wide host range are unlikely to have a significant impact on any individual pest species, because it is only one of a large spectrum of prey attacked. Of the many predators recorded only a few species are considered to be of any significance.

### *Parasitoids*

A few parasitoids from the family Braconidae have been recorded attacking the brown peach aphid (Kairo and Poswal 1995). Of particular importance are those in the genera *Pauesia*, which almost exclusively attack Lachninae (Mackauer and Chow 1986), and *Aphidius*. Most interesting of these are the parasitoids *Pa. antennata* and an *Aphidius* sp.

*Pauesia antennata* has not been studied in detail, but Starý and Ghosh (1983) provide a key to *Pauesia* species from India and surrounding regions. Early records of an *Aphidius* sp. from Central Asia reported that this parasitoid mainly attacked younger instars of the brown peach aphid (Archangelsky 1917). In the field, parasitism was reported to be about 25–30% in September and by mid-November only parasitized aphids could be found (Nevskii 1926). Thus it would appear that *Aphidius* sp. may be another important parasitoid, but further work is needed to identify the parasitoid species and research its host range.

Other reports of parasitoids attacking the brown peach aphid include *Lysiphlebus fabarum* (Marshall) (Vachidov 1974) and *Diaeretiella rapae* (McIntosh), but these do not appear to be as significant.

### *Entomopathogens*

The literature on biological control agents attacking the brown peach aphid also includes some pathogens. An unidentified disease causing significant mortality has been reported in Israel (Bodenheimer and Swirski 1957). In Central Asia a fungus, tentatively identified as a *Capnodium* sp., was observed attacking the aphid (Archangelsky 1917). More detailed research has however only been conducted on one pathogen found in Latvia, *Entomophthora thaxteriana*, which has now been renamed *Conidiobolus obscurus* (Tsinovskii and Egina 1972). This species was tested against several aphids and found to give substantial mortalities.

## Previous biological control programmes against the brown peach aphid

As a group, aphids have been the target of several successful classical biological control programmes (see above and Mills 1990). There are not however any recorded examples of

classical biological control against the brown peach aphid, or indeed of the use of *Pa. antennata*. Examples of similar programmes are detailed above.

## ***Pauesia antennata***

### **Characterisation**

#### *Taxonomy*

*Pauesia antennata* is a hymenopterous parasitoid from the family Braconidae, several species of which are highly specialised and only attack aphids. It was described from specimens collected in Baluchistan Province, Pakistan by Mukerji (1950) as a new parasitoid attacking the aphids, *Pt. persicae*, affecting peach. Mukerji placed this new parasitoid in the genus *Aphidius*, and named it *A. antennatus*. Few other *Aphidius* spp. were known from this area at this time and the report compares it with *A. pisivorus* Smith, *A. ervi* Haliday (= *medicaginis* Marshall) and *A. colemani* Viereck.

#### *Description*

*Pauesia antennata* is described as having 22 segmented antennae, including scape; dark brown propodeum; metacarpus nearly always as long as the stigma; second abscissa is sub-equal to the cross-vein, which is distally indistinct and incomplete; first abscissa of radius is somewhat longer than the second abscissa, while the intercubitus is slightly more than half compared with the first abscissa.

Length (adult) 3.5 mm; head smooth and shiny, brown with yellowish mouthparts, basal and first flagellar segments light brown remainder darker; thorax smooth, pro and mesonotum yellow, mesepisternum and metapleura brownish, remainder of thorax dark brown. Abdomen brownish except the petiole which is yellowish, ovipositor sheath greyish-brown. Wings hyaline, veins and stigma brownish-yellow.

#### *Source of culture material*

The cultures of *Pa. antenna* which shall be used for introduction into the Yemen are derived from material from Pakistan. The original collections of material have been made at sites around Quetta in the Baluchistan Province, where *Pa. antennata* was first collected and described.

### **Biology and ecology**

#### *Life history*

*Pauesia antennata* is a solitary endoparasitoid of the brown peach aphid, only one parasitoid completes its development which occurs within the body of the host. Usually only one parasitoid egg is deposited within the host aphid, although occasionally there may be two or more. When this does occur, the supernumerary eggs or larvae are eliminated, either by physical attack or by physiological suppression, to leave a single surviving parasitoid larva.

The act of oviposition is very short in *Pa. antennata* lasting only a few seconds, during which the egg is deposited inside the host aphid. This parasitoid is reported to preferentially attack final stage nymphs and early adults of the brown peach aphid. First instar parasitoid larvae hatch from between 43 and 47 hours after the egg is deposited (Poswal 1996). It passes through a further two or three instars before reaching the pre-pupal stage, the exact number is not yet known. Aphid parasitoid larvae are thought to feed on host haemolymph in early stages. Not until the last larval stage do they begin feeding on host tissues, particularly the reproductive organs. Parasitised aphids are therefore still capable of some reproduction before they perish, the amount is dependant on the stage of host attacked and if they manage to reach the adult stage before perishing.

By the time the parasitoid pre-pupal stage has been reached the aphid host has been completely consumed and has perished, and only its outer cuticle remains. Just before the larva passes into the pre-pupal stage it spins a silken cocoon on the inside of the host cuticle. This has now become slightly darkened and considerably hardened into a pupal case commonly referred to as a “mummy”. With experience these mummies can easily be distinguished. Pupal and adult development takes place within the mummy, and a fully formed parasitoid emerges from the host via a circular emergence hole it cuts in the mummy. This emergence hole can be in any part of the host’s abdomen. Studies at IIBC Pakistan have shown that at  $24\pm 4^{\circ}\text{C}$  the time taken from deposited egg through to adult is between 13 and 14 days, with males emerging slightly earlier than females. A simplified diagram of the life cycle is given in figure 1.

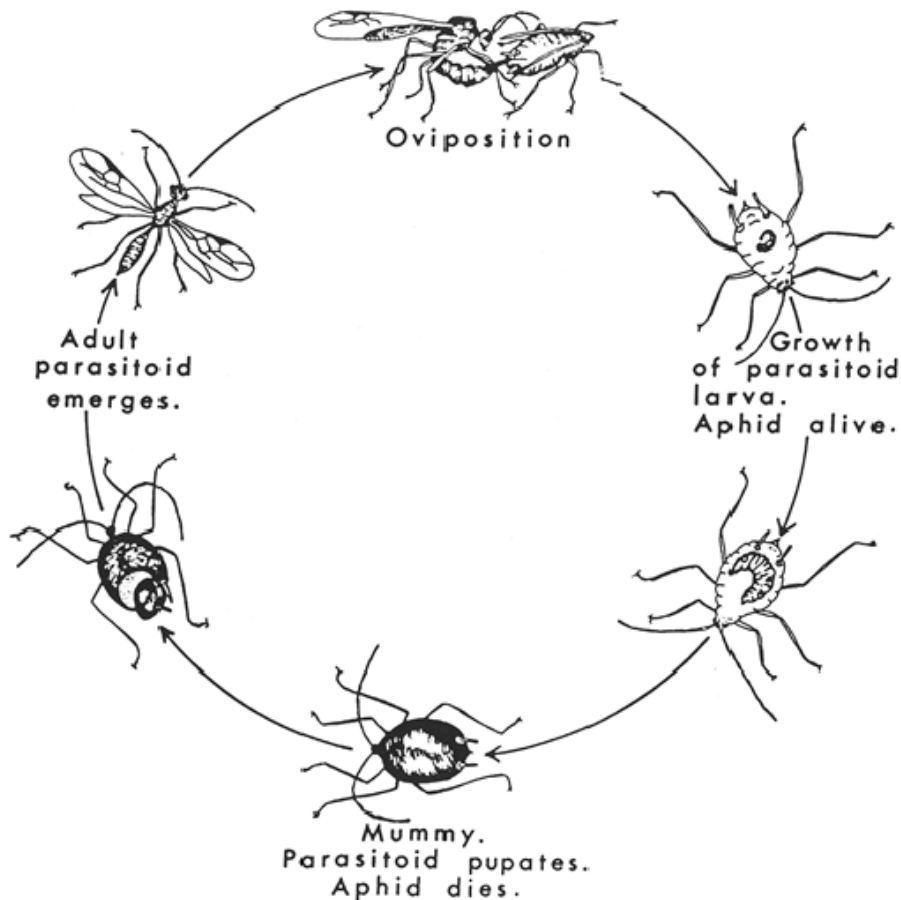


Figure 1. Life cycle of *Pauesia antennata* in the brown peach aphid.  
(Reproduced from Hågvar and Hofsvang 1991)

*Pauesia antennata* adults show a positive phototactic response, and need a few hours to fully mature. Following this they are able to mate, which typically takes from between 31 and 55 seconds (Poswal 1996), the females only mating once, in contrast to males which mate several times. Females can however begin ovipositing before mating, the progeny produced being solely haploid males. This is because reproduction in *Pa. antennata* is arrhenotokous or biparental, which means that unfertilised eggs produce these haploid males and fertilised eggs produce diploid females. It is known that in related parasitoid species males tend to be produced at the very beginning and then at the very end of the reproductive cycle, presuming the female has been successfully mated.

Female *Pa. antennata* are synovigenic, i.e. at emergence their oviducts contain some mature eggs but further eggs are produced throughout their reproductive life. Usually they continue parasitising throughout their adult life, with maximum activity occurring a couple of days after emergence and then tailing off. The fecundity of individual females is known to be dependant on their size, with larger ones being more fecund. The average fecundity of *Pa. antennata* has not yet been elucidated.

Adult parasitoids feed on host honeydew, which is an essential food source for them, and in addition to this their longevity is dependant on factors such as temperature, water and the presence of hosts. At IIBC Pakistan initial research has shown that *Pa. antennata* is very short lived (just a matter of 5–6 days), and that they can be kept alive on honey or glucose solution for an equivalent period of time to host honeydew.

Adult *Pa. antennata* locate host aphids using a mixture of mechanisms, the first step being location of host habitat followed by location of hosts and finally selection of suitable hosts. They detect chemical and physical cues which enable them to progressively decrease the area of search until suitable hosts are found. Final host selection follows a series of behavioural steps, progressing from external to internal examination, and rejection can occur at any point. Parasitoids in this family are known to be able to find high populations of their host very rapidly, indicating that they should be very effective biological control agents

#### *Culture methods*

Some aphid parasitoids are very amenable to simple rearing methods allowing them to be mass produced as commercial products for biological control. However those aphids occurring on more difficult host plants e.g. trees have often proved to be more difficult. One rearing programme which has been successful is that of *Pa. bicolor* on *Cinara cronartii* this scheme was based on the use of cut branches or logs, and more details are available in Kirsten and Kfir (1991). Starý stated that the rearing of aphid parasitoids is generally rather simple, however this has not proved to be the case for *Pa. antennata*.

The following summary gives details of the methods currently being used at IIBC for the rearing of brown peach aphids and the parasitoid. Similar schemes are in place in both Pakistan and the UK, differences will be indicated where necessary. A general scheme can be devised at any site but it may not work as well at some sites and thus there will always be a need for local adaptations. Brown peach aphids only feed on mature material, it has been noted in the field that trees under 4 years old are not attacked. Thus the use of small young peach trees is not possible. The scheme developed at IIBC is thus based on cut branches.

The host plants used are: peach (*Prunus persica*) in Pakistan, and sloe (*Pr. spinosa*) in the UK. Both of these are susceptible and thus make good rearing hosts. Studies in Pakistan have shown that there are differences in performance of the aphids on different peach varieties (Poswal 1996). For culturing purposes it has been found that, of those varieties tested, Florida King and Florida Prince are the best culture medium. Generally the selection of medium has been largely based on what is readily available at the sites.

Branches are cut from mature trees in the field, those selected are between 2 and 4 cm thick. These are brought back to the laboratory and cut into sections about 30–35 cm long. Once cut the sections are cleaned off, with mild detergent, to remove all organic matter, insects etc. If pathogens are thought to be present they can also be sterilised by washing with a 10% solution of bleach to destroy any fungal contamination. Finally they need to be rinsed with clean water.

Next the branches are set up as follows. The basal end is placed in a jar, for example a jam jar or similar drinking container. In the UK an inert potting material, perlite, is then put into the container, this material absorbs water and provides support for the cut branch keeping it vertical. The container can however just contain water, the important thing in either case is to keep the basal cut end submerged at all times. The top of the perlite is covered with cotton wool, which provides a surface from which fallen aphids can climb back onto the branch.

In the UK the cuttings are stored in the rearing room for a few days, to allow them to acclimatise, before aphids are added. The rooms run at various temperatures; in Pakistan a temperature of  $24\pm 4^{\circ}\text{C}$  is used for all rooms, in the UK a temperature of  $26\pm 1^{\circ}\text{C}$  is used for the aphids and  $21\pm 1^{\circ}\text{C}$  for the parasitoids. The aphids have been found to develop and reproduce successfully at a range of temperatures in the UK. As there is a need for as many aphids as possible a temperature towards the top of their optimum range was chosen to shorten develop time, thus increasing production. Temperatures above this are thought to be detrimental and are avoided. For the parasitoids maximum longevity is desired to ensure as many suitable aphids as possible have been encountered, and so hopefully maximising parasitism. The lighting regime in use is 16Light:8Dark, although a scheme of 13L:11D would be perfectly adequate.

When cuttings are ready for infestation aphids are added. The aphid culture in the UK is based on material received from Yemen. Dependant on how the aphids are sent, various methods can be used to infest the cuttings. If the aphids are free of plant material and walking around they can be gently lifted on with a moistened fine paintbrush. However if they are still feeding the branch can be placed alongside the cutting, in contact, and the aphids will transfer over themselves. Allowing natural transfer prevents high mortalities occurring as a result of the stylets being damaged during manual transfer. The aphids are very mobile and will quickly search over the cutting to find a suitable feeding site. They tend to aggregate and thus form a few small colonies on the cutting.

Whilst the cutting is viable the aphids remain fairly stationary at their feeding sites, although the colonies as a whole can move around to new feeding sites. Once the cutting is becoming spent, an increasing proportion of the aphids become restless and begin walking around the plant. If fresh material is not provided at this time they will all leave the cutting within 1–2 days. For steady production of aphids all that is necessary is to place a fresh cutting (or cuttings if numbers are high) in contact with the infested one and allow natural transfer.

Once all have moved over the old cutting can be discarded. In Pakistan sections of peach branches have been found to sustain 80–120 aphids for between 15 to 20 days, in the UK however with the higher temperature the sloe cuttings last for 10 to 15 days – just enough to get one generation of aphids through. The cuttings are kept within cages 50x50x50cm to keep them contained and free of contaminants.

For parasitoid production the ideal aphid stage is the late third instar to early adult. Following the regime in the UK aphids are reaching this stage at about 7 to 8 days in age, whilst in Pakistan this takes about 10 to 14 days. Cuttings with colonies of fresh adults can be selected and left to develop, and when the majority of nymphs are at the appropriate stage the cuttings can be used for parasitoid rearing. This method of aphid production produces mixed aged colonies but keeps mortality low. However, a large proportion of these aphids are rejected by the parasitoids.

To increase the proportion of aphids attacked a more synchronised group of aphids needs to be produced, which can be done as follows. Recently moulted adult aphids are selected from infested cuttings and then very carefully removed with a fine paintbrush (experience is needed to do this without harming the aphid). These are then transferred to fresh clean cuttings (Day 1) and allowed to settle. They remain on the cutting for two days, producing nymphs, and are then removed again at the end of Day 3 and returned to the stock culture. On average each aphid produces 6 to 8 nymphs in this time and these are allowed to develop for about 7 days to reach the late third instar. As these colonies have aphids which are within two days in age then when the correct stage is reached the whole colony provides ideal material for parasitism, thus maximising parasitoid production.

Cuttings with a population of either mixed or synchronised age aphids are transferred to a separate room for parasitoid rearing. A different room is used to prevent the contamination, and thus the loss, of the aphid stock culture. Heavily infested cuttings are set up in cages and then adult parasitoids can be added. It is useful to provide honey if the cuttings do not contain much honeydew, e.g. as a solution in a small piece of cotton wool in a small dish. To ensure the parasitoids have tried to mate they are placed in vials 25mm wide by 75mm long after emergence (usually one female with two or more males). A small wad of cotton wool about the size of a pea moistened with some honey solution (50:50) is placed in the vial cap and the parasitoids are left for about 18 hours before being added to the cage. If the females have not successfully mated, then only male progeny will be produced. The parasitoids remain in the cage until they perish and the next generation is allowed to develop. After about 13–14 days the first adults begin to emerge, usually males with females coming a day or so later. They can either be left in the cage to produce a further generation and increase the population in the cage, or they can be transferred to new cages to increase the number of cages.

### *Origin and distribution*

There are no reports in the literature of a proposed area of origin for *Pa. antennata*. Examination of the lachnine aphid fauna and their associated parasitoid spectrum across the Palaearctic led Starý to conclude that the Far East is the most likely area of origin for the aphid parasitoid fauna. This study included the brown peach aphid and *Pa. antennata*. There is also the suggestion that the brown peach aphid has originated in China (Kairo and Poswal 1995). The specialised interaction between *Pa. antennata* and *Pt. persicae* would suggest



they have co-evolved for some time. It seems reasonable therefore to propose that *Pa. antennata* originates from South East Asia, possibly China.

There are confirmed records of *Pa. antennata* from a few countries in Central Asia where the brown peach aphid is also common. This suggests that *Pa. antennata* has managed to follow the brown peach aphid to some extent during the aphid's westward dispersal.

### *Ecology*

Although there are no data about *Pa. antennata* from the field the biology is thought to be identical to closely related species, some of which have been studied (Starý 1988). In the field the sex ratio in adult aphid parasitoids tends to be female biased, when there is a plentiful supply of preferred hosts and conditions are favourable. Changes in any biological or environmental factors can however vary this to quite a degree.

The seasonal parasitoid biology is entirely dependant on that of their host aphid. The aphid shows both holocyclic and anholocyclic life cycles depending on local environmental conditions. In areas where winter temperatures fall to, or below freezing, the aphid follows a holocyclic life cycle and overwinters as oviparae. In contrast in warmer climates they follow an anholocyclic life cycle and continue as parthenogenetic apterae. In addition to these differences some aphids have been found to adapt their rates of development to local environmental conditions (Campbell *et al.* 1974). Those adapted to colder conditions developing faster at cooler temperatures than those adapted to warmer conditions. The parasitoids and hyperparasitoids from these aphids also behaved in a similar manner. There are implications from this for the introduction of biological control agents. Natural enemies pre-adapted to different conditions from those in the intended release areas may not perform as well as those pre-adapted to similar or identical conditions.

The number of aphid generations per year is also variable depending on whether there is an overwintering stage and on temperature conditions during the summer months. The parasitoid mirrors these differences, overwintering in cooler climates with the aphid by entering diapause as a larva/pupa in a dead host (Archangelsky 1917). Adult parasitoids emerge the following spring once the first generation of aphid nymphs have hatched, from overwintering eggs, and begun developing. The parasitoids must delay their emergence until after the aphid to ensure there are hosts to parasitise, resulting in the parasitoid season starting after the aphid. In warmer climates where there is no overwintering stage then both aphid and parasitoid continue development all through the season. The only record for the brown peach aphid season on *Prunus persica* is in Egypt where 18 overlapping generations were found (Darwish *et al.* 1989). The number of parasitoid generations per season is dependant both on the number of aphid generations and environmental conditions. However as the parasitoid life cycle is shorter then it would be anticipated that in the same environment there would be more generations of the parasitoid during the year than its host.

Studies have been made on the lower temperature thresholds, i.e. the temperature below which development is arrested, of aphid species and their parasitoids (Campbell *et al.* 1974). The temperature thresholds found in these studies (3.5–7°C) were below the means recorded in Sana'a in Yemen (between 12 and 22°C), suggesting that *Pa. antennata* will not have any problems establishing in the field and may continuously develop and reproduce throughout

the year. In the upland mountain areas however temperatures fall much lower, and thus *Pa. antennata* may need to go into diapause to overwinter.

The parasitoids have many mechanisms of dispersal, occurring either as larvae within live aphids, pupae in mummies or as free-flying adults. Dispersal as pupae in mummies is negligible, unless they become detached and are carried away e.g. by man. Similarly dispersal as larvae in living apterous aphids only occurs over short distances. In contrast dispersal as larvae in living alate aphids can occur over much greater distances, when the alates become airborne and are subject to prevailing air currents. The rapid dispersal of *Pa. bicolor* in South Africa, introduced for the control of *Cinara cronartii*, was attributed to it parasitising alate aphids (Kirsten and Kfir 1991). Adult parasitoids are also dispersed long distances by aerial drift, during periods of flight. The rate of such aerial dispersal is entirely dependant on prevailing conditions and thus can vary considerably.

#### *Use in biological control*

Although *Pa. antennata* has not been recorded from any biological control programmes, it has always been found in association with the brown peach aphid. It is believed to be the principle parasitoid of the aphid and as a consequence has been recommended as the candidate biological control agent of choice (Kairo and Poswal 1995).

#### **Safety and likely impact**

##### *Natural enemies and host range*

Brown peach aphids produce copious amounts of honeydew. Several species of ants are commonly found feeding on this, including *Linepithema humile* (Mayr) (= *Iridomyrmex humilis*) (Patti and Maniglia 1980). Usually in return for this honeydew the ants act as defenders, preventing parasitoids from attacking the aphids. In some cases however this has developed further and the ants are reported to be actually removing parasitoid larvae and pupae from parasitised aphids and destroying them (Archangelsky 1917, Plotnikov 1915). The impact of this type of activity has not however been quantified.

Of more importance are hyperparasitoids which might attack larvae and pupae of *Pa. antennata*. A number of species in the families Pteromalidae, Encyrtidae, Megaspilidae and Cynipidae are commonly found attacking aphid parasitoids in many areas. Several authors have examined the hyperparasitoid complexes of aphid parasitoids around the world. The most commonly reported species are *Alloxysta* spp., *Pachyneuron* spp., *Asaphes* spp., *Dendrocerus* spp. and *Syrphophagus* (= *Aphidencyrtus*) spp. The former species is endoparasitic on braconid larvae in live aphids, *Syrphophagus* spp. are also endoparasitic but can attack either live aphids or mummies, and the remainder are all ectoparasitic on parasitoid mummies. There is little if any host specificity in the ectoparasitic species and thus they can easily shift from one aphid-parasitoid complex to another in the same habitat. However there is some host specificity in the endoparasitic hyperparasitoids so they will not transfer over onto new aphid-parasitoid complexes as readily (Sullivan 1987) as ectoparasitic ones.

Where hyperparasitoids are abundant they are frequently reported to be affecting the control achieved by the aphid parasitoid. For example in China the hyperparasitoid *Dendrocerus*

*laticeps* (Hedicke) was reported to be limiting the parasitism by *Aphidius* spp. of aphids in wheat fields (Zong *et al.* 1986). Similarly the hyperparasitoid complex of *Trioxys* sp. greatly reduced its parasitism of *Aphis pomi* DeGeer in Canada (Bouchard *et al.* 1982). Hence the presence of such hyperparasitoids in the area of intended release for *Pa. antennata* could be very detrimental to its establishment and/or impact. However the only record of aphid parasitoids in the Arabian Peninsula is for *Syrphophagus aphidivorus* (Mayr) and *Pachyneuron* sp. from *Aphis fabae* Scopoli on broad bean in Iraq (Selim 1977). There are no reports of hyperparasitoids in the survey of aphid parasitoids in the Yemen by Starý and Erdelen (1982), which would suggest there is little to no threat posed by hyperparasitoids to the establishment of *Pa. antennata* in the Yemen.

Two hyperparasitoid species of *Pa. antennata* have been recorded in Central Asia, *Pachyneuron flavipes* (Förster) (= *syrphi* Ratzeburg) and *S. aphidivorus* (Archangelsky 1917), and three in Pakistan *Dendrocerus carpenteri* (Curtis) and *Pachyneuron* sp. (Poswal 1996), *Euneura lachni* (Ashmead) (= *Pachyneuron nazeeri* Mani) (Mani 1939). Studies by IIBC in Pakistan have found hyperparasitoids to be present throughout the season, gradually increasing towards the end of the year with a peak of 33.6% recorded in December. *Syrphophagus aphidivorus* has been recorded as a hyperparasitoid of *Aphidius colemani* in Brazil (Gravena 1979), and *Dendrocerus aphidum* (Rondani) in Chile. *Aphidius colemani* was reported to be the key species attacking the main pest aphids in the Yemen in 1982 (Starý and Erdelen). The accidental introduction and release of any hyperparasitoids from Pakistan into the Yemen must therefore be avoided, to prevent any interference of these parasitoid-aphid complexes, hence the need for third country quarantine and rearing at IIBC Silwood Park in the UK.

Parasitoids in the genus *Pauesia* with similar biology often attack other closely related aphid species in addition to their preferred host. It is also reported that less than one third of all parasitoids are restricted to one host species (Starý 1982). This suggests that the parasitoids have closely coevolved with their hosts, to become specialised natural enemies totally dependant on their hosts for survival, a prerequisite for successful classical biological control agents. Use of such alternate hosts however, for example in laboratory rearing, frequently causes a temporary or even permanent reduction in parasitoid fitness and performance.

*Pauesia antennata* is reported to be the primary parasitoid of the brown peach aphid. Therefore it is highly probable that the brown peach aphid is the primary host for *Pa. antennata*. Although evidence from closely related *Pauesia* species would suggest that *Pa. antennata* should have a few secondary aphid host species. Reports in the literature have presently only recorded the brown peach aphid as a host (Starý 1971).

### *Impact*

The adult female parasitoid can detect already parasitised aphids to some extent, this decreases the number of eggs lost in such hosts and so increases chances of successful development of their progeny.

When the parasitoid attacks aphids, inserting its ovipositor, it punctures the host's cuticle. It does not however paralyse the host. Many hosts are attacked and then rejected for oviposition during internal probing, due to a variety of factors. Such punctured hosts are usually killed by the internal examination, particularly when probed a number of times. As a

considerable number of aphids are rejected for oviposition then the total mortality in the aphid population as a result of parasitoid attack is higher than figures for parasitism would suggest.

Studies in Pakistan at sites in Quetta, Baluchistan, have shown parasitism by *Pa. antennata* in the field to vary from 2.7 to 20.1% (Poswal 1996). Figures for total mortality, which would be higher, are not however available. It is worth noting that brown peach aphid populations showed a peak occurring from late November to mid December, which went into sharp decline at the same time as the peak of parasitism is noted. This would suggest that besides other factors such as decline in temperature the parasitoid is exerting control over the brown peach aphid during its winter peak. The abundance of parasitised aphids obviously provides a resource for the hyperparasitoids, which also peak in December, although this does not interfere with the control as the populations of aphids remain low well into the new year.

## **Assessment of Potential Risks**

### **To non-target organisms**

Aphid parasitoids in the family Braconidae are specifically adapted for attacking hosts in the family Aphididae. They usually attack a few closely related aphid species, having a primary host and some secondaries. Thus *Pa. antennata* will be specific to closely related aphids in the subfamily Lachninae, as it is known to be specialised for locating, selecting and parasitising brown peach aphids on *Prunus* spp. and a few other host plants.

Although at present 74 aphid species are known from mainland Yemen, four from the island of Socotra, the only other recorded species from the family Lachninae is *Tuberolachnus salignus* Gmelin (van Harten *et al.* 1994). This aphid is also a new introduction to Yemen and attacks willows (*Salix* spp.). There are no records in the literature of *Pa. antennata* attacking this species, or any others in the same genus, suggesting it would not be able to develop in *T. salignus* in the very unlikely event it had oviposited in one. The lack of any records of *Pa. antennata* attacking aphids on *Salix* spp. would suggest that the parasitoid is not even attracted to these host plants to search for hosts.

Therefore the risk of *Pa. antennata* attacking *T. salignus* is extremely small, while other aphid species would be even more unlikely to be attacked. All of the aphid species present in Yemen are at least occasional pests. Another aphid occurring on peach is the green peach aphid, *Myzus persicae* (Sulzer), which is not that closely related to *Pt. persicae*. There is thus little risk of this being attacked by *Pa. antennata*, although should this occur it would be expected to be beneficial as this aphid is a common pest. Similarly in the very unlikely event that *Pa. antennata* attacked any other aphid species it would be expected to be beneficial.

As reported above this family of parasitoids have co-evolved with their hosts to such an extent that it would be impossible for them to try and attack other species. There is therefore no risk of attack by *Pa. antennata* to other organisms in Yemen e.g. beneficial insects.

### **To human and animal health**

As the possibility of *Pa. antennata* attacking non-target organisms is extremely remote, because they are specifically adapted to attacking in the presence of the brown peach aphid, then they would not be expected to be a danger to other animals. Even in the extremely unlikely event that they attempted to attack either humans or livestock there would not be an effect as the parasitoids do not have the ability to sting mammals. Therefore there would be no risk at all from speculative attacks on man, livestock or other animals and so *Pa. antennata* will not pose a threat to human or animal health.

### **To those handling the parasitoid**

There will be no direct risk at all to personnel from the parasitoid during handling or any other routine tasks, for the reasons stated above. It is however possible for certain individuals to develop allergies in situations where insects, and plants, are kept in confinement. Such an environment can often allow concentrations of organic matter to become unusually high. If this were to be the case in a facility used for rearing *Pt. persicae* and *Pa. antennata*, on suitable host plants, there may be an extremely small risk of sensitisation. The risk could be from either the host plants, the aphids or even the parasitoids and thus it would be advisable to maintain high standards of cleanliness and hygiene in the rearing environment.

### **Contaminants**

If care is not adequately exercised there is a possibility that when exotic biological control agents are introduced into a new country, or area, that contaminants will also be introduced.

One potential source of contamination to be particularly avoided is hyperparasitoids from the source of the parasitoid. These could jeopardise the effectiveness of the parasitoid and could also even adapt to attacking other indigenous (or introduced) parasitoids. This in turn could destabilise an existing system and create an additional pest problem. As reported above there are two recorded species of hyperparasitoids in the source material in Pakistan. These are both generalists and would be capable of transferring onto other aphid-parasitoid systems in the Yemen. An accidental introduction of these hyperparasitoids would therefore pose a risk to the existing aphid-parasitoid systems. The procedures for eliminating these hyperparasitoids by using a third country quarantine will however avoid this risk.

A second potential source of contamination is other pests organisms, in particular plant and insect pathogens, which do not occur in the Yemen. Spores of such pathogens could be carried with and survive in the shipment, procedures are required to prevent such an occurrence. The routine procedures employed at IIBC in the UK should avoid this eventuality.

Another important potential source of contamination is of different biotypes of the aphid pest itself, the introduction of which must be prevented to avoid the problem becoming worse. Such a hazard would definitely arise and pose a serious risk if insects were imported directly from the field, and so this type of importation must be avoided. By following simple quarantine procedures it is possible to eliminate all these problems, such methods are used at the Quarantine Facilities of IIBC in Silwood Park and details of specific points are given below.

## **Procedures for eliminating contaminants**

The importation and release of *Pa. antennata* will be conducted in accordance with the FAO Code of Conduct for the Import and Release of Exotic Biological Control Agents and recommendations laid down by the Ministry of Agriculture and Water Resources. To deal with specific problems of contamination the following procedures will be carried out prior to introduction.

### *Hyperparasitoids*

These can be eliminated simply by initiating and routinely setting up cultures using only identified adult *Pa. antennata*. Collections of parasitoid pupae can be set up individually, in e.g. separate vials, and each parasitoid identified as it emerges. At this stage only those parasitoids known to be *Pa. antennata* are extracted from the vials and used for culturing, and hyperparasitoids are removed and destroyed.

### *Pathogens*

Problems with plant pathogens can be effectively prevented by only using clean plant material. The cuttings used for culturing are cleaned up and sterilised prior to being used. As a precaution regular monitoring of the cuttings used in the culture is also carried out. This involves visual inspection and microbiological screening for disease. An additional practical measure taken during rearing is to keep the relative humidity below 60%. This prevents any spores being produced and thus plant pathogens being spread.

Suspect aphids, or any which show definite signs of disease can be removed from the culture and screened. If insect pathogens are identified the most practical method of preventing any disease spreading is again to keep humidities below 60%. This measure also prevents insect pathogens from sporulating, and thus infecting other aphids. As an additional measure host aphids for parasitoid rearing are only taken from cultures free of disease. Continual maintenance of such cultures in clean conditions will prevent the occurrence of diseases in the cultures, and thus contamination of material for shipment.

### *Different pest biotypes*

To ensure that different pest biotypes are not introduced, all parasitoid rearing is carried out using aphids which have originated from the Yemen. No live aphids are received in shipments from the IIBC station in Pakistan. As a final precaution adult parasitoids are separated from the mummies after emergence.

To ensure that all these measures are effective the positively identified parasitoids are reared through in clean culture in quarantine at IIBC Silwood Park for at least one generation.

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**Annex 1.** Confirmation of identifications of the pest and the parasitoid for introduction.

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**INTERNATIONAL INSTITUTE OF ENTOMOLOGY***An Institute of CAB INTERNATIONAL***Identification Services Report\***

56 Queen's Gate, London, SW7 5JR, UK.



14 June, 1995

List No. 7121 Europe

Country of Origin: PAKISTAN

Collection No. 23301

Sender: Dr Moses T.K. Kairo, Entomologist, International Institute of Biological Control,  
Silwood Park, Buckhurst Road, Ascot, Berkshire, SL5 7TA.

IDENTIFICATIONS	No. of Specimens	
	IDENTIFIED	RETAINED

**HOMOPTERA****APHIDIDAE** [G.W. Watson (IIE) det.]

<i>Pterochloroides persicae</i> (Cholodkovsky)	5290,5294	10	10
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This large, dark brown aphid occurs on the undersides of stout branches or on the trunk of *Prunus* species and occasionally on members of the Pomoidea and citrus, attended by ants. It occurs in the Mediterranean region, the Middle East, Central Asia, India, Pakistan, and has recently spread into Europe and the Yemen. Heavy populations on peach may kill branches. [GWW]

\*While all reasonable care is taken to ensure the accuracy and reliability of an identification report prepared by the Institute, no liability can be accepted by the Institute, its members, staff or agents in respect of any loss, damage or injury (whether fatal or otherwise), howsoever caused, which may be suffered as a result of the identification report.



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*An Institute of* CAB INTERNATIONAL**Identification Services Report\***

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14 August, 1996

List No. 7190 Europe

Country of Origin: PAKISTAN

Collection No. 23491

Sender: Dr Tony Cross, International Institute of Biological Control, Silwood Park, Buckhurst Road, Ascot, Berkshire, SL5 7TA.

IDENTIFICATIONS	No. of Specimens	
	IDENTIFIED	RETAINED
HYMENOPTERA		
BRACONIDAE [A.K. Walker (IIE) det.]		
<i>Pauesia antennata</i> Mukerji	10	10

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## contact CABI

### Africa

#### Kenya

**CABI**, ICRAF Complex  
United Nations Avenue, Gigiri  
PO Box 633-00621  
Nairobi, Kenya  
**T:** +254 (0)20 7224450/62  
**E:** africa@cabi.org

#### Ghana

**CABI**, CSIR Campus  
No. 6 Agostino Neto Road  
Airport Residential Area  
P. O. Box CT 8630, Cantonments  
Accra, Ghana  
**T:** +233 (0)302 797 202  
**E:** westafrica@cabi.org

### Americas

#### Brazil

**CABI**, UNESP-Fazenda Experimental  
Lageado, FEPAF (Escritorio da CABI)  
Rua Dr. Jose Barbosa de Barros 1780  
Fazenda Experimental Lageado  
CEP:18.610-307  
Botucatu, São Paulo, Brazil  
**T:** +5514-38826300  
**E:** y.colmenarez@cabi.org

#### Trinidad & Tobago

**CABI**, Gordon Street, Curepe  
Trinidad and Tobago  
**T:** +1 868 6457628  
**E:** caribbeanLA@cabi.org

#### USA

**CABI**, 38 Chauncey Street  
Suite 1002, Boston,  
MA 02111, USA  
**T:** +1 800-552-3083 (Toll free)  
**E:** cabi-nao@cabi.org

### Asia

#### China

**CABI**, Beijing Representative Office  
Internal Post Box 56  
Chinese Academy of Agricultural Sciences  
12 Zhongguancun Nandajie  
Beijing 100081, China  
**T:** +86 (0)10 82105692  
**E:** china@cabi.org

#### India

**CABI**, 2nd Floor, CG Block,  
NASC Complex, DP Shastri Marg  
Opp. Todapur Village, PUSA  
New Delhi – 110012, India  
**T:** +91 (0)11 25841906  
**E:** cabi-india@cabi.org

#### Malaysia

**CABI**, PO Box 210,  
43400 UPM Serdang  
Selangor, Malaysia  
**T:** +60 (0)3 89432921  
**E:** cabisea@cabi.org

#### Pakistan

**CABI**, Opposite 1-A,  
Data Gunj Baksh Road  
Satellite Town, PO Box 8  
Rawalpindi, Pakistan  
**T:** +92 (0)51 9290132  
**E:** sasia@cabi.org

### Europe

#### Switzerland

**CABI**, Rue des Grillons 1  
CH-2800 Delémont, Switzerland  
**T:** +41 (0)32 4214870  
**E:** europe-CH@cabi.org

#### UK

**CABI**, Nosworthy Way  
Wallingford, Oxfordshire, OX10 8DE, UK  
**T:** +44 (0)1491 832111  
**E:** corporate@cabi.org

**CABI**, Bakeham Lane  
Egham, Surrey, TW20 9TY, UK  
**T:** +44 (0)1491 829080  
**E:** microbiologicalservices@cabi.org  
**E:** cabieurope-uk@cabi.org