DETERMINATION OF INTENSITY OF SEED INHIBITORY DORMANCY BY LACTUCA TEST

Grbic Mihailo, Skocajic Dragana
Faculty of Forestry, Landscape Architecture and Horticulture Departmant
University of Belgrade

Abstract: Lettuce, as a plant that germinates readily and promptly, is used as an indicator to determine the intensity of inhibitory dormancy and the effect of dissolved pulp of the four selected species on the germination. Cotoneaster, (*Cotoneaster horizontalis Decne.*), holly (*Ilex aquifolium L.*),Chinese juni per (*Juni perus chinensis PFITZERIANA*) and wild rose (*Rosa canina L.*) are the species characterised by combined dormancy with the presence of inhibitory substances in the fleshy coat.

The study confirms the presence of inhibitory substances in the pulp in all the analysed species. The pulp of holly and Chinese juni per completely blocked the germination,rose pulp to a lower extent, and cotoneaster pulp to the lowest extent. The proof of the effect of inhibitors on lettuce seed is also in the direct proportion of the germination results and the applied concentration.

INTRODUCTION

Fleshy seed coats, whether they are pericarp (mesocarp), hypanthium, aril, or berrylike cones, as a rule, more or less inhibit germination. The effect of germination inhibitors from the fleshy coat is usually combined with another form of exogenous or endogenous dormancy. Much more rarely, the fleshy coat has no effect on germination, so it becomes a part of the sowing material, i.e. the fraction of pure seed (pure seed definition No 55), which is the case with the species in the genus Cornus. The coats without inhibitors are also pointed by the rare occurrences of vivi pary of the species in the genus *Skimmia,Citrus,Malus,...*,as well as the implementation of deferred extraction of maclura and walnut seeds in practice. As the inhibitory substances in the fleshy parts are most often formed during the period of the complete ri pening,or it is then that they turn into soluble forms available to the embryo, the collection in the period of physiological maturity, maceration, and immediate sowing are the only efficient method. On the other hand, multi ple dormancy in some species does not emphasise sufficiently the role and intensity of individual involved forms, so the applied procedures often remove only some, but not all forms of dormancy. This does not produce good results, and the sown seed does not germinate promptly and uniformly, or does not germinate at all.

According to literature data and our own experience, cotoneaster, (*Cotoneaster horizontalis* Decne.),holly (*Ilex aquifolium L.*),Chinese juni per (*Juni perus chinensis PFITZERIANA*) and wild rose (*Rosa canina L.*) are the species characterised by combined dormancy with the presence of inhibitory substances in the fleshy coat. Chinese juni per has the postponed germination because of embryo dormancy, impervious seed testa and inhibitors in the pulp of the berrylike cones. It is

similar with rose and cotoneaster, in which the pericarp is impervious, and the inhibitors are in the hypanthium. According to Nikolaevas (1977) classification, this complex form is a combination of two exogenous (A) types: physical (Aph) and chemical (Ach) dormancy, and an endogenous type (C) deep physiological dormancy (C_3). In holly, in addition to the above three types (Ach in mesocarp, Aph in endocarp), the embryo has two types of dormancy C_3 and B (morphological dormancy - undeveloped embryo which has to complete its growth and development).

Table 1 presents the methods of dormancy breaking, by the types of obstacles in the four study species.

The recommendations for the collection of cotoneaster (Anonymus, 1948) fruits clearly indicate the insufficient attention given to the problem of inhibitory dormancy. Although the collection of still greenish fruits in mid summer and prompt maceration is recommended, the possibility of fruit drying and seed extraction by crushing is also described. It is similar with holly, for which the storage of dry berrylike cones is recommended, and the practice of sowing the berrylike cones is described by Stilinovix (1987), although the prompt maceration is advocated by Hartmann et al., 1990.

It is only for the rose that the clear identification of inhibitory dormancy is reported by most authors. (Schopmeyer, 1974; Stilinovix, 1987; Grbix et al., 1996; Hartmann et al., 1990).

Although seed inhibitory dormancy, compared with other types, is relatively simply and promptly removed in practice, it causes problems if the time of seed collection is not correctly defined, which should be as close as possible to the technical maturity, in which seed has the highest germinative energy. On the other hand, it should also be sufficiently in advance because of the penetration of inhibitory substances to the embryo when the seed reaches the technical (complete) maturity. If not removed, it considerably increases the length of stratification, by which the term of sowing can be exceeded (Table 1).

The aim of the study is to determine the effect of dissolved pulp of the four selected species on the germination of lettuce seed (*Lactuca sativa L.*), as an indirect method for the

Table 1

The methods of dormancy breaking, by the types of obstacles in the four study species

species	types of dormancy			
	exogenous (para-) dormancy		endogenous (endo-) dormancy	
	chemical (inhibitory) Ach	physical Aph	morphological B	physiological C
Cotoneaster horizontalis Decne.	?	1.5-3 h H_2SO_4 stratification on 15-24°C, 3-4 months rana jesenja setva		stratification in soil on 5°C, 3-4 months
Ilex aquifolium L.	?	stratification on 30°C by day and 20°C by night, 1 month		stratification on 5°C, 1 month
Juniperus chinensis 'PFITZERIANA'	?	30 min H_2SO_4 stratification on 21-30°C, 2-3 months		stratification on 4°C, 4 months
Rosa canina L.	sakupljanje u fiziološkoj zrelosti i brza maceracija	stratification on room temperature, 2-3 months		stratification on 2-5°C, 2-5 months

determination of the intensity of inhibitory dormancy. Lettuce, as a plant that germinates readily and promptly, is widely used as an indicator (standard plant) in different biological experiments. It is recommended by U.S. and applied in numerous papers, especially in those dealing with the issues of pollutants. (Bowers et al., 1997; Rathbun, 1996; Wang, 1986; 1987; Wang at al., 1988).

MATERIALS AND METHODS

The fruits of cotoneaster (*Cotoneaster horizontalis* Decne.), holly (*Ilex aquifolium L.*),) and wild rose (*Rosa canina L.*)as well as the cones of Chinese juni per Chinese juni per (*Juniperus chinensis PFITZERIANA*, were collected in the Arboretum of the Faculty of Forestry (seed year 2001). As all varieties of lettuce are equally recommended for biological experiments, we applied the variety MAJSKA KRALJICA (germinative energy 80%, Agrocoop Declaration No 13, Declaration validity till 30.6.2002).

After the dry manual maceration, the crushed pulp was immersed in distilled water. Water solutions were prepared in the ratio 1:2, 1:3 and 1:4 (pulp mass to distilled water). Each mixture was mixed in the magnetic mixer for 30 minutes, after which the pulp was recrushed in water and left at 3-5°C in the airtight glass containers. After 24h, the mass was re-mixed and then strained. The solution was used for soaking blotting paper with lettuce seed. The solution was kept in the refrigerator throughout the duration of the germinative energy tests. The seed was placed on the blotting paper soaked with the respective solution. Each treatment consisted of 4x100 grains. The control was also established in four repetitions, 100 grains each, but the blotting paper was soaked in distilled water.

The testing of seed germinative energy was performed according to the regulations of the International Seed Testing Association (ISTA) for lettuce. The test lasts for seven days, at the constant temperature of 20°C; the substrate is blotting paper, germination energy is calculated based on 4 days, with pre-chilling. Because of the potential seed germination during the pre-treatment, chilling was omitted. To enable the more precise assessment of the effect of different concentrations of the pulp extracts on germination, the test was prolonged to 14 days, and the results are presented for the 7^{th} and 14^{th} day.

The result was presented by means of nine indicators of germination, some of which reflect only the quantitative value of germination, germinative capacity (GC), real germination (RG), and germinative energy (GE), others only the dynamics, mean germination period (MGP), germination intensity (GL), coefficient of the rate of germination (CRG), and coefficient of uniformity of germination (CUG), and still others refer to both groups (germination value by Chabator (Gv(Chab)) and germination value by Djavachir (GV(Djav)). The first five indicators are widely used and common, and their formulas are available in references. The coefficient of the rate of germination, coefficient uniformity of germination and have been calculated according to the formulas from the papers by Bewley, Black (1985), Chabator (1962), Djavashir, Pourbeik (1976).

RESULTS AND DISCUSSION

The technical germinative energy of the control group was the same both after seven days and after 14 days, and it amounts to 86%. Because of the presence of a low percentage of empty grains (2.5%), the absolute germinative energy is somewhat higher and amounts to 88.21. Both indicators after both test terms show the statistically justified difference compared to other treatments.

The treatments with the solution of holly and juni per pulps resulted in not one germinated grain in any of the repetitions. In the treatment with the solution of rose hypanthium in two stronger solutions (*Rosa 1:2* and *Rosa 1:3*) there were no germinated grains. In the mildest treatment *Rosa 1:4* after 7 days of the total 4 repetitions of 100 grains each, 5 grains germinated in the first and 1 grain in the second repetition (1.5%) which is a significant difference compared to the control and compared to all other treatments. Another 4 grains germinated during the following 7 days, accounting for altogether 2.5 % grains. The solutions of cotoneaster pulp block the germination of lettuce seed completely only in the strongest concentration (*Cotoneaster 1:2*). The germination of seeds treated by the two weaker concentrations was induced in 0.5-4%. The results after 7 days differ from the control significantly, but not from the treatment *Rosa 1:4* and those where the germination was absent. Till the 14th day, in the treatment *Cotoneaster 1:4,* the number of germinated grains increased and it was significantly higher than that in all other treatments, which indicates the weakest form of inhibitory dormancy in the cotoneaster. Germination energy was high only in the control. In all treatments it was 0%, i.e. germination did not occur in the period based on which the parameter was calculated, which indicates that, if germination did occur, the process was slowed down (Tables 2 and 3).

The parameters which indicate the germination dynamics (MGP GI and CRG) show similar tendencies - the treatments *Rosa 1:4, Cotoneaster 1:3, Cotoneaster 1:4* differ both from the control and from other treatments. The coefficient of coefficient of uniformity of germination (CUG), deviates from the above, because the tests in which a small number of grains germinated in one day have much higher values than the tests with a great number of germinated grains, which germinated during several days. Therefore, this parameter is not considered suitable for the assessment of dormancy.

The parameters of germinative energy which simultaneously indicate the quantity and quality of germination (assessment of germination after Czabator (OK(Czab)) and the assessment of germination after Djavanshir (OK(Djav))) point out that all cases of germination are below the level of significant differences, compared to the tests in which germination is absent, which confirms the effect of the inhibitors present in the pulp of the analysed species.

The morphology of germinated embryos - seedlings is also interesting. In the treatment *Rosa 1:4* all germinated embryos had a heavy deformation of root, the rootlet top had a distinctive darkening which prevented the elongation of the primary root at the darkened point. For this reason, the appearance of the root system was specific - compressed and with a greater number of lateral roots in individual seedlings. As the evaluation of the effects of toxic substances, e.g., on seedlings, is also carried out based on the development of vital structures, especially of roots, the

Table 2

Germination treatments with the different concentrations of the pulp extracts solution rose hypanthium, cotoneaster, holly and juniper pulps after 7 days

treatments	GC	RG	GE	MGP	GL	CRG	CUG	Gv (Czab)	Gv (Djav)
control	86.00^{a}	88.21^{a}	83.00^{a}	4.674^{a}	402.25^{a}	43.28^{a}	0.72^{b}	339.21^{a}	171.70^{a}
Cotoneaster 1:2	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Cotoneaster 1:3	0.50^{bc}	0.52^{bc}	0.00^{b}	0.125^{b}	0.25^{b}	3.85^{bc}	1.00^{b}	0.020^{b}	0.011^{b}
Cotoneaster 1:4	0.75^{bc}	0.77^{bc}	0.00^{b}	0.00^{c}	0.00^{b}	7.14^{b}	7.2E11^{ab}	0.025^{b}	0.018^{b}
Ilex 1:2	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Ilex 1:3	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Ilex 1:4	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Juniperus 1:2	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Juniperus1:3	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Juniperus1:4	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Rosa 1:2	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Rosa 1:3	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
Rosa 1:4	1.50^{b}	1.54^{b}	0.00^{b}	0.00^{c}	0.00^{b}	7.14^{b}	1.5E12^{a}	0.132^{b}	0.093^{b}

* Mean values denoted by the same letter are not significantly different at the level of 1% (by Duncan test of multiple scope)

Table 3

Germination treatments with the different concentrations of the pulp extracts solution rose hypanthium, cotoneaster, holly and juniper pulps after 7 days

treatments	GC	RG	GE	MGP	GL	CRG	CUG	Gv (Czab)	Gv (Djav)
control	86.00^{a}	88.21^{a}	83.50^{a}	11.674^{a}	1004.25^{a}	43.28^{a}	0.72^{b}	169.66^{a}	120.66^{a}
Cotoneaster 1:2	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Cotoneaster 1:3	0.75^{d}	0.77^{d}	0.00^{b}	2.625^{b}	4.50^{bc}	6.12^{c}	2.2E11^{b}	0.026^{b}	0.003^{b}
Cotoneaster 1:4	4.00^{b}	4.12^{b}	0.00^{b}	3.725^{b}	14.75^{b}	9.74^{b}	0.52^{b}	0.102^{b}	0.105^{b}
Ilex 1:2	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Ilex 1:3	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Ilex 1:4	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Juniperus 1:2	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Juniperus1:3	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Juniperus1:4	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Rosa 1:2	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Rosa 1:3	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}
Rosa 1:4	2.50^{c}	2.55^{c}	0.00^{b}	3.50^{b}	12.00^{b}	10.08^{b}	1.9E12^{a}	0.077^{b}	0.080^{b}

* Mean values denoted by the same letter are not significantly different at the level of 1% (by Duncan test of multiple scope)

observed phenomenon is another proof of the chemical effect of pulp on embryo development.

After the test of germinative energy, the remaining seeds treated with rose pulp, declared as fresh non-germinated seeds, were rinsed in distilled water and placed on the blotting paper soaked in distilled water, for germination. After 24 hours, 96% of the grains previously treated with rose pulp 1:3, germinated. In the variant Rosa 1:2, 7% of the grains germinated the first day, and 38% of the grains germinated in seven days, but there was no further germination till the 14th day. This unofficial continuation of the experiment indicates a relatively ready evacuation of inhibitors from the treated seed, which is proportional to the applied concentration.

CONCLUSION

The study confirms the presence of inhibitory substances in the pulp in all the analysed species. The pulp of holly and Chinese juni per completely blocked the germination,rose pulp to a lower extent, and cotoneaster pulp to the lowest extent. The proof of the effect of inhibitors on lettuce seed is also in the direct proportion of the germination results and the applied concentration.

On the other hand, lettuce seed, which is widely used as an indicator (standard plant) in different biological experiments, confirmed its usability for the assessment of inhibitory dormancy of ornamental tree and shrub seeds.

The study experiments also show the potential more precise evaluation of the effect of inhibitors by the analysis of vital structures, especially of root, which in some cases has an atypical growth and development. Further investigations should, consequently, include the evaluation based on the seedling morphology.

As the development of inhibitory dormancy depends on the stage of maturity of the fruit at the moment of collection and seed extraction, all the experiences of the study experiments indicate that this method can also be used in the determination of these important terms. This is going to be checked by the continued research of the study species, but also of the new species of fruits characterised by the indications of inhibitory substances present in the fleshy coat.

REFERENCES

Anonymus.1948. Woody Plant Seed Manual. Forest service of U.S. Department of Agriculture. Miscellaneous publication no. 654.

Bewley, J. D., Black, M. 1985. Seed, Physiology of Development and Germination. Plenum Pres. New York and London.

Bowers, N., Pratt, J.R., Beeson, D., Lewis, M. 1997. Comparative evaluation of soil toxicity using lettuce seeds and soil ciliates. Environmental Toxicology and Chemistry, 16, 207-13.

Czabator, F. J. 1962. Germination value: An index combining speed and completeness of pine seed germination. For. Sci. 8(4), 386-96.

Djavanshir, K., Pourbeik, H. 1976. Germination value - a new formula. Silvae Genetica 25(2), 79-83.

Grbix, M., Mijanovix, O., Jeftix, S. 1996. Different presowing treatments of *Rosa canina SCHMIDTS IDEAL* seeds and their impact on production cycle shortening. 2nd International Rose Symposium. Antibes, France. Acta Horticulturae 424, 99-101.

Hartmann,H. T.,Kester,D. E.,Davies,F. D. 1990. Plant Propagation Princi ples and Practices. Prentice-Hall International,Inc.

Nikolaeva, M. G. 1977. Factors affecting the seed dormancy pattern. In The physiology and biochemistry of seed dormancy and germination, A. A. Khan, ed. Amsterdam: North-Holland Publishing Co., 51-76.

Rathbun, J. 1996. A Simple Bioassay Using Lettuce Seeds. The Volunteer Monitor, The National Newsletter of Volunteer Water Quality Monitoring, 8, 1, Spring 1996.

Schopmeyer, C. S. (Techn.Coord.). 1974. Seeds of Woody Plants in the US. For.Serv. USDA. Washington, DC.

Wang, W. 1986. Comparative toxicology of phenolic compounds using root elongation method. Environmental Toxicology and Chemistry, 5, 891-6.

Wang, W. 1987. Root elongation method for toxicity testing of organic and inorganic pollutants. Environmental Toxicology and Chemistry, 6, 409-14.

Wang, W., & Williams, J.M. 1988. Screening and biomonitoring of industrial effluents using phytotoxicity tests. Environmental Toxicology and Chemistry, 7, 645-52.

E-mail: sf.bg@sezampro.yu