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Preface

The third ever ISTA, Forest Tree Seed Testing Workshop‘ was held in Prague, Czech Republic, from October 20 – 22, 2003. Previous meetings were held at Guildford, England, 1973 and Macon, Georgia, U.S.A, 1989. The Prague meeting was attended by 42 participants from 20 countries. The meeting agenda was an ideal mix of practical exercises, demonstrations, presentations and discussion sessions.

After the meeting there was an opportunity to choose between a 1-day trip to the State Tree Seed Centre in Týniště nad Orlicí or a 3-day tour dealing with seed testing, processing and storage facilities in both the Czech Republic and The Republic of Slovakia.

This publication contains most of the papers and one of the posters presented at the meeting. A more complete account of the practical exercises, formal discussions and lively debates has been presented elsewhere (Gosling, 2004 – Seed Testing International volume 127, pages 34 – 36). Some pictures taken during the meeting and the 3day tour are presented on inside pages on the cover.

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Further thanks go to the chairpersons of the various sessions, practical session leaders, speakers and demonstrators, plus all the attendees for their active participation and involvement throughout the meeting. The high quality of the meeting is a clear reflection of everyone's enthusiasm, expertise, and conscientious preparation and well developed communication skills.

Special thanks go to the authors of the articles included in this volume, i.e. for their willingness to prepare their papers and posters for publication.

Finally, thanks also to Ing. Yveta Sefrnova, Ing. Miroslav Houba and Dr. Zdenka Polanecka at the Agricultural Seed Testing Laboratory (CZDL03) where we visited; to Ing. Zdenek Kiesenbauer of the Dendrological Gardens of the Research Institute of Ornamental Gardening; Ing. Zdenka Hlavova and Ing. Martin Plasil from the Tree Seed Centre in Tyniste nad Orlici; Ing. Katarina Chvalova and her colleagues from Semenoles (Slovakian Tree Seed Centre); and Dr. Elena Foffova and her colleagues from the Slovakian Tree Seed Testing Station (SKDL02).

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Survey of seed-borne diseases of woody trees in Egypt

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Abstract

Routine and specific techniques were used to detect seed-borne microflora of five tree species in Egypt, i.e. *Acacia mangium, Araucaria angustifolia, Casuarina equisetifolia, Eucalyptus camaldulensis* and *Ficus bengalensis.* Seeds were collected from several commercial tree nurseries. The blotter test was the most acceptable method for detecting seed-borne fungi. Assays detected eight genera of fungi, i.e. *Botryodiplodia, Botrytis, Cephalosporium, Colletotrichum, Curvularia, Fusarium, Macrophomina,* and *Phoma* and two genera of bacteria, viz. *Pseudomonas* and *Xanthomonas. Botryodiplodia theobromae, Fusarium moniliforme* and *F. oxysporum* were the most prevalent fungi isolated. They caused seed rot, seedling diseases and mortality, wilt, growth suppression, and stem deformation. *Pseudomonas* spp. and *Xanthomonas* spp. also commonly caused various seedling diseases, including dieback. *Colletotrichum gloeosporioides, Fusarium moniliforme, F. oxysporum* and *Phoma* spp. might be seed transmitted as they were isolated from embryos, however, *Botrytis* spp., *Cephalosporium* spp., *Curvularia tuberculata, Macrophomina phaseolina* were found only on the seed coat. *Trichoderma* spp., when mixed with fertilizers, improved seedling growth and minimized seed-borne diseases.

Introduction

Although natural forests are uncommon in Egypt, woody trees and shrubs are often grown in orchards and gardens and along streets and agricultural roads. In nurseries, seedlings of such trees suffer from many damaging diseases. Because orchards contain many trees of the same species, they are more prone to disease problems than individual trees scattered across a farm or community. Seeds of softwood and hardwood trees are attacked by fungi and other microbes (Ivory, 1987). Seed-borne pathogens of wood trees affect nursery seedlings and reduce seed germination and seedling vigor. They also decrease the longevity of stored seeds. Several species of fungi which are generally considered as saprophytes behave as pathogens under certain circumstances including injury to the seed or seed coat, and conducive moisture and temperatures which favor fungus growth and increase the physiological and physical vulnerability of tree cones and fruits, seeds, and seedlings to infection (Mittal et al., 1990). Studies of the mycoflora of *Eucalyptus* spp. seeds showed that many fungi were associated with them. Although most were saprophytes, several of these fungi are pathogens or potential pathogens (Mittal et al., 1990; Mohanan and Sharma, 1991; Pongpanich, 1990; Sharma and Mohanan, 1991; Yuan et al., 1990). *Colletotrichum* and *Curvularia* are associated with damping-off of *Eucalyptus camaldulensis* in Malaysia (Ahmad, 1987). Gibson (1978) described *Botryodiplodia theobromae* as important in causing stem canker and die-back which killed young *Pinus* seedlings. This fungus also causes severe die-back of *Acacia mangium* (Wiley, 1993). Several fungi cause deformation, decay, and reduce germination of seeds or destroy seeds (Gravatt, 1931). Many tree seeds, like the seeds of agricultural crops carry fungus spores both internally and externally. Fungi that are inside the seed most often occur as mycelium. Seed-borne fungi, in general, may be classed as externally associated or internally seed-borne. Numerous methods are available by which fungi can be detected in seeds (ISTA 1981; Singh and Mathur, 1993). In some methods, the

actual presence of the fungus is detected. In others, the presence is detected as a result of the effects they cause on seeds, and yet in others, by the symptoms they produce on seedlings and young plants. Therefore, the present investigation was made to survey for seed borne diseases of woody trees in Egypt and to find possibilities to minimize their injurious effect.

Materials and methods

Seed samples of five major tree species i.e., *Araucaria angustifolia* (Bertol) Kuntze, *Acacia mangium* R. Br., *Eucalyptus camaldulensis* Dehnh, *Casuarina equisetifolia* Forster et G. Forester and *Ficus bengalensis*. L. were obtained from 20 commercial nurseries where seedlings are often damaged. Seeds of each species were assayed for health by different standard methods of seed health testing (ISTA, 1993). The presence and type of fungi were determined according to their development on seeds, which had been incubated on a substrate such as a water-soaked, standard blotter, deep freezing blotter or potato dextrose agar (PDA) medium.

Standard blotter method

Two hundred seeds of each tree species were plated on water-soaked filter papers, five seeds per 15 cm diameter Petri dish. The seeds were incubated at 22 ± 2 °C under 12 hours of alternating darkness and fluorescent light.

Deep-freezing blotter method

The preparation and procedures were the same as for standard blotter, except that after incubating at 20 °C for a day the dishes were transferred to -20 °C in darkness for a day and then incubated for 5 days incubation at 20 °C.

Agar-plate method

Two hundred seeds of each tree species were surface sterilized in 1 % sodium hypochlorite for 1 minute, then plated five seeds per 15 cm diameter Petri dish. Incubation conditions were the same as for the blotter test.

Isolation and identification of fungi

Fungi which grew from the seeds during the three assays were transferred to agar media. They were then identified based on colonies produced on the culture media after 7 days incubation at 25 °C in darkness. When identifying the fungi to species, the colonies were observed from the obverse and reverse sides. Based on spore and other characteristics, the fungi were then identified using stereo-and compound microscopes.

Isolation and identification of bacteria

Extraction of the bacteria was done using the liquid plating assay (Bolkan et al., 1997) by placing 400 seeds of each tree species in a doubled plastic bag (20 cm x 25 cm and 0.15 mm thick) containing 150 ml sterile phosphate-Tween buffer (7.75 g/l of Na_2HPO_4 + 1.65 g/l KH_2PO_4 + 0.2 ml/l Tween 20), pH 7.4. The plastic bags with their contents were then incubated in a refrigerator at 4 °C for 15 min. After refrigeration, the plastic bags with their contents were put onto a shaker for 15 min. Then 0.1 ml of 0, 1 : 10, 1 : 100 dilutions (prepared using phosphate buffer without Tween) of each sample were pipetted onto each of the three plates of a semi-selective medium (2 g K_2HPO_4; 0.5 g KH_2PO_4; 0.25 g $MgSO_4.7H_2O$; 1.5 g boric acid; 10 g sucrose, 0.1 g yeast extract and 15 g of agar) in 980 ml distilled water. After autoclaving the medium was allowed to cool to 45 – 50 °C in a water bath and then 100 mg nicotinic acid (dissolved in 20 ml

sterile distilled water) was added; 30 mg nalidixic acid (sodium salt, dissolved in 1 ml of 0.1 M NaOH); 10 mg potassium tellurite (1 ml of 1 % Chapman tellurite solution from Difco); and 200 mg cycloheximide (dissolved in 1 ml absolute methanol). Next, using an L-shaped glass rod, the material was spread onto the medium and incubated at 26 °C (Fatmi and Schaad, 1988). The bacteria were identified based on the Gram reaction (Suslow et al., 1982), tobacco hypersensitivity (Klement et al., 1964), spore formation (Bradbury, 1986), their ability to cause soft rot of potato, onion and cucumber slices (Friedman 1951) and levan production (Lelliot and Stead, 1987).

Pathogenicity tests

Pathogenicity tests were made to ascertain the disease causal pathogens. Four pots (20 cm internal diameter) for each treatment were filled with autoclaved, aerated sandy-clay soil. The pots were inoculated with each isolated fungus and bacterium, and then sown with sodium hypochlorite surface sterilized seeds of each tree species (five seeds/pot). The check treatment consisted also of four pots for each tree species, but without inoculation with any pathogen. The experiment lasted for 60 days.

Location of seed-borne fungal infection in seeds of *Araucaria angustifolia* and *Eucalyptus camaldulensis*

To determine the location of fungi in seed tissues of *E. camaldulensis* and *A. angustifolia*, 100 seeds of each tree species were soaked in tap water for 12 hours to facilitate the separation of seed parts viz. seed coat, and embryo. Then the tissues were surface sterilized and plated onto PDA. The Petri dishes were incubated under the conditions described earlier. The developing fungal colonies were examined and the percentages of infections were recorded.

Control of seed-borne fungi and bacteria

A commercial biopreparation of Promote based on the spores of *Trichoderma harzianum* 2×10^7/g and *Trichoderma koningii* 3×10^7/g was mixed with mineral fertilizers.

The fertilizers were new plant care (20, 20, 20) NPK (30 kg) + LAV (ammonium nitrate with limestone, 30 kg) + DASA (ammonium nitrate, ammonium sulphate, 30 kg). The biopreparation Promote was mixed together with the fertilizers at two different rates (0.5 g and 1 g per 1 kg of fertilizers). The health status of the seedlings was then determined.

Results and discussion

Detection and isolation of fungi and bacteria associated with tree seeds

Fungi detected from seeds of *Araucaria angustifolia, Acacia mangium, Eucalyptus camaldulensis, Casuarina equisetifolia* and *Ficus bengalensis* in the standard blotter, deep freezing blotter, agar plate media and liquid assay techniques are shown in Tables 1 and 2. Representatives of nine species of eight genera of fungi were detected in seeds of the five tree species (Table 1).

Based on the number of isolation attempts *Botryodiplodia theobromae* developed on blotter, deep freezing blotter and PDA media on average for 11.3, 3.5 and 5.9 % of the isolation attempts for each technique, respectively. The corresponding data were for (i) *Botrytis cinerea* for 5.6, 2.0 and 1.0 %, (ii) *Curvularia tuberculata* for 18.7, 2.8 and 9.4 %, (iii) *Cephalosporium* spp. for 6.7, 5.0 and 2.3 %, (iv) *Colletotrichum gloeosporioides* for 6.8, 1.7 and 2.0 %, (v) *Fusarium moniliforme* for14.6, 2.3 and 5.2 %, (vi) *F. oxysporum* for 6.3, 1.4 and 1.5 %, (vi) *Macrophomina phaseolina* for 5.5, 1.4 and 2.0 % and (vii) *Phoma* spp. for 5.1, 2.2 and 0.4 %.

The liquid assays resulted in the isolation of two bacteria i.e. *Pseudomonas syringae* pv. *syringae* and *Xanthomonas campestris* pv. *campestris* from seeds of the five trees with mean averages of 15.9 and 9.4 %

Table 1. Seed borne fungi detected in seeds of five tree species

Fungi	Percent seed-borne fungi																	
	Araucaria angustifolia			*Acacia mangium*			*Eucalyptus camaldulensis*			*Casuarina equisetifolia*			*Ficus bengalensis*			Mean		
	S. blotter	Deep freez	PDA	S. blotter	Deep freez	PDA	S. blotter	Deep freez	PDA	S. blotter	Deep freez	PDA	S. blotter	Deep freez	PDA	S. blotter	Deep freez	PDA
Botryodiplodia theobromae	11.5	5.0	2.0	3.3	1.5	0.0	14	0.0	10.0	5.8	2.8	1.3	22	8.0	16	11.3	3.5	5.9
Botrytis cinerea	3.5	3.0	1.8	1.8	1.0a	0.5	10	0.0	0.0	3.5	3.0	2.5	12	3.0	0.0	5.6	2.0	1.0
Curvularia tuberculata	4.8	1.5	1.0	5.3	1.5	0.0	48	0.0	32.0	11.5	4.8	2.0	24	6.0	12	18.7	2.8	9.4
Cephalosporium spp.	8.8	5.0	1.0	1.5	0.8	0.5	7.0	2.0	3.5	6.0	3.5	1.5	10	0.0	3.0	6.7	5.0	2.3
Colletotrichum gloeosporioides	1.0	0.8	2.8	5.3	1.5	0.0	10	0.0	0.0	3.5	3.0	1.8	14	3.0	5.0	6.8	1.7	2.0
Fusarium moniliforme	5.5	5.5	3.0	8.0	2.0	5.8	38	0.0	8.0	11.5	4.0	3.0	10	0.0	6.0	14.6	2.3	5.2
F. oxysporum	5.8	2.8	1.3	5.0	0.0	3.8	10	0.0	0.0	4.8	1.5	1.0	5.8	2.8	1.3	6.3	1.4	1.5
Macrophomina phaseolina	5.0	1.3	3.5	2.8	2.0	0.8	8	0.0	0.0	5.5	3.0	2.0	6.0	0.8	4.8	5.5	1.4	2.0
Phoma spp.	6.5	3.5	0.0	2.0	0.0	0.0	2	0.0	0.0	10	5.5	2.0	7.0	2.0	0.0	5.1	2.2	0.4
Total frequency	52.4	28.4	16.4	35	10.3	11.4	147	2.0	53.5	62.1	31.1	17.1	110.8	25.6	48.1	80.6	22.3	29.7
Mean	5.8	3.2	1.8	3.9	1.1	1.3	16.3	0.2	5.9	6.9	3.5	1.9	12.3	2.8	5.3	9.0	2.5	3.3

S. blotter = Standard blotter test
Deep freez = Deep freezing blotter test
PDA = PD agar plating test

respectively. These results agree with those of Mittal et al. (1990), which showed numerous fungal species associated with *Eucalyptus*. Some of them infected seedlings after germination, for example, *Macrophomina phaseolina* and *Verticillium albo-atrum*. Fungi are considered to be the most important group of microorganisms causing loss of seed viability. Some soil borne pathogens have been found associated with seeds of *E. grandis* in India (Mohanan and Sharma, 1991).

In the present investigation, the standard blotter, deep freezing blotter and PD agar assays detected most of the fungi associated with the seeds of the five tree species. Among the different methods, the standard blotter method resulted in the highest percentages of fungi that were associated with the five tree species seeds with total frequencies of 80.6 % as compared to 22.3 and 29.7 % in the deep freezing blotter and agar plate tests. The standard blotter technique resulted in an isolation frequency of 52.4 % of the nine species of fungi for *Araucaria angustifolia* as against 28.4 and 16.4 % using the deep freezing blotter and agar plate methods, respectively. This technique also resulted in an isolation frequency of 35 % for *Acacia mangium* compared to frequencies of 10.3 % and 11.4 % for the other techniques. For *Eucalyptus camaldulensis* this was 147 % as compared with 2 and 53.5 %. For *Casuarina equisetifolia*, the standard method resulted in an isolation frequency of 62.1 % as compared to 31.1 and 17.1 %, for the other two techniques, respectively. It resulted in an isolation frequency of 110.8 % for *Ficus bengalensis* compared to 25.6 and 48.1 % for the other two techniques.

These results are in agreement with those reported by Nath et al. (1970), Neergaard (1973), Bilgrami et al. (1979) and Abdelmonem and Rasmy (1996), who stated that the blotter test usually results in the detection of the most microbes associated with certain shrub seeds. They mentioned that the standard blotter, deep freezing blotter and PDA plate methods were favorable for the growth of various species of fungi when seeds were sized for health. They also recorded that the standard blotter and agar methods were the best for detecting *F. moniliforme* and *F. solani* from seeds of some crops.

The data in Table 2 show the significance of the liquid plating assay in detecting the two bacteria with total frequency of 25.3 %. *Pseudomonas syringae* pv. *syringae* and *Xanthomonas campestris* pv. *campestris* were found associated with the seeds of the five tree species with mean isolation frequencies of 15.9 and 9.4 %, respectively.

Table 2. Seed borne bacteria detected on seeds of five tree species

Bacteria	Percent seed-borne bacteria					
	Araucaria angustifolia	***Acacia mangium***	***Eucalyptus camaldulensis***	***Casuarina equisetifolia***	***Ficus bengalensis***	**Mean**
Pseudomonas syringae pv. *syringae*	16	9.5	14	20	20	15.9
Xanthomonas campestris pv. *campestris*	10.5	6.5	10	10	10	9.4
Total frequency	26.5	16	24	30	30	25.3
Mean	13.3	8.0	12	15	15	12.7

Pathogenicity of seed-borne fungi and bacteria

Pathogenicity tests were carried out to determine the main causal pathogens. These tests ascertained the seed borne infection of all isolated species causing typical seed and seedling disease symptoms.

Table 3 shows the different symptoms caused by the different test fungi and bacteria. *Botryodiplodia theobromae* caused root rot, stem canker, die-back and seed damage. *Botrytis cinerea* caused only seed decay. *Curvularia tuberculata* produced damping-off and seedling wilt. *Cephalosporium* spp. caused growth suppression and stem deformation. *Colletotrichum gloeosporioides* caused damping-

off, leaf spots, anthracnose and blight. *Fusarium moniliforme* and *F. oxysporum* produced seed rot, root rot, growth suppression, stem deformation, seedling mortality and wilt. Inoculation with *Macrophomina phaseolina* resulted in seed rot, seedling mortality and foliage yellowing.

Table 3. Seed borne organisms isolated and the diseases they caused in pathogenicity tests with seeds and seedlings

Seed-borne fungi and bacteria	**Diseases caused**
Botryodiplodia theobromae	root rot, stem canker, die-back and seed damage
Botrytis cinerea	seed decay
Curvularia tuberculata	damping-off and seedling wilt
Cephalosporium spp.	growth suppression and stem deformation
Colletotrichum gloeosporioides	damping-off, leaf spots, anthracnose and blight
Fusarium moniliforme	seed rot, root rot, growth suppression, stem deformation, seedling mortality, and wilt
F. oxysporum	seed rot, root rot, growth suppression, stem deformation, seedling mortality, and wilt
Macrophomina phaseolina	seed rot, seedling mortality and yellowing of foliage
Phoma spp.	seed rot, and growth suppression
Pseudomonas syringae pv. *syringae*	seed decay, watery root, blight and dieback
Xanthomonas campestris pv. *campestris*	root rot, water soaked and dieback

Phoma spp. caused seed rot, and growth suppression. Pathogenicity tests with the bacteria produced irregular-shaped (1 to 5 mm diameter), black or gray, and slightly sunken lesions developed on cotyledons and other leaves and to a lesser extent on the growing apical tip of shoots. Inoculation with *Pseudomonas syringae* pv. *syringae*, produced seed decay, watery root, blight and dieback. *Xanthomonas campestris* pv. *campestris* induced root rot, and dieback.

Location of fungi and bacteria in *Eucalyptus camaldulensis* and *Araucaria angustifolia* seeds

Table 4 gives the results of the location of fungi in the seed coats and embryos of *Eucalyptus camaldulensis* and *Araucaria angustifolia* seeds. The majority of fungi were found in the seed coats. *Colletotrichum gloeosporioides, Fusarium moniliforme, F. oxysporum* and *Phoma* spp. were also detected in embryos of seeds of both trees.

Table 4. Location of seed-borne fungal infection in seed tissues of two tree species

Fungal infection	***Eucalyptus camaldulensis***		***Araucaria angustifolia***	
	Seed coat	**Embryo**	**Seed coat**	**Embryo**
Botryodiplodia theobromae	++	-	+	-
Botrytis cinerea	+	-	++	-
Curvularia tuberculata	++	-	++	-
Cephalosporium spp.	+	-	+	-
C. gloeosporioides	+	+	+	+
Fusarium moniliforme	+	++	++	+
F. oxysporum	-	+	-	+
Macrophomina phaseolina	++	-	+	-
Phoma spp.	+	+	+	+

Table 5. Effect of soil treatments on seedling development and health status of five tree species

	Ficus bengalensis			***Casuarina equisetifolia***			***Eucalyptus camaldulensis***			***Acacia mangium***			***Araucaria angustifolia***		
	Untr. soil	**Trich. 1g/Kg F**	**Trich. 5g/Kg F**	**Untr. soil**	**Trich. 1g/Kg F**	**Trich. 5g/Kg F**	**Untr. soil**	**Trich. 1g/1Kg F**	**Trich. 5g/Kg F**	**Untr. soil**	**Trich. 1g/Kg F**	**Trich. 5g/Kg F**	**Untr soil**	**Trich. 1g/Kg F**	**Trich. 5g/Kg F**
Seedling development (%)	50	81	78	51	78	70	55	80	71	52	79	74	54	77	72
Healthy seedlings (%)	20	72	68	22	72	66	30	72	65	22	68	63	25	70	66

All data based on 100 seeds per treatment
Untr. Soil = Untreated soil
Trich. = *T. harzianum* + *T. koningii* (Promote)
F = Fertilizers

Improvement of seedling growth

The effect of the inoculation of *Acacia mangium, Araucaria angustifolia, Casuarina equisetifolia, Eucalyptus camaldulensis* and *Ficus bengalensis* seeds with certain fungi and bacteria and the influence of *Trichoderma harzianum* and *Trichoderma koningii* in reducing disease severity is given in Table 5. Both developed and healthy seedlings were recorded. Seedling development was calculated on the basis of 100 seeds.

The mixture of fertilizers and the antagonistic fungi i.e. Promote in both cases when applied as 1 kg fertilizer to 1g Promote (*Trichoderma harzianum* and *T. koningii*) or 1 kg fertilizer to 0.5g of Promote significantly increased the number of developed seedlings and disease free seedlings in all five tree species. Soil treated with 1.0 g Promote per 1 kg of the fertilizer seemed to be optimal for the most beneficial effect of the antagonistic fungi *Trichoderma harzianum* and *Trichoderma koningii* against pathogenic as they resulted in an increase in the mean number of developed seedlings in the five tree species by 77, 79, 80, 78 and 81 % for *A. angustifolia, A. mangium, E. camaldulensis, C. equisetifolias* and *F. bengalensis*, respectively. These results agree with those of Chet and Baker (1981), Duskova (1995), Lo et al. (1996) and De Meyer et al. (1998) who reported that *Trichoderma harzianum* suppressed many pathogenic fungi, including *Botrytis cinerea* and *Colletotrichum lindemuthianum*. In the present investigation, other fungi such as *Botryodiplodia theobromae, Curvularia tuberculata, Cephalosporium* spp., *Fusarium moniliforme, F. oxysporum, Macrophomina phaseolina* and *Phoma* spp. were suppressed by the biological control agents. The level of the suppression of individual fungi was very similar.

Conclusions

- The blotter test was the best method for detecting seed-borne fungi.
- The liquid plating assay detected the most bacteria.
- *Trichoderma harzianum* and *T. koningii*, when mixed with fertilizers, improved seedling growth and health and dramatically reduced seed-borne diseases.

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Breaking dormancy in tree seeds with special reference to firs (*Abies* species) - the 1.4x solution

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Abstract

As with most gymnospermous trees, seeds of fir *(Abies)* species generally display a wide range of dormancy. This dormancy level, or degree, may vary from one crop year to the next even for seeds from the same parent trees; among parent trees in the same stand in any one crop year; among cones on the same parent tree, and from seed to seed in the same cone. Thus, in any one seedlot, some seeds may be non-dormant, slightly dormant, somewhat dormant, while others are very dormant. Standard stratification/prechilling treatments to promote germination usually fail to accommodate such wide variations. A 3- or 4-week stratification treatment often fails to satisfy the requirements for breaking deep dormancy, so some seeds are lost for seedling production. If the stratification duration is doubled, or tripled, non-dormant and less-dormant fir seeds tend to chit in the refrigerator, again usually being lost for seedling production. The stratification-redry (strat-redry) procedure, first described in the 1980s, is reviewed. This method overcomes a wide range of dormancy levels by controlling seed moisture content during an extended chilling period. Subsequently, most, if not all, of the viable seeds germinate synchronously. A previously-unpublished, new, simplified method for applying the strat-redry procedure that relies only on changes in seedlot weight is described. Some nursery data are presented.

Experimental and discussion

Tree seed dormancy is an evolved trait that inhibits/prevents germination in most temperate forest coniferous species when seeds are first shed in the autumn with winter approaching. In any given year, or location, some seeds may lack a sufficient depth of dormancy to arrest their germination, and the germinants are unlikely to survive until the following spring. Seeds of several fir species have been observed to germinate in snow banks and studies have shown that such seeds will germinate at controlled, low temperatures in the laboratory (Edwards, 1969; 2004a). In nature, dormant seeds that are exposed to cold wet conditions over winter, germinate when temperatures rise in early spring. In the laboratory, seed analysts emulate this natural dormancy-breaking treatment by exposing moistened seeds to low temperature in a refrigerator, a process that has become known as stratification or prechilling; a more general term is pretreatment.

Most, if not all, fir species exhibit some degree of dormancy. Judging from international prescriptions for breaking dormancy in the laboratory, seeds of balsam *(Abies balsamea)*, Fraser *(A. fraseri)*, subalpine (*A. lasiocarpa),* California red *(A. magnifica)*, European white *(A. alba)*, Cilician *(A. cilicica)*, Japanese *(A. firma)*, Nikko *(A. homolepis)*, and Sakhalin *(A. sachalinensis)* firs are consistently dormant and always require a pretreatment to obtain good germination. Others, including Pacific silver *(A. amabilis)*, white *(A. concolor)*, grand *(A. grandis)*, noble *(A. procera)*, Grecian *(A. cephalonica)*, Caucasian *(A. nordmanniana)*, Spanish *(A. pinsapo)*, and Veitch *(A. veitchii)* firs exhibit varying degrees of seed dormancy and require a second test without pretreatment to determine how dormant they may be. However, even for those species showing persistent dormancy, in any given seed crop year the degree of dormancy may vary from one seed source to another, may vary among parent trees in any single seed source, may vary within the crown of any given parent tree, and may vary within a single cone.

The degree of dormancy, therefore, is a population phenomenon, and for any single seed collection it is not surprising to find a wide variation in this condition from one seed to the next in the same container. Thus, some seeds in the container may be relatively non-dormant and germinate with little or no pretreatment, while others may be very dormant and germinate only after a prolonged pretreatment. In between are seeds, some of which require less (or more) pretreatment than others. For these reasons, if a single pretreatment is applied to the seed lot, the dormancy breaking requirements may be satisfied for some seeds, but not all viable seeds will germinate because their dormancy-breaking requirements have not been satisfied. Judging the length of pretreatment, especially for nursery sowing, has been mostly an imprecise science. If the seedlot is very dormant, a longer pretreatment might be applied, during which chitting might begin while the seeds were still in the refrigerator (see above). This usually renders these germinants difficult to sow without damage to the emerging radicles, thereby making this proportion of the seedlot non-useable for seedling production. As already mentioned, if the pretreatment is not long enough, very dormant seeds will not germinate, again making this proportion of the seedlot non-useable for seedling production purposes.

In the 1980s, a procedure now known as the stratificiation-redry method was described that went a long way to solving some of these difficulties (Edwards, 1981; 1982a, b; 1986a, b; 1997). In this method, seeds were hydrated and chilled as for the standard stratification treatment. That is, seeds were soaked in water for 48 hours, drained, then placed in a closed container in a refrigerator set for 1-5 °C for 4 weeks. In practice, the duration of chilling might be extended to 8, or even 12 weeks, to promote higher germination in deeply dormant seeds.

Following the standard stratification treatment, when seed moisture content (mc) was between 45 % and 55 % depending on species and seed lot, the seeds were then redried to a mc of 35 %, placed in a closed container and returned to the same refrigerator for up to an additional 12 weeks. During this second period of chilling, this reduced mc of the seeds prevented non-dormant, and not-so-dormant seeds from germinating, while permitting the dormancy breaking requirement of deeply-dormant seeds to be satisfied more fully. At the termination of the treatment, dormancy had been broken in all viable seeds, but none could germinate because of the reduced mc. When placed in a germinator at favourable germination temperatures, the moisture constraint was removed allowing the seeds to germinate in a synchronized manner. The main effect was on germination rate. When grand fir *(Abies grandis)* seeds were stratified and redried in this manner, visible signs of chitting were observed a week earlier than in seeds that received stratification only; 80 % of the seeds germinated within the next 4 days (by day 9 of the test); over 86 % of the seeds germinated by the 14th day of the test. No more seeds germinated during the latter two weeks of the test. This compared with 60 – 70 % germination at the 28th day of the test for seeds that had received the standard 4-week pre-treatment. In other words, 8 out 10 seeds germinated within a period of four days, and a few more germinated during the following 5 days.

The stratification-redry method has been shown to improve germination in Pacific silver fir *(Abies amabilis)* (Edwards, 1981; 1982a, b; 1986; 1997; Leadem, 1986), subalpine fir *(A. lasiocarpa)* (Leadem, 1988; 1989), noble fir *(A. procera)* (Tanaka and Edwards 1986), and Nordmann fir *(A. nordmanniana)* (Jensen, 1997). However, controlling fir seed moisture content during stratification is not a new idea, having been recommended for prolonged pretreatment of seeds of hybrid firs (Wright, 1950) more than 30 years earlier. Additionally, reduced seed moisture content has been recommended for pretreatment of Guatemalan fir *(A. guatemalensis)* (Donahue et al., 1985). Recent anecdotal evidence from growers of ornamental fir trees have reported that seeds of Fraser, balsam, Canaan *(A. phanerolepis)*, California white, and Siberian *(A. sibirica)* firs have also responded well to the stratification-redry procedure.

To perform the redry portion of the method, stratified seeds must be spread in a one-seed thick layer to permit uniform drying; when first removed from the refrigerator the moist seeds are generally placed on an absorbent surface that may be changed during the drying period. Depending on ambient conditions (temperature and humidity), the drying step may take several hours until

the required 35 % moisture content has been achieved. Knowing when the correct moisture level had been achieved, that is, when to stop drying, was difficult to define at first. The problem was approached as follows.

Seed moisture content (mc %) is determined from the following formula:

$$\text{mc } \% = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100 \qquad \ldots.(1)$$

This may be transposed as follows:

$$\text{new fresh weight} = \frac{\text{dry weight x100}}{(100 - \text{specified mc } \%)} \qquad \ldots.(2)$$

From formula 2, if the average dry weight of several representative samples, say 50 seeds each, is determined, then the average new fresh weight for several other 50 seed samples could be calculated when they had reached the 35 % moisture content. When this calculated average new fresh weight had been achieved, drying would stop. Initially it was proposed that several samples (50 seeds each) of the drying seeds be identified at random positions among the spread-out drying mass and that their weight be monitored. The same 50-seed samples were to be used at each weight check point. Weight checks were to be made hourly for the first stages of drying, then more frequently as the new fresh weight was approached. This approach proved to be clumsy, especially with larger seedlots, especially because turning the seeds over, to permit all surfaces to dry, was fraught with difficulties in keeping the 50-seed samples used for monitoring the change in fresh weight correctly identified.

When describing this process to fir tree growers who had little if any laboratory experience, and had no access to equipment for determining the initial dry weights of seed samples, a new approach had to be sought. Recently it was realized that there is a simple and direct connection between the original fresh weight of the seeds when they are removed from storage and the fresh weight at which they have a mc of 35 %. An example will help to describe this relationship. Suppose 50 g of fresh seeds are taken from a container to determine dry weight. Following the correct drying procedure, suppose the weight is now 46 g. Thus, applying formula 1 (above), the mc would be 8 %. Using this dry weight in formula 2 (above) it can be calculated that to achieve a mc of 35 %, the fresh weight of those same seeds would have to be 70.8 g. That is, the initial fresh weight of the seeds will have to increase from 50 g to 70.8 g, or by 1.42 times the initial weight, to achieve a mc of 35 %. This does not require any knowledge of the actual seed mc %, Provided the starting fresh weight (50 g) of the seeds was known, following stratification they should be redried to 1.42 times this weight (70.8 g). This will be referred to below as the "multiplying factor".

For this sample, initial storage mc was 8 % (as calculated in the example). One immediate question was how would storage mc influence the calculation of the new fresh weight? Typically, fir seeds (and those of other conifers) are maintained between 6 % and 10 % mc to maximize their storage life. Calculations similar to those outlined above were undertaken using 6 % and 10 % as the initial, storage moisture content. The results are summarised in Table 1. Also, from operational experience with noble fir seeds (Tanaka and Edwards, 1986) it was found that it was expedient to redry large volumes seeds to an average mc below 35 %. This is because it is was impossible to uniformly dry large quantities of seeds (as used for nursery production of stock for reforestation purpose) to the very precise target of 35 % mc; some seeds in a large mass may not have dried to this point and would tend to chit during the second period of stratification. For this practical reason, it was recommended that the target new fresh weight should be 30 – 35 %. By drying to a wider target of 30 – 35 %, far fewer seeds in a large seed mass would remain above 35 % when the drying step was terminated. Additional calculations were made to determine the multiplying factors that would give the new fresh weight at 30 % mc (Table 1).

Table 1. The effect of initial storage mc on the multiplying factor to achieve 30 – 35 % mc

Initial seed mc (%)	Final seed mc (%)	Multiplying factor
6	35	1.45
6	30	1.34
8	35	1.42
8	30	1.31
10	35	1.38
10	30	1.29

It was found that the multiplying factors varied from 1.45 to 1.29, that is over a range of 0.16 (about 11 – 12 %). This was considered to be a small variation, and that the median value of 1.37 might be used throughout to simplify the calculations; and, further, that this value might be rounded to 1.4 for further simplification. To verify this, calculations were performed to determine if the new moisture contents fell into the required range of 30 – 35 % (Table 2).

Table 2. Final seed mc % using simplified multiplying factors of 1.37 and 1.4

Initial seed mc (%)	Final seed mc (%)	Multiplying factor
6	31.4	1.37
6	32.9	1.4
8	32.9	1.37
8	34.3	1.4
10	34.3	1.37
10	35.7	1.4

With the exception of using 1.4 as the multiplying factor when the initial mc is 10 %, which would provide a final mc nearer to 36 % than to 35 %, all other final moisture contents fell within the range of 30 – 35 % using either multiplier. Clearly, the use of 1.4 is justified. If seeds have been stored at 10 % mc, the simple solution is to dry them to a slightly lower new fresh weight to ensure they achieve a new mc no greater than 35 %. With this one exception, initial storage mc is of no consequence in achieving the 30 – 35 %, that is, the 1.4 multiplier will be appropriate at any storage mc between 6 – 10 %. This, then, greatly simplifies both large scale and small scale operational application of the stratification-redry method as summarised below.

A simplified strat-redry procedure

Seeds should be weighed at the outset, before hydration begins. After a 48-hour imbibition period, they are drained and placed in a closed container for chilling at 1 – 5 °C. After 4 weeks in the refrigerator, the seeds are removed, spread out on a level surface in a one-seed thick layer, and air-dried to 1.4 times their initial weight. As noted earlier, at the start of air drying, fir seeds typically have a mc of 45 % or higher, so redrying to 1.4 times their original weight (representing a mc between 30 – 35 %) usually takes several hours; longer when ambient conditions are cooler and wetter, less time when conditions are warmer and drier. A fan moving the air over the seeds aids the drying process. Seed weight should be checked at hourly intervals (or less) during the early stages

of the drying step, then more frequently as the new target fresh weight is approached. It is safer to err on the dry side, that is achieved a lower new fresh weight than that calculated, than to allow the seeds to be heavier/too moist.

When the new fresh weight has been reached, the seeds are replaced in a dry, closed container and returned to the refrigerator for an additional 12 weeks. During this second stratification period, they should be inspected weekly to see if any premature germination has begun; if this is the case the seeds should be removed, spread out and dried further. During these inspections it is advantageous to turn the container, or otherwise mix the seeds so that those nearer the centre of the bulk are brought nearer to the outer edges, and vice versa, to equalize moisture distribution. Also, briefly opening the containers allows some aeration to occur but the seeds should not be permitted to dry further.

At the end of the second stratification period the seeds should be sown into a moist medium and kept moist for the next several days or week to alleviate the moisture stress brought about by the redrying. There is evidence that stratified-redried seeds are less sensitive to the germination environment, especially temperature, meaning that they could be sown outdoors earlier than seeds given standard stratification. Other benefits of the method may be seen in more positive geotropism of the radicles in germinating seeds, another feature (together with reduced temperature sensitivity) that indicates greater seed vigour. Because most of the moisture removed during the redrying step comes from the seedcoat, there is less fungal activity also.

One detail in this procedure and with the standard stratification pretreatment also, is that the water used for the initial 48-hour imbibition is typically taken from the cold supply. Water temperature will vary from source to source, but it is usually not lower than 5 °C even during winter months, and is generally several degrees higher. As the seeds soak, water temperature slowly rises as it equilibrates with room temperature. The water is not kept cold. Thus, the initial hydration rate is lower than that at the end of the imbibition period when the water is typically warmer. (For a further discussion of this phenomenon see Edwards 2004b, "Kinetics of water absorption in fir seeds and some implications for tetrazolium tests and excising embryos", elsewhere in these Proceedings.) This is an important issue because one report (Jones et al., 1991) concluded that soaking noble fir seeds in water can cause damage in a majority of seedlots, although no details were provided on the nature of the damage, where it occurs, or its extent. No other reports of coniferous seeds being damaged by water soaking are known. It needs to be pointed out that Jones et al. (1991) compared the effect on noble fir germination of soaking seeds in water at 4 °C for 2 days, with allowing the seeds to hydrate on moistened filter paper at the same temperature for 21 days. That is, they compared the effect of a more than ten-fold difference, 2 days versus 21 days. The moisture contents reported indicate that a 2-day soak at very low temperature is inadequate to hydrate the seeds sufficiently for the cold treatment to have its effect. As mentioned above, for a further discussion of the phenomenon of thermo-kinetics of water absorption by noble fir seeds, see Edwards elsewhere in these Proceedings.

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Kinetics of water absorption in fir seeds and some implications for tetrazolium tests and excising embryos

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Abstract

Water uptake by empty seeds of noble fir (*Abies procera* Rehd.) was used as an expression of water absorption by the seedcoats of filled seeds. The initial rate of water absorption by empty and filled seeds was measurably faster at 25 °C (non-stratifying seeds) than at 5 °C (stratifying seeds). By the third day, water uptake by empty seeds had essentially ceased at 25 °C, and the asymptote was maintained over the duration of the study (28 days). However, water uptake by empty seeds was still ongoing after 28 days at 5 °C, when moisture contents averaged nearly 60 % higher than for empty seeds absorbing at 25 °C. For filled seeds, water uptake continued for 10 days before leveling off (the lag phase). In general, water uptake by filled seeds at 25 °C followed the three traditionally recognized phases. When germination was first seen at 25 °C (after 17 – 20 days), the seedcoat contained 60 % of the total seed moisture. When empty seeds that had ceased to take up moisture at 25 °C were transferred to 5 °C water uptake resumed. However, moisture contents did not achieve the same levels as for seeds continually imbibed at 5 °C, suggesting that the imbibant had undergone some change that modified the forces of hydration. A theory of hydration is presented to explain these effects. The implications for tetrazolium and excised embryo testing are discussed.

Experimental and discussion

Water absorption is part of the basic physiology of seed germination of angiospermous and coniferous forest trees. In non-dormant, readily-germinable seeds, the first stage in the process of germination is the absorption of water through the process of imbibition, which occurs in dormant and dead seeds as well. From studies extending over more than 80 years it is accepted that there are three phases of absorption, viz., A) a rapid increase in moisture content due to imbibitional hydration that begins immediately when contact is made with water; B) a much reduced, often difficult to detect, perhaps non-existent, lag phase when the absorbing tissues become saturated; and C) a second, more gradual (than in A) increase that begins as cellular components and energy sources become actively mobilized (Toole et al. 1956; Mayer and Poljakoff-Mayber, 1963; Bewley and Black, 1978; Farmer, 1997). For a review of the earlier literature see Edwards (1969). Thus, when water uptake is plotted over time, these three phases are manifest as a rapid initial rise, followed (but not always) by a leveling off of the curve, and then a further, usually more gradual, increase as the seeds begin to germinate. This is the triphasic pattern of water uptake, a graphic representation of which can be found in many textbooks, such as Bewley and Black (1978) or Farmer (1997).

Imbibition (phase A) is a physical (as compared to a metabolic) process related to the properties of colloids, and the extent to which it occurs is determined by seed composition, seedcoat permeability, the availability of a source of water, either liquid or vapour, and temperature. It occurs in live and dead seeds, and because it is a diffusion process it is positively related to temperature (Farmer 1997). This presentation will focus on the effects of temperature on phase A, and as this relates to phases B and C. Whereas the vast majority of the literature supports the view that more water is absorbed

as temperature increases, some earlier reports contradicted this. More recent treatises (Bewley and Black, 1978; Farmer, 1997) agree that imbibition occurs faster at higher temperatures. The reverse then can be considered to be true: compared to the rate of water uptake at temperatures favouring germination, imbibition will occur more slowly at lower temperature. These statements focus on the rate of imbibition, but much more needs to be said about the total amount of water absorbed at higher versus lower temperatures. This is the primary objective of this paper.

Theoretical principles of tissue hydration dictate that the final volume of water absorbed at high temperature will be less than that at low temperature; in contrast, the rate of imbibition is greater at high temperature, than at low temperature. There are two features of water molecules that need to be kept in mind. One is that they carry a very weak ionic negative charge, and also that they are constantly in motion due to their kinetic energy. This second feature, kinetic energy, is the more important in that it is significantly altered by changes in temperature, whereas the weak negative charge is not. The importance of these two features can be easily explained as follows.

Water absorption relates to the orientation of water molecules into shells surrounding the micelles (or particles) of the hydrating substrate (in this case, the seedcoat). Consider the particles/micelles of the absorbing seedcoat as spheres; diagrammatically these could be drawn as circles. In comparison to the water molecules, the substrate particles are positively charged. Thus there is a weak attractive force between the substrate particles and water molecules. Whereas the substrate particles are stationary, the water molecules are able to move toward the substrate particle due to the attractive force between them. This force obeys the inverse square law, meaning that it decreases rapidly with distance from the centre of the absorbing particle.

On contact with water molecules at temperatures that favour seed germination, for example 25 °C, the water molecules closest to the substrate particle are quickly attracted to form a shell surrounding it. Over the next several hours, water molecules that were originally further from the particle are also attracted, and form additional shells around the particle. Thus, because the water temperature is fairly high (25 °C), several shells of water molecules form around the attracting/absorbing substrate particle relatively rapidly. However, there is a limit to how many shells of water molecules can be attracted to, and remain attracted to, the absorbing substrate particle. This is because beyond a certain distance from the centre of the particle the attractive force has decreased to a point where is too weak to overcome the kinetic energy of the water molecules. It is this kinetic energy that, as mentioned earlier, keeps the water molecules in constant motion; because they are all negatively charged they are constantly trying to repel each other. So, beyond a certain distance from the core of each hydrating particle, no more water molecules can remain attracted; even if they come close enough, their kinetic energy causes them to move away again. At germination-favouring temperatures (such as 25 °C), water molecules rapidly form shells around the absorbing substrate particles, i.e., imbibition occurs rapidly, creating a rapid increase in moisture content (phase A), then absorption ceases, and moisture content reaches a plateau (phase B). To an observer, it would appear that the substrate is now "saturated" with water. Consider now what happens at low temperature, for example, 5 °C.

At 5 °C, the kinetic energy of the water molecules is greatly reduced. This decrease has a $Q_{10} = 2$; that is for each 10 °C decrease, the kinetic energy decreases by one half. For a 20 °C decrease (from 25 to 5 °C, as in this discussion), the kinetic energy of the water molecules will be only one fourth that at the higher temperature. When surrounded by water molecules, the same attractive force as described earlier exists, that is, the water molecules begin to move toward the substrate particle. Because their motion is now reduced, it takes longer for water molecules to form the first shell, followed by several more shells around the hydrating particle. Thus, the rate of water absorption is considerably reduced at low temperature. However, because the kinetic energy of the water molecules is now greatly reduced, those molecules further away from the particle remain attracted, and stay attracted to the

particle. Given sufficient time, more shells of water molecules remain attracted, that is, absorbed by the substrate particles at low temperature than at high temperature. So, at lower temperatures, imbibition occurs slowly, creating a slower increase in moisture content, but this increase continues over a much longer time span ending in a higher moisture content. To an observer, over the same time span as at the higher temperature it would appear that the substrate is not "saturated", and that water uptake (phase A) is continuing.

If this theoretical discussion holds true, then at a temperature favouring germination tree seeds could be expected to become quite rapidly "saturated", and not to take up any more water until germination began, while at a lower temperature, given sufficient time, they would more slowly reach a higher moisture content. Data to support this theory have been published (Edwards, 1971).

Seeds from three individual noble fir (*Abies procera* Rehd.) parent trees were placed on moist filter paper at 25 and 5 °C and allowed to take up moisture over 28 days. Empty seeds, identified and separated by x-ray techniques, were studied to express the role of the seedcoat of filled seeds in water absorption. At 25 °C, empty seeds became essentially saturated between 3 – 5 days at approximately 10 – 14 ml of water per 1,000 seeds, and showed little or no change in moisture content thereafter. At 5 °C, absorption in empty seeds proceeded at a measurably slower rate for the first 2 – 3 days, but uptake continued throughout the test period, and after 21 days the moisture content varied between 16 – 23 ml/1,000 seeds (among the three trees), and was still rising. Seed moisture content after 21 days at 5 °C (the stratification period normally prescribed for the species) averaged 56 % higher than after 21 days at 25 °C.

For all trees, empty seeds absorbing at 25 °C had entered the lag phase B by day 10; they were left at 25 °C until day 15 when they were transferred to 5 °C. Almost immediately (by day 17) their equilibrium moisture content was disturbed, and water absorption resumed. Although moisture contents of the transferred seeds continued to rise throughout an additional 45 days, they did not absorb as much water as empty seeds in contact with water at 5 °C continuously for 30 days. After 60 days of hydration, empty seeds transferred (at 15 days) from 25 to 5 °C had made up less than one half of the difference in moisture content after 30 days between seeds continuously absorbing at the two temperatures (Edwards, 1971).

Similar patterns of water uptake were observed for filled seeds. Water was absorbed quite rapidly at first (more rapidly at 25 °C than at 5 °C) and at 25 °C the seeds had reached an apparent equilibrium moisture content after 10 – 12 days; at 25 °C the equilibrium moisture level for filled seeds averaged at least 50 % higher than in empty seeds. In contrast, moisture levels in seeds absorbing at 5 °C continued to increase throughout the study period and after 21 days their moisture level averaged 14 % higher than at 25 °C. Filled noble fir seeds absorbing at 25 °C exhibited visible signs of germination between 17 and 20 days, which disturbed the moisture equilibrium (phase B) and resulted in further absorption (phase C), but at a lower rate than initially (phase A). Thus, they displayed the three phases of absorption described earlier; phase A occupied the first 5 – 7 days, phase B (the lag phase) began some days later, and phase C (the second increase in moisture level) began some 7 – 10 days later still. In comparison, filled seeds imbibing at 5 °C had not reached the lag phase (B) at the end of the 28-day observation period.

Using the absorption by empty seeds to indicate the ability of the seedcoat of filled seeds to take up water, two distinct relationships were discerned: I - during the first 2 – 3 days at 25 °C the seedcoat moisture comprised 60 – 70 % of the total water absorbed by filled seeds; II – after 10 – 11 days at 5 °C the seedcoat moisture comprised essentially the entire amount of water taken up by filled seeds. These differences are due to the more rapid transport (at 25 °C) of water molecules across the megagametophyte tissue to the embryo. The major site of imbibition is clearly the seedcoat, this structure absorbing water much more rapidly (at either temperature) than the megagametophyte; seedcoat moisture comprised not less than 60 % of the total seed moisture when germination was

first observed. As discussed in the theoretical considerations, the rate of water absorption was clearly greater at 25 °C (than at 5 °C), but the total amount of water absorbed was greater at 5 °C (than at 25 °C).

Early investigators (such as Shull, 1913) proposed the theory that high temperature would tend to increase the dispersion, and thus the water holding capacity, of the organic colloids of the seeds. Whereas such a relationship appeared true for filled noble fir seeds for imbibitional periods not exceeding 10 – 15 days, such changes within the imbibant with increasing temperature did not occur in empty noble fir seeds. Two alternatives should be considered. First, it is possible that alterations within the imbibant occurred at 5 °C, that is, water absorbing capacity was increased, because this is the temperature at which most tree seeds are stratified to overcome dormancy. Stratification is known to aid the conversion of less-active into osmotically-active materials in the seeds of many tree species (Toole et al., 1956; Mayer and Poljakoff-Mayber, 1963). Alternatively, it is possible that changes in the particles of the imbibant during hydration were such that water absorbing capacity at 25 °C was reduced. If no alteration had occurred at the higher temperature, then it would have been expected that when transferred to the lower temperature, a further build up of water molecules would have occurred to the same degree as measured for tissues imbibed continuously at 5 °C. The limited increase observed following transfer from higher to lower temperature is manifest that the particles of the imbibant absorbing at 25 °C had been modified in some way that limited the forces attracting the orientating water molecules. Thus, one of the main features of stratification of noble fir seeds, which produces more prompt and uniform germination, is the large increase in water absorption by the seedcoats. This effect is not related to the seed embryo which was found to be non-dormant (Edwards, 1969; see below). The increases in water content likely aid in weakening the mechanical restraint to the developing embryo imposed by the seedcoat and megagametophyte tissue. Although no other such detailed studies on the thermo-kinetics of water absorption by other coniferous seeds is known, the principles discussed here are of a general nature and are likely to function for other tree species.

It has been reported that soaking noble fir seeds in water causes damage when subsequent germination was compared with that of seeds hydrated on moist filter paper (Jones et al., 1991) as in the study above. No details were provided on the nature of the damage, where it occurs, or its extent, and no other reports of coniferous seeds being damaged by water soaking are known. It needs to be pointed out that Jones et al. (1991) compared the effect of soaking seeds in water at 4 °C for 2 days with hydrating them on moistened filter paper at the same temperature for 21 days. That is, they compared the effect of a more than ten-fold difference, 2 days versus 21 days, in hydration period. The moisture contents reported indicated that a 2-day soak at a very low temperature is inadequate to hydrate the seeds sufficiently for stratification to be effective (see Edwards, 2005, "Breaking dormancy in tree seeds with special reference to firs (*Abies* species) - the 1.4x solution" elsewhere in these Proceedings). As has been discussed above, water uptake at low temperature is slower than that at higher temperature. The water temperature, 4 °C, chosen by Jones et al. (1991) is the point at which water is at its densest (it expands anomalously above and below this temperature) so it might have been expected that rate of water uptake would have been at its lowest. It is difficult to conceive how this might have damaged the seeds. In operational practice, when more than a few grams of seeds are to be stratified, they are typically soaked for 48 hours in water taken from the cold supply. Initial water temperature will vary from source to source, but it is generally not below 5 °C even during winter months, and may be several degrees higher. As the seeds soak, water temperature slowly rises as it equilibrates with room temperature, which may remain cool. However, in operational practice the water is not kept cold. This means that the hydration rate during the early hours will be slower than that at the end of the imbibition period, when the water has become typically warmer. That is, seeds begin to take up water slowly at first, then more quickly toward the end of the imbibition period.

The effect of the method of hydration when stratifying western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) seeds has been reported (Edwards, 1973). It was found that there was no significant difference in germination whether the seeds were placed on moist filter paper (dipping into a water bath at 1 – 4 °C, similar to the process preferred by Jones et al., 1991) and stratified for 0 – 16 weeks, or if they were hydrated directly in water at room temperature for 48 hours, after which the water was decanted, the seeds lightly surface dried then placed in closed plastic bags and stratified at 1 – 4 °C for 0 – 16 weeks. For durations of chilling longer than 8 weeks, germination was slightly less from seeds chilled in plastic bags because the bags were not adequate to maintain the moisture level/humidity within. In all other aspects, there were no differences in germination due to hydration method. Western hemlock seeds have been found to be generally more susceptible to moisture stress during the stratification/germination processes than other coniferous seeds, and they do not respond well to the stratification-redry method (Edwards, 1986). Whereas they germinate more slowly and less completely unless they were kept fully moistened, they exhibited no effect of being hydrated in liquid water for 2 days, and the same number of normal germinants were recorded (Edwards, 1971). Based on these observations alone, it is doubtful that noble fir seeds, or those of any other North Temperate Zone conifer species are "damaged" by soaking in water.

As has been explained, noble fir seeds (and probably those of other firs – perhaps other conifer species – also) absorb water over several days, the rate of absorption being highly temperature dependent. Even at a higher temperature (25 °C) filled noble fir seeds became fully saturated only after some 10 days of hydration. At low temperature (5 °C) water uptake by filled and empty seeds continued over several weeks. When germination began at 25 °C the seedcoats contained 60 % of the total moisture in the seeds. Noble fir seeds are considered to be dormant and require stratification to promote prompt and complete germination. A separate study was conducted to determine the location, or cause, of this dormancy (Edwards, 1969). Intact embryos were excised from stratified and unstratified seeds and cultured on agar to determine if there were any differences in growth. No differences were found, embryos from unstratified seeds growing as quickly as those from stratified seeds. It was concluded that dormancy in noble fir seeds (again, likely also in other firs) does not reside in the embryo. However, this study revealed a detail given little attention elsewhere, viz., that even at 25 °C 48 – 72 hours of hydration are required for the embryos (of non-stratified seeds) to become sufficiently pliable for placing on the culturing agar. If hydrated for 48 hours or less, the embryos were consistently too brittle (like dry spaghetti) to be removed intact from the surrounding seed tissues. No definitive hydration period was determined, but it was estimated that at least 60 hours hydration at "room temperature" is necessary to achieve the pliable state. All embryos were easily worked when the intact seeds had been soaked for 72 hours. This observation has implications for tetrazolium testing. Current prescriptions (ISTA, 1999) call for the seeds to be "cut transversely at both ends to open the embryo cavity" before "premoistening at 20°C". It is suggested that some of the inconsistencies found in tetrazolium testing of fir seeds may be due to a hydration period that is too brief. However, if any "damage" does occur when noble fir seeds, or those of other conifers, are hydrated in warm or cold water, as contended by Jones et al. (1991), it cannot occur in the embryo. The excised embryo observations discussed above, and the nature of the international prescription to "open the embryo cavity" when conducting viability testing (presumably to achieve rapid hydration of the internal tissues at a fairly high temperature), clearly indicate that soaking in water does not harm the embryo. Together with the principles of hydration discussed, these conclusions are likely to have general application to (at least) seeds of other firs. However, further detailed studies on other tree species are needed to confirm this.

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Comparative results from nineteen laboratories testing forest tree seeds during 2001

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Abstract

Nineteen laboratories took part in comparative tests done to determine the consistency of inter-laboratory results of tests done for purity analysis and weight determination and seed germination. The tests were done using one seedlot of European larch (*Larix decidua*) and two seedlots each of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The laboratories also had the option of determining the viability of *Fagus sylvatica* seeds using a tetrazolium staining test and an ISTA-approved germination test.

The tests to determine the percent of pure seeds, weight of 1,000 seeds, and percent normal seedlings resulted in quite comparable results. The main source of variability in the purity analysis results were difficulties in interpreting PSD definitions 47 and 51. In the germination test the results of the first counting of germinants and the evaluation of ungerminated seeds differed among the participating laboratories.

The tetrazolium and germination tests done on the *Fagus sylvatica* samples produced variable results, suggesting that the differences in interpreting viability resulted from differences in interpreting staining patterns, or in evaluating normal and abnormal seedlings and ungerminated seeds.

Introduction

Only a few ISTA accredited laboratories test tree seeds. The ISTA proficiency test programme does not cover tree seeds and the last referee test for this group of seeds was organised in 1995 by Mrs. Samuel from the Forestry Commission, Research Division, Alice Holt Lodge (now ISTA laboratory GBDL02) as a preauthorisation referee test. Consequently, it is impossible to compare the results of tests from different laboratories and so laboratory SKDL02 in collaboration with the ISTA Forest Tree and Shrub Seed Committee decided to do a comparative test of commonly-used seed tests to determine the reliability of the results from different tree seed testing laboratories.

Participating laboratories

The 19 laboratories listed below took part in the test. Thirteen of the laboratories were accredited ISTA- laboratories (for anonymity these were designated as ISTA 01 – 13). The other six were non-ISTA laboratories (designated as S 14 –19), who voluntarily participated in the referee test. The accredited ISTA laboratories were:

CZDL02 Výzkumný ústav lesního hospodářství a myslivosti, Výzkumná stanice Uherské Hradiště, Czech Republic

DEDL04 Staatliche Landwirtschaftliche Untersuchungs- und Forschungsanstalt Augustenberg, Karlsruhe, Germany
FRDL01 Centre de Nancy, Unité Recherches Semences Forestiers, Champenoux, France
FRDL02 Station Nationale d´Essais de Semences, Beaucouzé, France
GBDL02 Official Seed Testing Station for Tree and Shrub Seed, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, United Kingdom
JPDL04 Forestry and Forest Product Research Institute, Ibaraki, Japan
NODL02 Norwegian Forest Seed Station, Hamar, Norway
RODL02 Institutul de Cercetari si Amenajari Silvice, Laboratorul de Seminte Foresti, Bucuresti, Romania
SEDL05 Seed Laboratory, Dept. of Silviculture, The Swedish University of Agricultural Science, Umea, Sweden
SEDL06 Skog Forsk, The Forestry Research Institute of Sweden, Sävar, Sweden
SIDL01 Kmetijski institut Slovenije, Seed Testing Labolatory, Ljubljana, Slovenia
SKDL02 Lesnícky výskumný ústav, Výskumná stanica Liptovský Hrádok, Slovakia
USDL03 National Tree Seed Laboratory, Dry Branch, Georgia, U. S. A.

The six, non-ISTA laboratories were:
Bayerische Landesanstalt für Forstliche Saat- und Pflanzenzucht, Teisendorf, Germany
Danish Tree Improvement Station, Humlebaek, Denmark
National Forest Genetic Resources Centre, Natural Resource Canada, Canadian Forest Service - Atlantic, Fredericton, New Brunswick, Canada
Forstliche Bundesversuchsanstalt, Abteilung für forstliches Vermehrungsgut, Wien, Austria
Instytut badawczy lesnictwa, Warsaw, Poland
Landesforstanstalt Eberswalde, Forstsaatgutprüfstelle Waldsieversdorf, Germany

Tests and samples

All the participating laboratories were obligated to do the purity and weight tests on seed samples from the seedlots listed below. As well, tetrazolium and germination tests were done on a voluntary basis on two samples of *Fagus sylvatica* seeds.

Sample No.	Species	Test			
Obligatory test					
1	*Larix decidua* Mill.	Purity	Weight	Germination	
2	*Picea abies* Karst.	Purity	Weight	Germination	
3	*Picea abies* Karst.			Germination	
4	*Pinus sylvestris* L.			Germination	
5	*Pinus sylvestris* L.			Germination	
Voluntary tests					
6	*Fa*gus *sylvatica* L.			Germination	Tetrazolium
7	*Fagus sylvatica* L.			Germination	Tetrazolium

When making the tests the laboratories were asked to adhere to the valid ISTA Rules and complete the evaluation sheets provided by the organiser.

Test results

The detailed results of this comparative test have been given to all the participating laboratories. In the following we present some of the general findings and conclusions. The results of the tests done in the participating laboratories are in:

1. Histograms showing percentages of the evaluated categories for specific fractions of purity or categories of ungerminated seeds (hard, fresh, empty, dead) obtained by each laboratory.
2. Histograms produced by the statistical package Statistica which show the distribution of the results obtained by the various laboratories. The x-axis shows the values obtained by testing the samples (e.g. the percent pure seeds or normal seedlings) and the y axis gives the frequency of the value, i.e. the number of laboratories which obtained that result. The vertical lines indicate the marked limits of ± s (standard deviation) and ± 3.s in the curve of normal distributions shown in the histogram. Results lying outside the first interval (± s), but especially those on the border or outside of the second interval (± 3.s), suggest possible difficulties in carrying out the test or in reporting the results of a specific laboratory. The normal distribution curves in these diagrams can be misrepresentative, when there are large differences in the results or extreme values obtained by some of the laboratories. For this comparative test the histograms are used only in reasonable cases.

Purity

Most of the laboratories made their purity test using the entire working sample, while five laboratories divided the sample and used two sub-samples.

The distribution of the determined purity percentages is rather close to the mean value and only one laboratory (Fig. 2) exceeded the ± 3s interval for determining the purity of spruce sample 2.

The ISTA Rules 1999 introduced a new category for winged and dewinged seeds in the pure seed definitions 47 (*Picea*) and 51 (*Larix*). These definitions were not fully accepted by all seed testing laboratories. Three laboratories (ISTA 12, S 15 and S 19) did not distinguish between winged and dewinged seeds. Although the others made this distinction the differences in the results, e.g. the percentage of winged seeds in sample 1of *Larix* seeds, show that in the case of species belonging to the PSD 51 it can be difficult to decide, if the seeds have wings or not (Fig. 1).

Fig. 1 Results in purity analysis on the sample 1- *Larix decidua* obtained in participating laboratories (the sum of percentages in categories winged seeds and seeds without wing represents the pure seed percentage)

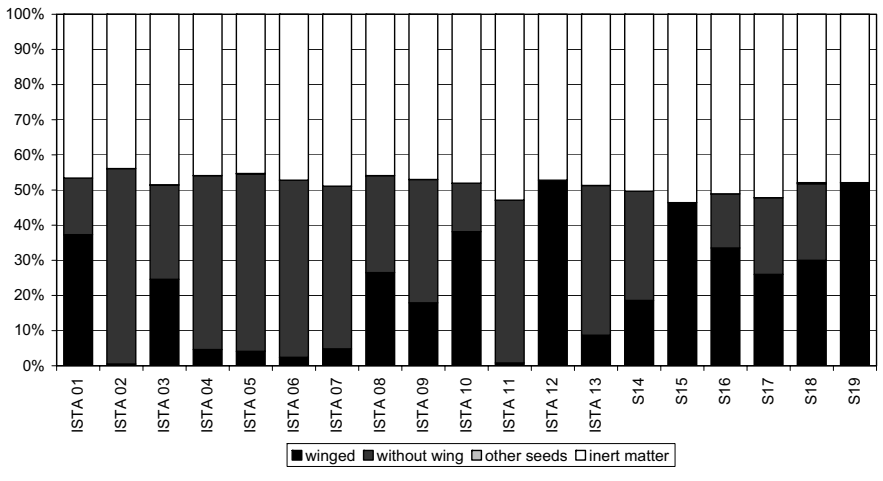
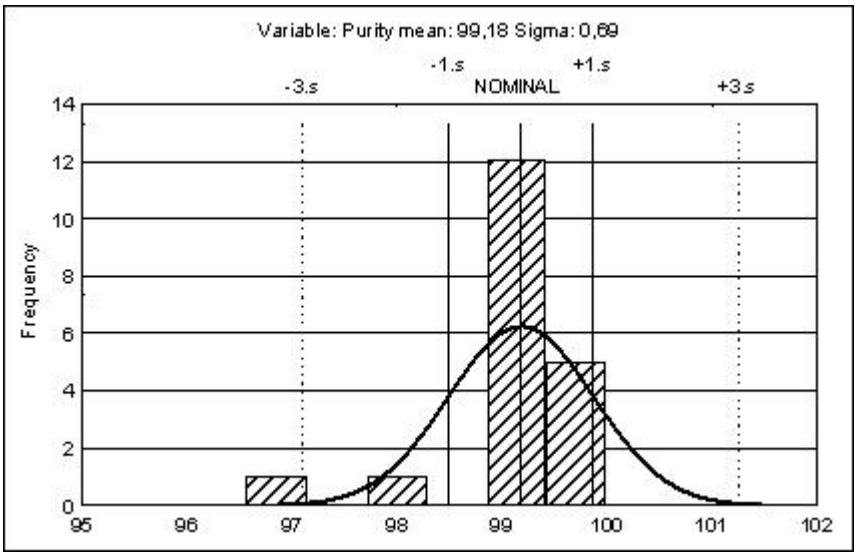


Fig. 2 Distribution of purity percentages obtained in participating laboratories in purity analysis on the sample 2 – *Picea abies* (X-axis – the purity percentage, Y-axis – the number of laboratories with the particular purity percentage)



Problems detected:

- The original weight of the sample and the weights of component parts was not reported.
- The calculation was not done in accordance with rule 3.6.2.2. The percentage of the purity fractions was not calculated from sum of weights of component parts, but from the weight of the working sample.
- Small calculation errors.
- When purity was determined by using two sub-samples, instead of using the intact sample, the variation between the samples exceeded the tolerances given in the Rules, A 15, Table 3.1.

Weight of 1,000 seeds

The weight was determined in 14 laboratories by weighing eight replicate samples of 100 seeds each and in four laboratories by counting the entire working sample. In laboratory S 15, the weight was determined as a mean value of two replicates, of 1,000 seeds each, but this method is not described under Weight determination in the ISTA Rules, chapter 10.

For *Larix decidua* there was little variation in the determined weights (3.78 – 4.32 g), except for the data from laboratory ISTA 10 (7.53 g), which was probably a mistake. In this case the weight was determined by using the entire working sample. The lowest and highest weight values for *Picea abies* (7.37 and 8.43 g) seeds are near to the boundary for the ± 3s interval; the other values ranged from 7.70 to 8.24 g (Fig. 3).

Detected difficulties included:

- Coefficient of variation not given or not calculated correctly.
- Rounding procedure was wrong.

Germination of *Larix, Picea*, and *Pinus* seeds

Regarding the parameters compared in the germination test, the results in category of most importance, i.e. determination of normal seedlings, were the most consistent. However, there was considerable variation in the results from the different laboratories, especially when medium quality seeds were tested, e.g. in sample 1 of *Larix decidua* (Fig. 4). In some cases extreme germination

values reached or exceeded the ± 3s interval. This occurred, for example, for sample 3 of *Picea* seeds where the germination percentage from ISTA 6 laboratory was of 88 % (the mean value from all laboratories in this sample was 93 %), and for sample 5 of *Pinus* seeds (Fig. 5) where the germination percentage from the laboratory ISTA 13 was 60 % (the mean value was 73 %). In the other categories, the germination test results show a high level of variability.

Fig. 3 Distribution of weights of 1,000 seeds obtained in participating laboratories in weight determinations on the sample 2 – *Picea abies* (X-axis – the weight of 1000 seeds, Y-axis – the number of laboratories with the particular weight of 1,000 seeds)

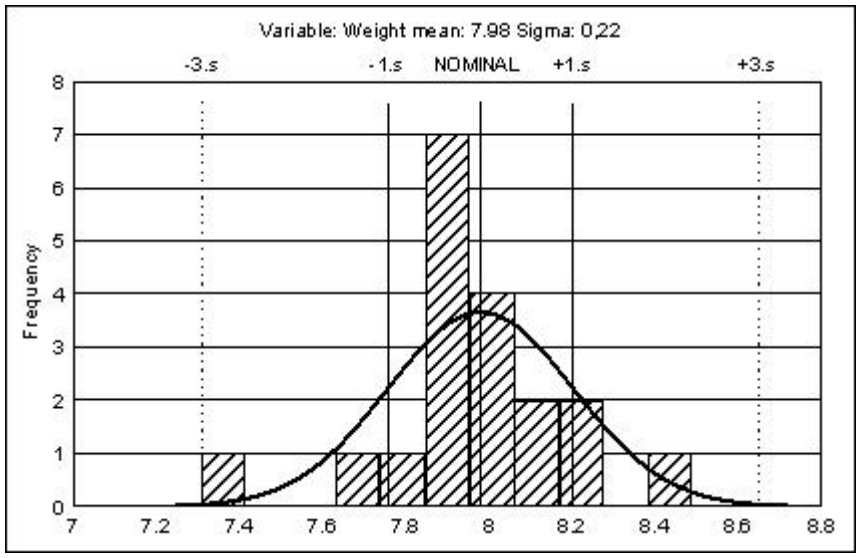
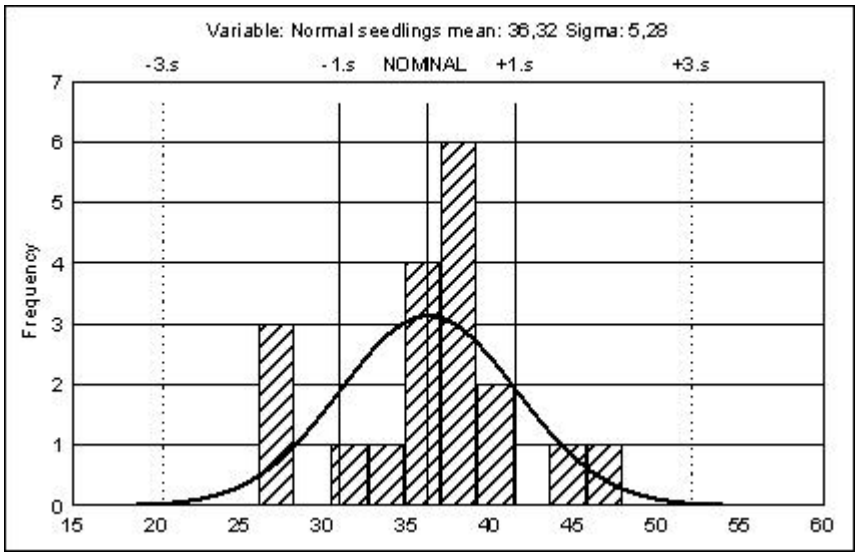


Fig. 4 Distribution of normal seedlings percentages obtained in participating laboratories in germination tests on the sample 1- *Larix decidua* (X-axis – the percentage of normal seedlings, Y-axis – the number of laboratories with the particular percentage of normal seedlings)



There were big differences in the percentages of seeds germinated by day 7 of the test (i.e. the first counting). European laboratories often report this parameter as germination energy. The big differences in these values (from zero to the percentage at the end of the germination test) show that

the laboratories did not evaluate the germinants in the same way. Laboratories with zero germination after 7 days probably consistently wait for whole seedlings to develop. The others used the criterion that germinated seeds must have a radicle 4 or 2 times longer than the seed, which is an often-used procedure for conifer seeds.

Fig. 5 Distribution of normal seedlings percentages obtained in participating laboratories in germination tests on the sample 5 - *Pinus sylvestris* (X-axis – the percentage of normal seedlings, Y-axis – the number of laboratories with the particular percentage of normal seedlings)

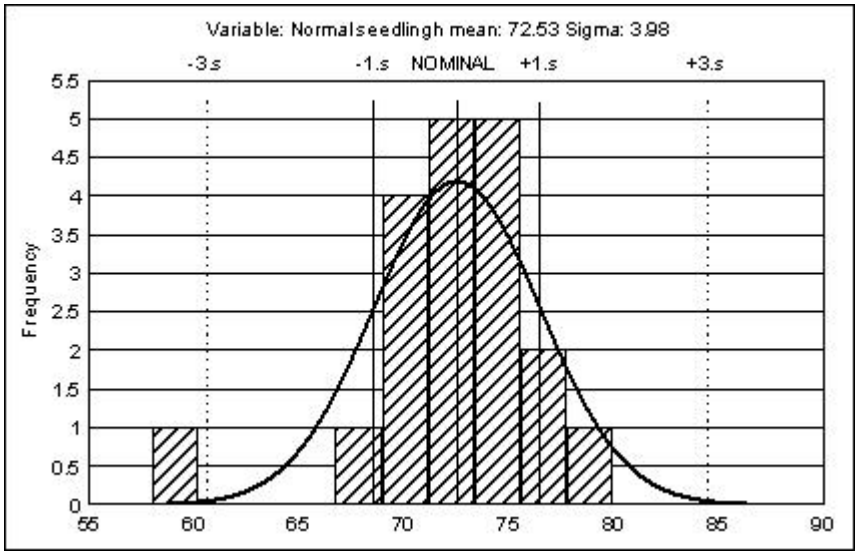
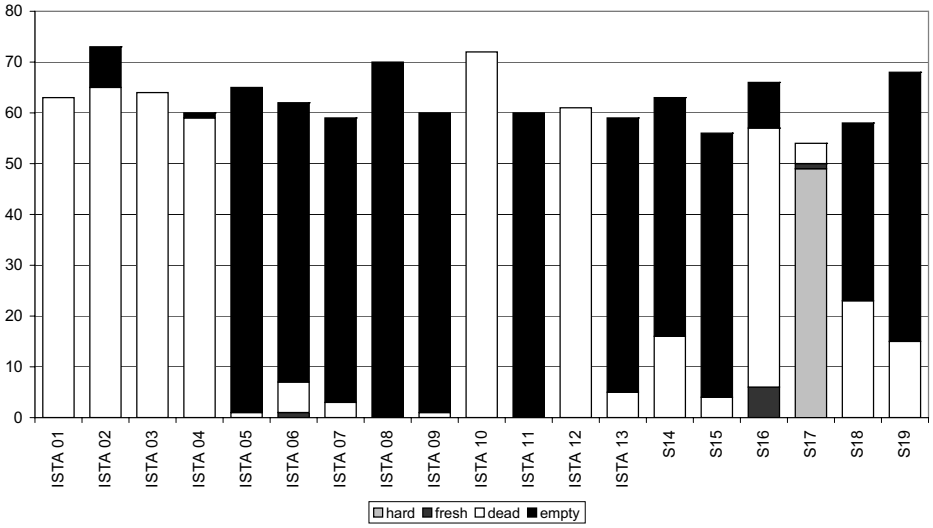


Fig. 6 Classification of the ungerminated seeds in germination tests on the sample 1 - Larix decidua carried out in participating laboratories



Not uniformity was found in the data obtained for classification of ungerminated seeds, i.e. hard, fresh, dead, or empty. Some of the laboratories recorded only dead seeds; others only empty seeds while many of the laboratories used both categories (Fig. 6, 7 and 8). Sometimes, e.g. laboratories ISTA 03, S 16 and S 17 for *Picea* seeds (Fig. 8) and especially *Pinus* samples (Fig. 7) most of the ungerminated seeds were classified as fresh seeds. Some of participating laboratories classified a part of the ungerminated seeds as the hard seeds. However, the word "hard" describes quite eloquently

the quality of some ungerminated conifer seeds. Nevertheless, they are not hard seeds in the exact sense of ISTA Rules definition 5.2.7: *Hard seeds: seeds which remain hard at the end of the test period, because they have not absorbed water*. The hard seeds referred to by laboratory S 17 for *Larix* seeds (Fig. 6) are undoubtedly empty seeds which have a much harder seed coat than filled seeds and the hard seeds reported by laboratories S 19 an S 18 for seeds of *Picea* and *Pinus* (Fig. 7 and 8) should be classified as fresh or dead seeds.

Detected difficulties:

- The results were not rounded or small errors occurred in the rounding procedure, e.g. the sum of the percentages was not 100.
- Variation between four replicates exceeded the tolerances given in the Rules, A 15, Table 5.1.
- For the germination tests the required four replicates of 100 seeds each were not used. For example laboratory ISTA 02 used unequal numbers of the seeds for the test, and they used the mean weighed for the final result. Consequently, while the calculation was correct it was not possible to check tolerances between replicates.

Optional tests (tetrazolium, germination) with *Fagus sylvatica* seeds

In both tests there was a wide distribution of the results (Fig. 9 – 12). Variation among the four test replicates also often exceeded the tolerances given in the ISTA Rules. In the germination test, some laboratories mentioned the high number of abnormal seedlings and fresh seeds.

There was also a clear difference in results of both common tests used for determining seed quality: the germination test and the biochemical test for viability (Tetrazolium test). The mean germination percentage from all laboratories doing the tests was 37 % for sample 6, and 28 % for the sample 7. These two values are much lower than the mean viability percentage determined by the Tetrazolium test, which was 58 % for the sample 6 and 57 % for the sample 7.

Detected difficulties:

- For the TTC test four replicates of 100 seeds each were not used.
- The results were not rounded or there were small rounding errors so that the sum of the percentages was not 100.
- Variation between the four replicate samples exceeded the tolerances given in the Rules, Annexe 15 Table 5.1. In the case of germination test, which can last more than 20 weeks it is difficult to repeat the test.

Fig. 7 Classification of the ungerminated seeds in germination tests on the sample 4 - *Pinus sylvestris* carried out in participating laboratories

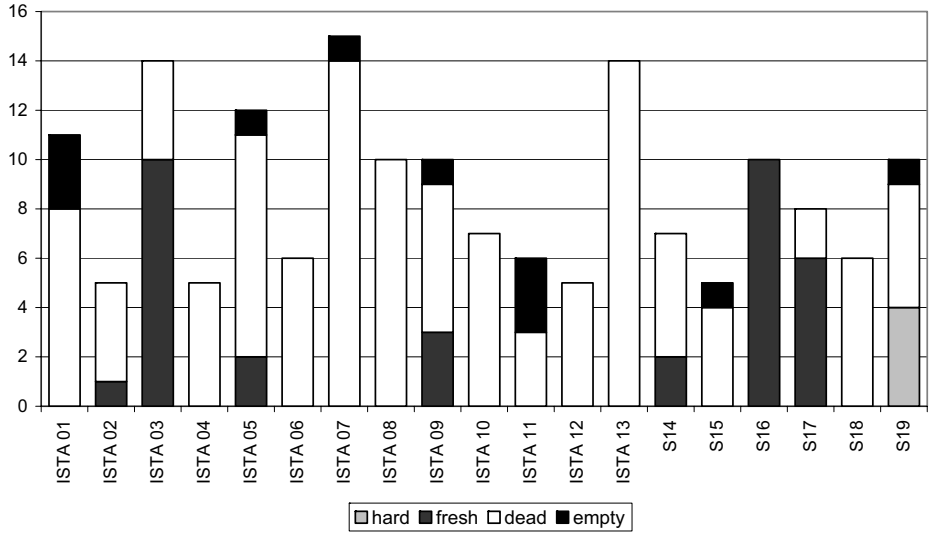


Fig. 8 Classification of the ungerminated seeds in germination tests on the sample 3 – *Picea abies* carried out in participating laboratories

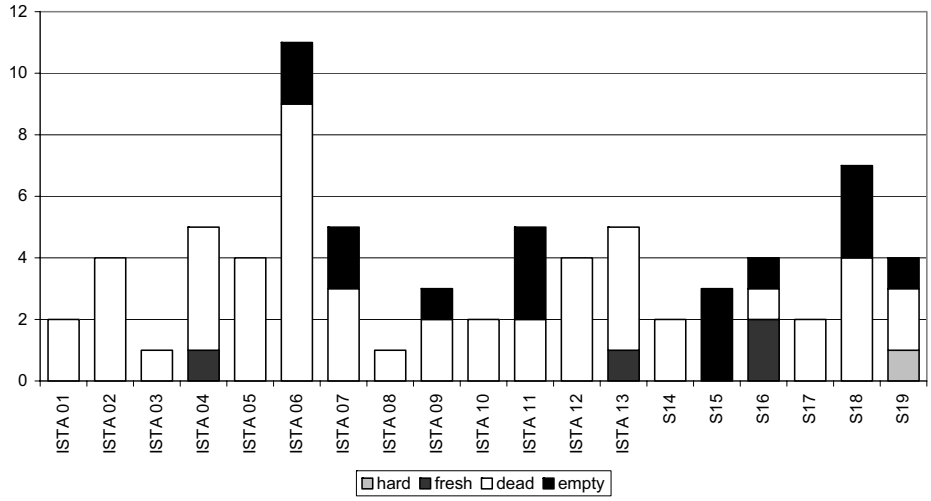


Fig. 9 Results in germination tests on the sample 6 – *Fagus sylvatica* obtained in participating laboratories

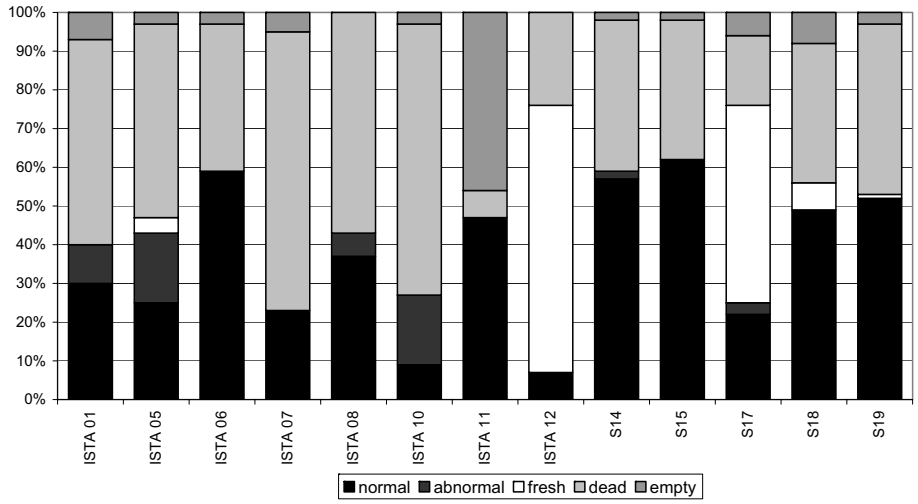


Fig. 10 Results in tetrazolium tests on the sample 6 – *Fagus sylvatica* obtained in participating laboratories

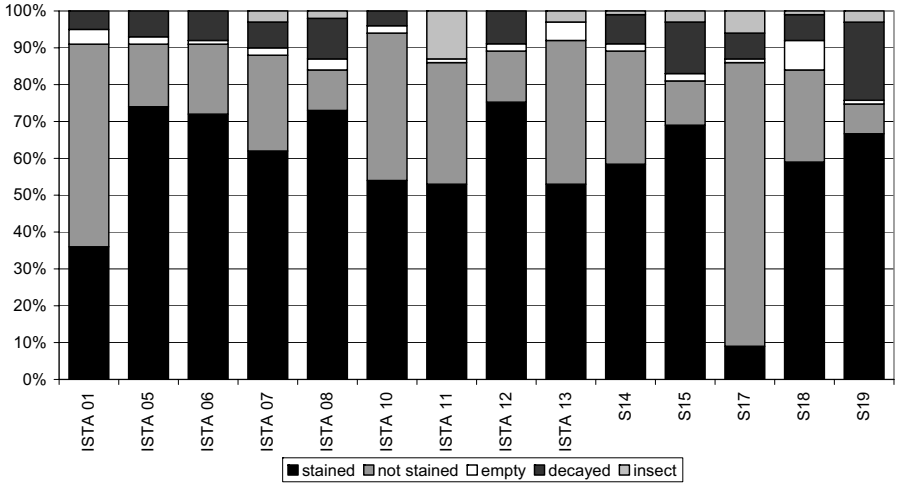


Fig. 11 Results in germination tests on the sample 7 – *Fagus sylvatica* obtained in participating laboratories

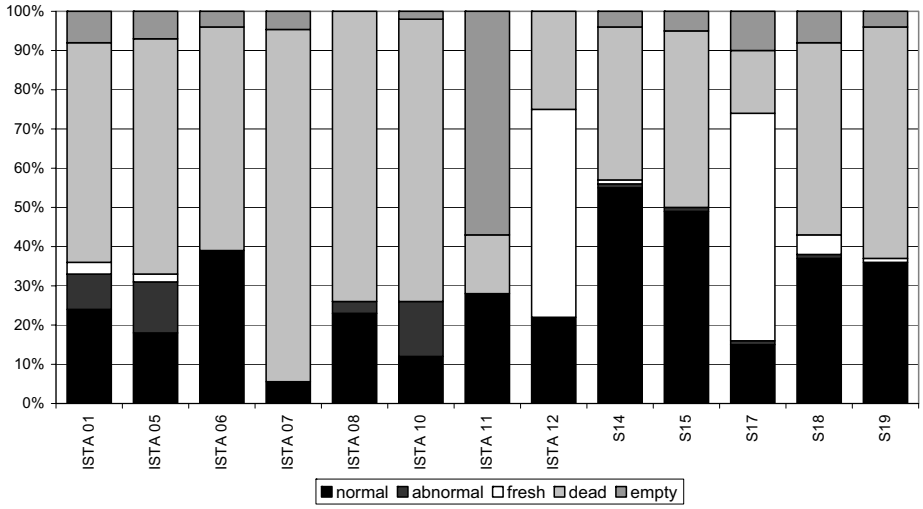
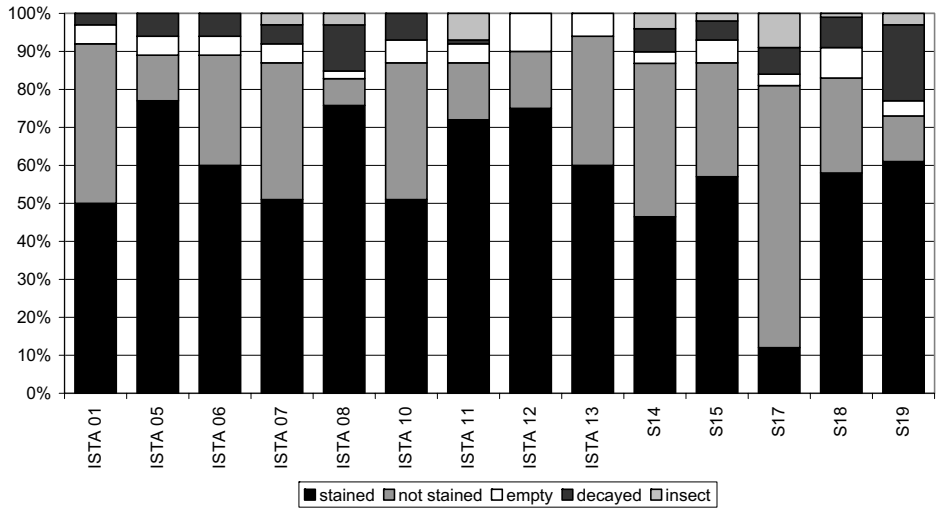


Fig. 12 Results in tetrazolium tests on the sample 7 – *Fagus sylvatica* obtained in participating laboratories



Discussion and conclusions for next FTS Committee activities

The main aim of this comparative test was to obtain results showing the variation in tree seed test results and allow the participating laboratories the chance to compare their results. It was not a referee test in the sense of the ISTA Referee Test Programme introduced in 2001 (Ednie, 2001) and there was no intention to do a laboratory performance rating. Nevertheless, the participants individually received feedback from the study leader regarding possible difficulties in doing the tests and in evaluating and reporting the test results.

Based on the results of these tests the following recommendations were made to the next FTS Committee regarding activities where work is needed:

- ***Purity:*** revise PSDs of conifer seeds
- ***Germination:*** revise the definition of "normal seedling"
- ***TZ and germination test of dormant seeds:*** discuss in more detail testing and evaluation procedures

The results obtained in our test indicated a need for more widespread exchange of information among laboratories testing tree seeds. The results also supported the FTS Committee goal: to revise the "Tree and shrub seed handbook" and to organise this workshop with practical training.

Acknowledgements

We thank all the laboratories listed above for their willingness to participate in this co-operative test and to all the contact persons for their excellent collaboration.

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How to obtain more from ISTA 'double' germination tests

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Summary

The ISTA 'Rules for Seed Testing' prescribe a 'double' germination test (one with and one without pretreatment) to assess the quality of many temperate tree species. Untreated and pretreated germination tests both receive interim germination counts (for example at 7, 14, 21 and 28 days of a 35d test) – but only the final germination percentage from each test must be reported on a test certificate.

This paper reports comparisons of interim and final germination percentages between untreated and pretreated seeds for a particular seedlot of Corsican pine (*Pinus nigra* var. *laricio*) in i) an ISTA 'double' germination test; ii) laboratory germination tests over a range of temperatures and iii) a broadcast sown, nursery seedbed. The ISTA 'double' germination test for this particular seedlot showed a very rare combination of characteristics - prechilling significantly stimulated early germination but ultimately resulted in significantly fewer normal germinants. Both effects were reproduced in the nursery, and consequently although prechilled seeds led to significantly fewer nursery seedlings, the earlier emergence (and hence longer growing season) of prechilled seeds resulted in significantly bigger plants.

It is concluded that not only can the germination capacity from an ISTA 'double' germination test be used to determine the maximum germination potential of any seedlot, but even very rare responses to pretreatment can be analysed and interpreted to offer practical sowing advice to plant producers.

Key words: Corsican pine; seed; pretreatment; ISTA 'double' germination test; nursery performance

Introduction

Since 1966, the ISTA 'Rules for seed testing' have prescribed 'double' germination tests (one with and one without pretreatment) to assess the quality of many temperate tree species. Although 'double' tests receive interim germination counts (for example at 7, 14, 21 and 28 days of a 35 days test) only the final germination percentage from each test must be reported on the test certificate. However, the ISTA Rules contain no guidance on how to compare, analyse or interpret the two sets of results. Nor do they explain how 'double' tests might be used to provide useful, practical advice to plant producers, for example on how to achieve the best field performance from individual seedlots.

There are numerous papers on the mathematical analysis and representation of cumulative germination data (Brown and Mayer, 1988a, 1988b; Czabator, 1962; Hunter et al., 1984; Nichols and Heydecker, 1968; Scott, Jones and Williams, 1984). But they are all intended to be applied to research data collected at much more frequent intervals than are required for ISTA germination tests on tree species where successive germination counts are usually separated by at least 7 days. As a consequence, Gosling and Peace (1990) proposed a simple statistical comparison between the respective interim and final germination percentages in such tests. In their paper, 'double' germination test results could be classified into 10 'pretreatment response (ptr) codes'. An analysis of the

ptr codes from 'double' germination tests on almost 1,000 seedlots showed that the majority of seedlots responded beneficially to pretreatment but that a significant minority was harmed by pretreatment. Instances of pretreatment harming tree seed germination in laboratory germination tests had been previously identified by Swofford Thomas (1958), Hellum (1968), Hocking (1972) and Hellum and Dymock (1985) but the significance of this to field emergence has been ambiguous.

This paper extends some of the general observations of Gosling and Peace (1990) on the analysis, classification and interpretation of ISTA 'double' germination tests, and specifically examines the laboratory germination and nursery performance of one seedlot of Corsican pine (*Pinus nigra* var. *laricio*) with a rare response to moist chilling at 4 °C.

Materials and methods

Seedlot

A Corsican pine seed lot [Identity number 80 (4599)] was obtained from the Forestry Commission, UK, where it had been stored for 6 years at 7 % moisture content (fresh weight basis) and +2 °C.

Laboratory experiments

ISTA germination test

The seedlot was tested using International Seed Testing Association (ISTA) 'double' germination test methods. It was selected for this particular study because of its very rare response to pretreatment.

Laboratory prechill treatment

Seeds were incubated on moist filter paper at 4 °C for 3 weeks.

Laboratory germination over a range of constant temperatures

Four replicates of 100 seeds were incubated on moist filter paper over a range of constant temperatures (10, 15, 20, 25, 30, 35 and 40 °C) in the dark (as described by Gosling, 1988).

Laboratory germination assessments

Germination was assessed at regular intervals according to the definitions of the ISTA Rules (1985). Seeds proved slowest to germinate at 10 °C and took 42 days to reach their maximum percentage germination. All germination tests were extended to this duration. Discoloured, rotting, soft, unhealthy and empty seeds were all classified as 'dead'.

Nursery experiment

Sowing

Unchilled and prechilled seeds were sown into separate plots on seed beds at Headley Nursery, Surrey, UK on 21st April 1987. There were eight replicates of each treatment combination and plots were arranged in a completely randomised block design. Each plot was sown with 100 seeds in a 0.02m wide strip across a 1.2 m seed bed and 0.01 m was left between plots. After sowing, the seedbed was gently rolled, covered with a layer of light coloured grit and sprayed with a pre-emergent herbicide (diphenamid 4 kg a.i. ha^{-1}) within one day of sowing. All plots were covered with bird netting until seedling emergence had stopped. Baited traps were placed inside the netting to trap mice and the seedbeds were subsequently fertilised and irrigated according to the conventional practices for the nursery.

Nursery assessments

Cumulative emergence and survival counts were made weekly from the date of first emergence until germination peaked (i.e. either three identical counts were made on consecutive occasions or for at least one month after the maximum emergence had been recorded, if seedling numbers were declining).

At the end of the growing season, 20 random plants were sampled from each plot and seedling height and root-collar diameter were measured.

Statistical analysis

Differences in laboratory germination percentages were tested using standard statistical methodology as detailed by ISTA (Miles, 1963). Unchilled and prechilled means were compared by computing a normal deviate derived from the normal approximation to the binomial distribution.

Nursery performance was analysed by sowing seed in a randomised block design and recording percentage germination, percentage survival, seedling height and root collar diameter.

Analysis of variance (ANOVA) was used to test for significant differences between prechilled and unchilled seed. Significant differences were identified at the probability level 0.025 as this is the tolerance level adopted by ISTA unless otherwise shown.

Results

Table 1 summarises the analyses, classification and interpretation of 'double' germination tests proposed by Gosling and Peace (1990). It can be seen that column 4 offers practical nursery sowing advice for the nine simplest, commonest and most well defined ptr code responses. However, it was acknowledged that 24 out of 995 'double' tests fell into ptr code 10 and it was not possible to suggest practical advice for this response at that time. In this paper I specifically examine the laboratory and nursery emergence of a Corsican pine seedlot with a ptr code 10. But in order to study this more meaningfully, it is necessary to subdivide ptr code 10 into a further three ptr codes which are defined in Table 2.

Table 1. The analysis, classification and interpretation of ISTA 'double' germination tests into pretreatment response (ptr) codes (after Gosling and Peace, 1990)

Maximum % germination after pretreatment	Interim % germinations after pretreatment	Pretreatment response code	Practical advice
Significantly higher	Significantly faster	1	See 'A' below
	Not significantly different	2	
	Significantly slower	3	
Not significantly different	Significantly faster	4	See 'B' below
	Not significantly different	5	
	Significantly slower	6	See 'C' below
Significantly lower	Significantly faster	7	
	Not significantly different	8	
	Significantly slower	9	
Higher, the same or lower	Alternately significantly faster then slower or vice-versa	10	*"Unknown"*

A This seed is dormant and should be pretreated before sowing.
B This seed is not dormant but field emergence will benefit from pretreatment.
C This seed will not benefit from pretreatment.

Table 2. The classification and renumbering of ptr code 10's

Maximum % germination after pretreatment	Interim % germinations after pretreatment	Pretreatment response code	Practical advice
Significantly higher	Alternately significantly slower then faster	10a	To be determined
Not significantly different	Alternately significantly faster, then slower or vice-versa	10b	To be determined
Significantly lower	Alternately significantly faster then slower	10c	To be determined

Figure 1 shows the course of germination of the Corsican pine seedlot during the ISTA 'double' germination test. At day 7 of the test, prechilled seeds (■) have germinated to a significantly higher ($p \leq 0.025$ an ISTA convention) percentage than unchilled seeds (□). However, at day 14, the unchilled seeds have overtaken the prechilled seeds and by the end of the germination test the maximum percentage germination of the unchilled remains significantly higher ($p \leq 0.025$). When the interim and final germination percentages (unchilled versus prechilled seeds) are compared with Table 1 it is clear that the seedlot has a ptr code of 10. And when the results are further compared with Table 2 it is clear that it is more precisely ptr code 10c. Prechilling has surprisingly stimulated significantly faster early germination (day 7), then significantly slower germination (day 14) and ultimately a lower maximum percentage germination (day 21). It is important to note that the majority of ungerminated seeds at the end of both tests were dead – the number of viable seeds remaining with the potential for later germination was therefore insignificant.

Fig. 1 Course of germination of Corsican pine seeds on moist filter paper during an ISTA 'double' germination test carried out at a daily alternating 20/30 °C following 0 and 3 weeks moist prechill at 4 °C (□ – 0w prechill; ■ – 3w prechill)

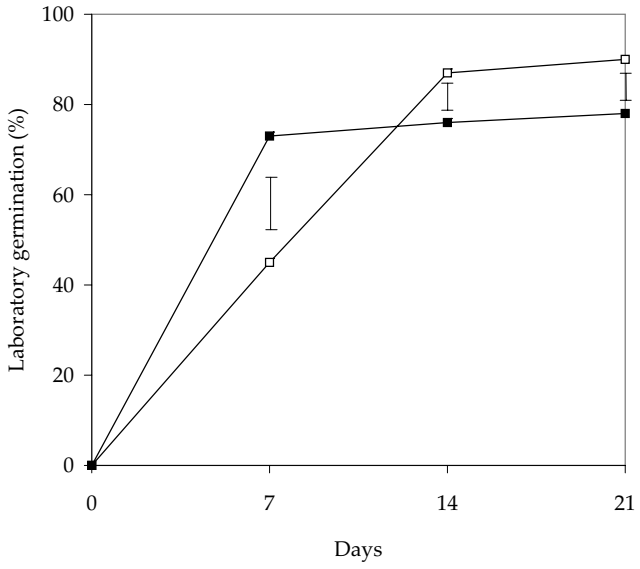


Figure 2 shows the maximum percentage germination of the same Corsican pine seedlot when it was incubated over a range of constant temperatures between 10 – 40 °C. The results are extremely interesting. Clearly, the significantly detrimental effect of prechilling to germination capacity that was observed in the ISTA double germination test at an alternating 20/30 °C is also reproduced at constant temperatures of 15 and 20 °C. But at constant temperatures of 10 and 35 °C prechilling was

actually significantly beneficial ($p \leq 0.025$). In other instances where the germination of unchilled and prechilled tree seeds have been compared over a range of constant temperatures, (Allen, 1960; Gosling, 1988; Jones and Gosling, 1994; Poulson, 1996; Corbineau, Bianco, Garello and Come, 2002) prechilling has usually benefited seed germination at several temperatures - and left it unaffected at the others; occasionally it has been shown to harm germination at several temperatures - or leave it unaffected at the others. This is the first time that prechilling has been demonstrated to bring about a beneficial effect at some temperatures and a detrimental effect at others on the same seedlot. It was therefore important to observe the nursery performance of this seedlot to see how it responded to pretreatment when broadcast sown in a traditional bare-root nursery system.

Fig. 2 Germination capacity of Corsican pine seeds incubated for 42 days at constant temperatures following 0 and 3 weeks moist prechill at 4°C (inset shows course of germination during 21day ISTA 'double' germination test) bars signify 97.5 % confidence limits (□ – 0w prechill; ■ – 3w prechill)

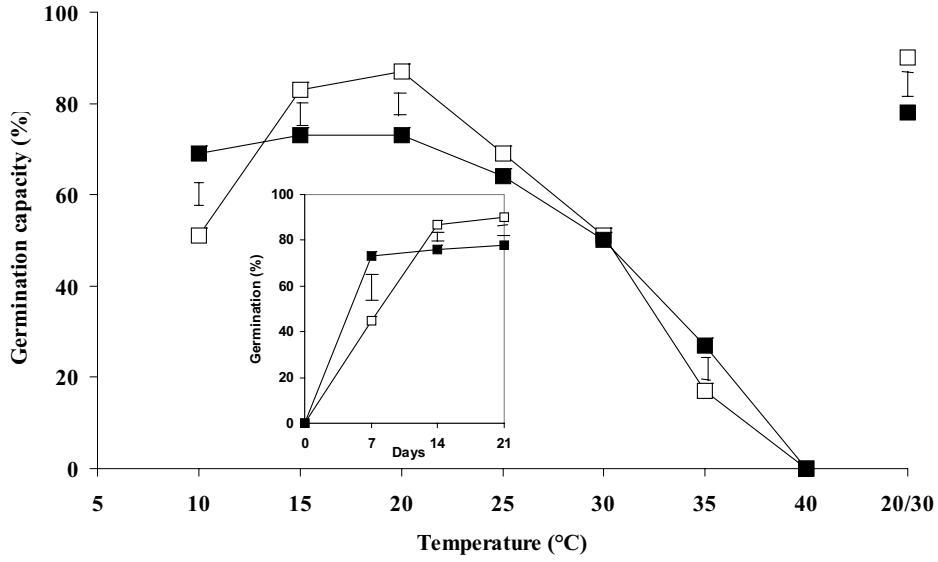


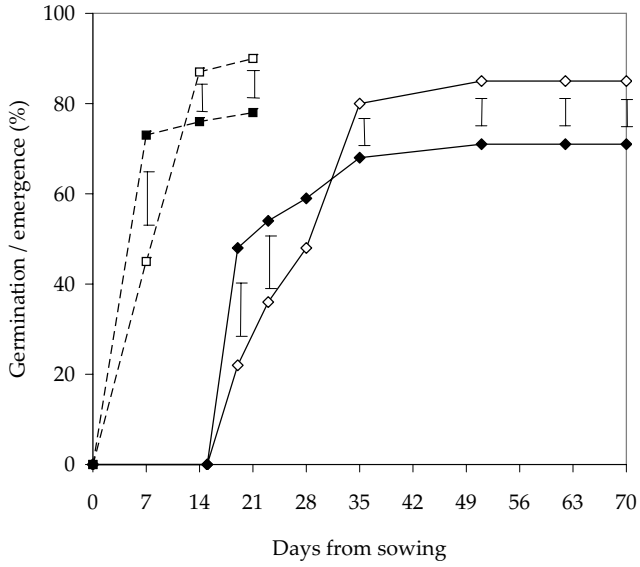
Figure 3 shows the course of emergence of Corsican pine in the nursery following 0 weeks (◇) and 3 weeks prechill (◆), (and for comparative purposes the ISTA laboratory germination results). The graph shows that the nursery emergence of both chilled and unchilled seeds was much slower than in the laboratory. Secondly, it is noticeable that the final nursery emergence percentages for chilled and unchilled seeds are only slightly lower than the laboratory equivalents. But the most important observation from this comparison is that the rather surprising combination of an early stimulation of prechilling to germination rate, coupled with the eventual harmful effect of prechilling on final emergence, was consistent between ISTA laboratory test and nursery.

But in the nursery it is not only seedling numbers that are important, seedling size is also a consideration. Table 3 shows several of the nursery performance parameters for this Corsican pine seedlot, and allows comparison with the ISTA laboratory germination results. Columns 5 and 6 present the end of season seedling heights and root collar diameters of unchilled and prechilled seeds. It is clear that although prechilling significantly reduced the end of season seedling numbers ($p \leq 0.01$) it significantly increased end of season seedling size ($p \leq 0.01$). In other words, sowing untreated seeds resulted in significantly more, but smaller seedlings, whereas prechilling led to significantly fewer, but bigger plants.

This paper concludes by considering whether there is any advice that can be generally offered to nursery managers about other seedlots exhibiting these rather unusual germination characteristics in an ISTA 'double' germination test. In particular, whether such seedlots should be pretreated before sowing or not?

The first point to make is that seedlots with the above response to prechilling in a 'double' germination test are very rare. A seedlot where prechilling significantly stimulates early germination, then significantly slows it down and ultimately yields significantly fewer normal germinants (ptr 10c) is unusual. It is therefore particularly noteworthy that this very rare laboratory response was consistent between the laboratory seed test and the nursery in all respects. The ISTA 'double' germination test accurately predicted three important nursery characteristics: i) the final, nursery seedling numbers for both unchilled and prechilled seeds; ii) the faster nursery emergence of prechilled seeds in comparison to unchilled; and iii) the lower final nursery emergence of prechilled seeds.

Fig. 3 Course of emergence of Corsican pine seeds in Headley nursery following 0 weeks (□) and 3 weeks prechill (■) and during ISTA 'double' germination test (□ – 0w prechill; ■ – 3w prechill)



The only doubt about whether these characteristics would be reproduced under all nursery conditions is generated by Figure 1. Although nursery temperatures were not monitored in this experiment, it is likely that in this season they were between 15 – 20 °C (where prechilling harmed germination capacity). But if nursery temperatures had been consistently 10 °C, or the seed had been sown in a polythene tunnel, glasshouse, or under cloches, then seedbed temperatures may have approached 35 °C and prechilling could have significantly benefited seedling numbers as well as seedling size.

Finally, it must also be acknowledged that it was not immediately obvious from the ISTA 'double' germination test that prechilled seeds would lead to 10 % bigger seedlings at the end of the growing season in the nursery (Table 3). There are three possible explanations. Either, the larger seedlings resulted from the earlier emergence of prechilled seeds and the consequently longer growing season; or they were a result of the 20 % lower seedling density permitting a faster growth rate; or a combination of the two. But whatever the cause it seems likely that other seedlots exhibiting ptr 10c would probably respond similarly in the nursery.

Table 3. Cumulative nursery emergence and seedling survival, height and root collar diameter

Treatment	ISTA germination capacity (%)	Cumulative emergence (%)	Survival (%)	Seedling height (mm)	Seedling root-collar diameter (mm)
Unchilled	90	85	84	52	2.06
Prechilled	78***	71***	67***	57**	2.29**

The overall advice about any seedlot exhibiting ptr 10c would therefore be:

- If seedling size is more important than seedling numbers – prechill the seedlot.
- If seedling numbers are more important than seedling size - do not prechill the seedlot.

This paper demonstrates that it is possible to compare, analyse, interpret and offer practical sowing advice even from very rare, and at first sight surprising results of a 'double' germination test. This seed testing information would be of significant benefit to nursery managers, who could consider it in relation to the specific nursery regime that will be used, likely seedbed conditions and whatever compromise is required between seedling size and seedling numbers.

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What is the relationship between a 'germination' test and a 'viability' test?

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Summary

This paper explores whether there are significant differences between germination and viability tests. It also discusses the concepts of germinability and viability.

A germination test is defined as a technique to measure the proportion of seeds that are capable of becoming normal seedlings (i.e. they are germinable). In contrast, there are a number of viability tests which permit seeds to be classified as alive (i.e. they are viable). Germination and viability tests can both be used to identify unproductive seeds (e.g. dead, empty and various other categories).

An important distinction between the two tests is that the former is a measure of actual performance (i.e. a seed which has germinated) whereas the latter is more a measure of likely potential performance. This means that although viable seeds may be capable of germinating into normal seedlings, they are not necessarily able to germinate and they have certainly not been demonstrated to have germinated. Hence a germination test does not measure the same property as a viability test and germinable and viable are not synonymous.

Seed testers must accept that most users of seed test results are under the false impression that germination and viability are synonymous - the customers of seed test results need greater education. It is also impractical to expect seed test users to avoid wanting to draw comparisons between the results of germination and viability test results. Therefore it is necessary for seed testers to define a relationship between the two properties. An acceptable proposal to seed testers, their customers, researchers and seed conservation establishments might be:

viable seeds (at the end of either a germination or viability test) ≈
% normal seedlings + % abnormal seedlings + % fresh (or fresh stained) seeds.

Key words: Seed, germination, viability, normal seedling, ungerminated, fresh, alive

Introduction

One of the primary aims of the International Seed Testing Association (ISTA) is to promote the development and use of accurate, reproducible and standardised seed sampling and testing procedures (Anon, 2004). The 'International Rules for Seed Testing' (Anon, 2004) plus numerous handbooks are published and regularly updated to fulfil this role. Unfortunately, even the latest edition of the ISTA Rules (Anon, 2004) does not make it clear whether 'germination' and 'viability' tests measure the same, similar or different seed properties.

The purposes of this paper are to explore these anomalies; stimulate discussion (or maybe even provoke argument!) on the subjects of 'germinability' and 'viability', and finally to propose a relationship between the two concepts.

Discussion

'Chapter 5: The germination test' (Anon, 2004) begins with ISTA Rule 5.1 and the statement:

"The object of the germination test (my underlining) is to determine the maximum germination potential of a seed lot."

ISTA Rule 5.2.2 defines this further and states:

"The percentage germination indicates … seeds which have produced seedlings classified as 'normal'".

In the context of this paper, it is therefore important to note firstly, that no other category except 'normal seedlings' contribute towards the 'percentage germination'; and secondly that ISTA Rule 5.6.5 goes on to state that:

"The viability of ungerminated seeds at the end of a germination test, may be determined by a tetrazolium test (my underlining)."

Together these rules make it perfectly clear that a germination test measures 'maximum germination potential' (Rule 5.1) which is the same as 'percentage germination' which is the same as the percentage of 'normal seedlings' (Rule 5.2.2). The rules also make it fairly clear that this property, (which we could call 'germinability'), is distinctly different to the property called 'viability' which may be determined by the tetrazolium test, but only for ungerminated seeds. By implication this means that seeds that have already produced either a 'normal-' or 'abnormal-' seedling cannot be 'viable'.

So, let us look at the object of the tetrazolium test. 'Chapter 6: The topographical tetrazolium test' begins with ISTA Rule 6.1 and the statement:

"The objects of biochemical tests are:

1. To make a quick estimate of the viability of seed samples in general and those showing dormancy in particular.
2. In the case of particular samples which at the end of a germination test reveal a high percentage of dormant seeds (5.6.5), to determine the viability of individual dormant seeds or the viability of a working sample."

Frankly, it is much less clear what this means. So I shall interpret it to mean the same as all my seed testing colleagues, i.e. that a 'viability' test can be used to determine the percentage of seeds that are 'viable', either i) in a working sample which has not undergone a germination test, i.e where all seeds are 'ungerminated'; or ii) from amongst the ungerminated seeds at the end of a germination test.

Unfortunately, both parts of this sentence also reinforce the notion created by ISTA Rule 5.6.5 that if a seed has already germinated it therefore cannot be 'viable'! However, all of these examples are in direct contradiction of Rule 6.5.2.A.4 which states that:

"Viable seeds relate to those that show the potential to produce normal seedlings".

To complete my confusion, ISTA Rule 5.6.5.A.3 states:

"If fresh seeds are to be reported at 5 % or more, it must be verified that they have the potential to produce a normal seedling. This may be done with a tetrazolium test, dissection, embryo excision or x-ray …"

Let me summarise what this means in practice. A seed analyst does a germination test to determine the percentage of seeds capable of producing a normal seedling (Rule 5.2.2). If there are ungerminated seeds at the end of the germination test and more than 5 % of them appear 'fresh', then a viability (e.g. tetrazolium) test must be used to determine whether the fresh seeds 'have the potential to produce a normal seedling' (Rule 5.6.5.A.3). This is a completely circular argument – if a seed has failed to germinate at the end of a germination test, it is necessary to do a tetrazolium test on it to 'prove' that it has the potential to germinate!

However, because Rules 5.6.5 and 6.1 state that only ungerminated seeds can ever be 'viable' they both imply that any seed that has already produced a normal seedling cannot be 'viable' – even more confusing.

There is obviously a degree of ambiguity about the use of the terms 'germination' and 'viability' in the current ISTA Rules. In my opinion, it is important for these to be resolved.

In reaching a solution, it is important for ISTA to not only take account of the immediate customers of their seed test results but also the wider seed research community and the increasing number of seed conservation establishments. In seed research and seed conservation it is almost universally accepted that a 'germination test' does not measure the same property as a 'viability test' and that 'germinable' and 'viable' are not synonymous (Copeland and McDonald, 1995; Gosling, 2004). A germination test (as the name implies) is understood to measure the proportion of seeds that have actually germinated. Whereas the plethora of viability tests, such as topographical tetrazolium, excised embryo, conductivity etc., all use completely different methods to determine whether seeds are alive (='viable') or not.

Both of these communities would acknowledge that the main limitation of the 'germination' test is that it can significantly underestimate 'viability'. There are several reasons for this. Firstly, because there may not be a suitable dormancy breakage pretreatment available for some species. Secondly, because optimum germination conditions are unknown. Thirdly, because some seeds that are alive at the outset of a germination test can die during its course.

These two communities would also acknowledge that the greatest mistake that can be made with viability test results is to use them as an unqualified estimate of the percentage of 'normal germinants'. Viability tests were never intended to measure this property, they measure the proportion of seeds which are alive. It is simply inconceivable to expect a 'viability' test to differentiate between living seeds that are 'dormant' or 'non-dormant'. It is equally inconceivable to expect a 'viability' test to reliably differentiate between seeds that will germinate into either 'normal seedlings', 'abnormal seedlings' or simply remain 'fresh' at the end of a germination test.

In contrast, many of the users of ISTA seed test results are under the false impression that 'germination' and 'viability' are synonymous – they therefore need re-education. But it is also vitally important for ISTA to recognise that the users of ISTA results must be able to compare 'germination' and 'viability' test results. A sui-table equation for ISTA to adopt would be:

viable seeds (at the end of either a germination or viability test) ≈
% normal seedlings + % abnormal seedlings + % fresh (or fresh stained) seeds.

Acknowledgements

I would like to acknowledge the many enjoyable discussions I have had with numerous colleagues over several decades on the subject of 'germinability' versus 'viability'. Any misconceptions I still have are entirely my own fault!

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Excised embryo tests of peach, apple and pear

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Abstract

The Excised Embryo Test (EET) method for determining the viability of dormant seed was developed over 50 years ago by Florence Flemion at the Boyce Thompson Institute for Plant Research, Inc, and by Claude Heit at the New York State Agricultural Experiment Station at Geneva, NY. Over 50 years of experience in laboratories has accumulated since. The EET method has proved especially valuable for testing seed quality in the fruit trees peach, apple and pear. This method is a viability test that overcomes dormancy in some species by the removal of embryo from the seed coverings that may restrict or inhibit embryo growth. The excised embryo may germinate, may remain firm and white or become green and photosynthetic, depending upon the depth of dormancy. Seed preparation, excision, and evaluation can usually be completed in 14 days or less.

The ISTA rules for the excised embryo method for a number of genera, including *Malus, Prunus,* and *Pyrus,* were adopted more than 10 years ago. The purpose of focusing on the excised embryo method at this workshop is to bring the benefits of this method to the attention of tree seed testers and to demonstrate the techniques of the method.

Introduction

The planting value of seed is determined by means of a viability test. With the germination test, the most widely used viability test, seed is tested using prescribed temperatures, substrates and test duration. The seed must actually germinate and produce specific functioning seedling parts. However, the Embryo Excision Test (EET) is an indirect method of determining viability. The test procedure involves moistening the seed, which aids in the removal of the embryo from the surrounding tissues as well as leaching out germination inhibitors if present. The seed embryos are removed from seed coverings and placed on a moist substrate for observation. The viable embryo may develop into a seedling or it may remain firm and white. One of more of the cotyledons may exhibit growth or greening. Non-viable embryos appear discolored and decay. The EET can be utilized on fresh or dormant lots and results can be obtained in a week or less, but the procedure is labor intensive and requires a certain level of skill. Peach, apple, and pear seed do not germinate immediately when planted, either in the field or in the laboratory. Dormancy in these species, which may last for several months, can be by-passed by removing the embryos from the surrounding tissues, hydrating and incubating the embryos.

Over 70 years of experience has accumulated in excised embryo seed viability testing. Florence Flemion found that excised embryos of peach *(Prunus persica)*, apple (*Malus* sp.) and hawthorn (*Crataegus* sp.) form dwarf seedlings that grow into normal seedlings. She incubated the embyos in peatmoss at 25 °C (Flemion, 1934). Tukey grew seedlings of peach, apple, pear (*Pyrus* sp.), plum

and cherries (*Prunus* spp.) from excised embryos in liquid, sand, and agar media. The agar with nutrients media became the standard (Tukey, 1935). He then tested peach samples from six states and Japan and found the results correlated with field emergence (Tukey, 1936). Flemion achieved agreement with the normal testing method with peach samples from five states and four crop years in moist peat moss at 25 °C. She found that agar with nutrients is not needed (Flemion, 1936). Later she tested a number of samples from nine species on wet filter paper at 21 to 23 °C. She included a table comparing the excised embryo results with normal germination and a very informative figure with embryo photographs of nine species (Flemion, 1938). Later she extended her work to include 24 additional species from 11 families, also with tables comparing excised embryo results with germination (Flemion, 1941).

Claude Heit and Carrie Nelson excised and grew a number of species, including peach and plum, on moist filter paper and cotton. The best results were in closed petri dishes at room temperature (Heit and Nelson, 1941). Tukey summarized ten years of data on peach, tightening and refining the methodology. Excised embryo results compared favorably with germination in very many lots (Tukey, 1944). Flemion then presented data for 92 samples of 58 species in 19 families, with tables showing the comparison of excised embryo tests with germination tests, and also listing the 62 species in 24 families tested by the excised embryo method in the extensive bibliography (Flemion, 1948). Heit wrote about the history and value of the excised embryo test, with a table listing the 89 species tested successfully and illustrations of embryos, including peach. He separated the peach embryos into four categories: strong seed with vigorous growth, good reliable seed with fair vigor, old seed of weak doubtful value, and dead seed (Heit, 1955). Heit explained benefits of the excised embryo test to nurserymen in a 1966 report (Heit, 1966). Lee Chao and David Walker reported that peach seed germinated after removal of the pericarp and seed coat. Seed coat extract inhibited germination (Chao and Walker, 1966).

Methods and materials

The methodology of embryo excision is reported in several manuals. Ellis, Hong, and Roberts detailed the procedures in the Handbook of Genebanks (Ellis et al., 1985). In addition there is the chapter on the excised embryo test in the International Seed Testing Association Tree and Shrub Handbook (Chirco and Waters, 1991).

Hydrating the seed before excision makes the seed more pliable and less susceptible to injury and lubricates the embryo. Nevertheless, some embryos may be damaged. Extra seed should be prepared to replace damaged embryos. Excision damage produced by the analyst can readily be distinguished from naturally occurring damage to the embryo. Slight excision marks can be overlooked, especially those associated with extensive cotyledon tissue.

Peach (*Prunus persica* Batsch.)

The peach (*Prunus persica* Batsch.) embryo is removed from the seed in two steps: first the hard endocarp (peach pit), then the seed coat and remnant of the nucellus. The epicotyl, hypocotyl, and radicle form a small embryo axis loosely attached to the cotyledons near one end of the seed. The two large cotyledons are part of the embryo.

Crack the endocarp open by inserting a knife blade in the suture and applying pressure or a blow from a hammer to force the two halves of the endocarp apart (photographs 1 and 2). Alternately, use a large vise to apply steadily increasing pressure to the seed placed sideways in a vise (suture edges against the vise jaws). Soaking the seed several hours or overnight reduces shattering of the endocarp and damage to the embryo.

Photo 1

Photo 2

Photo 3

Photo 4

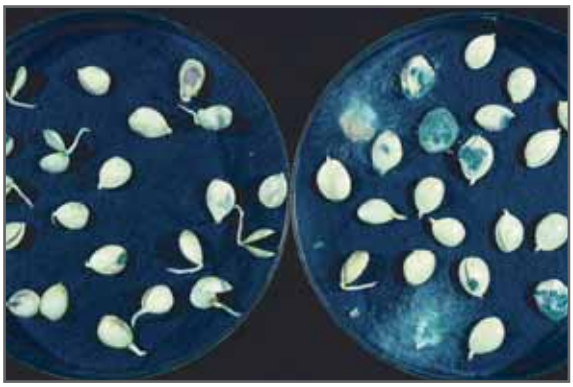

Photo 5

Photo 6

Photo 7

Photo 8

Scratch or cut a small opening in the seed coat to allow water to enter the seed and soak the seed in room temperature water for two or three days. Use water at least five times the volume of the seed and change the water once or twice a day. Remove the seed from the water and, while still wet, remove the seed coat with scapulas, or use friction between the thumb and seed to remove the seed coat (photograph 3). Hold the seed firmly with the left hand thumb and first finger and the first finger of the right hand, and push against the seed and outward with the right hand thumb. If the seed is hydrated, the seed coat will adhere to the thumb skin and slide off the embryo. Turn the seed over and repeat on the other side. Then remove the seed coat from the end of the seed by pinching the seed between the right thumb and first finger. Be careful to not separate the small embryo axis from the cotyledons.

Incubate the excised embryos 10 to 14 days on top of blotter or filter paper at 20 °C in a Petri dish or covered with a glass on a Jacobsen Apparatus, or in a wet germinator. Evaluate the embryos every second or third day beginning at four days. Radicles elongate or cotyledons grow or turn green on viable embryos. Embryos that are firm and white at the end of the test period are also viable. Non viable embryos turn off color and may also become soft or infected with fungi or bacteria (photographs 4 and 5).

Apples *(Malus)* and **pears** *(Pyrus)*

Soak the seed in room temperature water 12 to 18 hours, with water at least five times the volume of the seed. The seed coats produce a mucus layer when hydrated and become slippery. Tukey suggests washing the seed in calcium hypochlorite to remove this covering (Tukey, 1935; 1944). Drain the water and cut off and discard the distal one-quarter of the seed. Return the seed to water and soak an additional 12 to 18 hours at room temperature. Drain the water again and use forceps or dissecting needles to remove the embryo from the seed coat. For embryos that are difficult to remove, make a small cut on the seed coat perpendicular to the first cut at the top or bottom of the first cut. This will reduce the seed coat around the embryo and allow the embryo to slide out easier. Incubate and evaluate the same as for peaches (photograph 6)

Conclusions

The excised embryo test is well documented and correlates well with germination of after ripened peach, apple and pear seed. It is accepted as on official testing method by both of the major testing associations, AOSA and ISTA, and the International Genebanks program. In addition to the fruit species discussed here there is excision information for use on flowers, shrubs, trees, ornamental vines and vegetables species (Heit, 1955). This technique has also been used as a research tool for determining specific types of dormancy (seed coat growth restrictions, gas exchange, moisture absorption or the presence of inhibitory substances) (Stimart, 1981) and is receiving renewed attention in the field of propagating hard to germinate ornamental species (Wiegrefe, 1999). Seed analysts that test these species should be aware of and use this valuable test.

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Germination and dormancy breaking treatments in *Chamaerops humilis* (Dwarf Fan Palm) seeds

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Summary

Different treatments for promoting germination of dwarf palm seeds were investigated. Pretreatments applied were: seed soaking in distilled water with few drops of bleach, boiling water, hot water, soaking in sulphuric acid and manual scarification. Germination tests were performed under controlled conditions at 15/25 °C and a 16-h light photoperiod. Some seeds were stored at 15 °C for 80 days - when soaking in sulphuric acid resulted in the highest final germination percentage. However, other seeds were stored at 15 °C for 300 days during which time they lost their dormancy and no longer benefited from pretreatment at all.

Introduction

Dwarf fan palm (*Chamaerops humilis* L.) is the only wild European species of the Arecaceae family, with distribution in the Mediterranean rim. It resists low temperatures, drought and can grow on poor and rocky soils. Little is known about the germination behaviour of this species (Piotto, 2003). Therefore, the effect of various treatments on seed germination was studied.

Material and methods

Fruits were collected in September 2001 in the region of Murcia (SE Spain) and stored at 15 °C for approximately 30 days in open polythene bags. During that time epicarp and mesocarp were removed by means of a coffee-grinder. The seed surrounded by endocarp was treated as a unit (and hereafter termed as seed). After removal of epicarp and mesocarp, seeds were stored in inside plastic boxes at 15 °C for 80 days before the first trial, and 300 days before the second trial. In both trials and all treatments, four replicates of 25 seeds each were tested for germination in trays with vermiculite, and incubation took place at 15/25 °C (8-h dark/16-h light photoperiod). Final germination percentage (± SD) on the 130th day was calculated. The time necessary (in days) to reach 50 % of the final germination percentage (T_{50}) was calculated by linear interpolation from the two germination values closest to median germination.

Trial 1

Several seed pretreatments were applied to seeds stored at 15 °C for 80 days:

- Soaking in distilled water: seeds were soaked in distilled water for 3 days, or in water with few drops of commercial bleach for 1, 3 or 7 days, at 25 °C.
- Boiling water: seeds were immersed in boiling distilled water (100 °C) and then left to cool at room temperature for 24h.

- Hot water: seeds were immersed in distilled water heated to 80 °C for 15, 30 and 60 min.
- Sulphuric acid: seeds were immersed in H_2SO_4 (96 %) for 15 min, and then washed thoroughly with tap water.
- Manual scarification: endocarps were sanded with a metal file or broken with a clamp.

Trial 2

Several acid scarification treatments were studied in more detail after seeds had been stored 300 days at 15 °C.

- Sulphuric acid: seeds were immersed in H_2SO_4 (96 %) for 0, 5, 15, 30 and 60 min, and then washed thoroughly with tap water.

Results and conclusions

In the first group of trials, carried out with seeds that had been stored for 80 days at 15 °C, the best treatment for enhancing germination was soaking in sulphuric acid (68 % final germination) (Table 1). Soaking seeds in water, with drops of bleach, for 1 and 3 days also increased the final germination percentage (53.0 % and 53.8 %, respectively) with respect to the control seeds (34.0 %).

Table 1. Germination percentages of dwarf fan palm seeds (after 130 days of incubation at 15/25 °C) after different pretreatments. Seeds had been stored for 80 days at 15 °C before the experiment was performed.

Treatment		Germination (%) (mean ± SD)
Control (untreated seeds)		34.0 + 8.24
Soaking in water for 3 days		31.2 ± 11.86
Soaking in water with drops of bleach	1 day	53.0 ± 12.44
	3 days	53.8 ± 17.40
	7 days	27.5 ± 17.50
Soaking in sulphuric acid for 15 min		68.0 ± 17.20
Boiling water		25.0 ± 15.00
Soaking in water at 80 °C	15 min	10.0 ± 5.00
	30 min	10.0 ± 0.00
	60 min	2.5 ± 2.50
Sanded with metal file		12.5 ± 2.50
Broken with a clamp		5.0 ± 5.00

Table 2. Final germination percentages (after 120 days of incubation at 15/25 °C) and T_{50} values (days) of dwarf fan palm seeds after different scarification times with sulphuric acid. Seeds had been stored for 300 days at 15 °C before the experiment was performed.

Treatment	**Germination (%) (mean ± SD)**	**T_{50} (mean ± SD)**
Control (untreated seeds)	89 ± 3.31	16.1 ± 1.84
H_2SO_4 5 min	76 ± 4.89	17.6 ± 3.82
15 min	79 ± 5.19	16.8 ± 2.10
30 min	77 ± 8.66	15.3 ± 3.09
60 min	82 ± 3.46	12.8 ± 1.06

However, a dramatic removal of seed dormancy was observed after 300 days storage at 15 °C as a high germination percentage (ca. 90 %) was obtained without any scarification treatment (Table 2). In this second group of trials, the scarification of seed coat with sulphuric acid did not increase germination (Table 2). Besides, the T_{50} values of untreated and scarified seeds were very similar (Table 2).

Therefore, it is sufficient to store the seeds for 300 days at 15 °C to obtain a high germination percentage, without the need for any pretreatment. If it is necessary to germinate seeds shortly after collection, pretreatment with sulphuric acid should be performed.

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Poor seeding in Junipers may depend upon non-specific pollination mechanisms

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Abstract

In most Gymnosperms, pollen lands on the micropyle drop and is drawn into the ovule as the drop is reabsorbed. Pollen from different species as well as abiotic material (dust and inorganic particles) may also reach the micropyle drop. If protected from pollen and/or dust, the pollination drop persists on the micropyle for 10 – 15 days.

This paper demonstrates the effect of live pollen, dead pollen and different sized silica particles on the reabsorption process of *Juniperus oxycedrus* subsp. *oxycedrus*.

The average volume of unpollinated micropyle drops was considered the control treatment, and the drop volume after the following treatments was measured – addition of:

- viable pollen of the same species
- dead pollen of the same species
- silica gel of different sizes (10 – 15 μm, 40 – 63 μm and 63 – 200 μm)

Results suggest that the pollination mechanism in *Juniperus oxycedrus* subsp. *oxycedrus* is highly non-specific. The micropyle drop was drawn towards the inner part of the ovule even in presence of dead pollen and all but the largest sized inorganic particles - thus leading to failures in the ovule fertilization.

Such an inability to recognize suitable pollen represents a weak point in the pollination process especially if plants are exposed to pollution sources (particulate, powder, dust, etc). This vulnerability may explain poor seed production in *Juniperus.*

Introduction

Gymnosperms lack specialized receptive areas for pollen, which has more direct access to the ovule than in angiosperms. In most gymnosperms, pollen lands on the micropylar drop and is immediately drawn into the ovule as the drop is reabsorbed (Gelbart and von Aderkas, 2002) (Fig. 1A-C). The apical part of the nucellus of each ovule produces its own micropylar drop (Singh, 1978). The micropylar liquid is a dilute sugar solution, the composition of which has only been analysed in a limited number of species (Singh, 1978). If shielded from pollen, the drop may persist on the micropyle for several days.

Under natural conditions, pollen from various species as well as abiotic material (such as dust) may land on the micropylar drop. Sarvas (1962) noted that any airborne particles, including insect eggs, may be drawn into the ovule. Here we studied the effect of particles of different size on the pollination mechanism of *Juniperus oxycedrus* subsp. *oxycedrus*. The study is part of a research project (sponsored by APAT, the Italian environmental protection agency) on the reproductive efficiency of three *Juniperus* species growing in Italy.

Materials and methods

Branches with mature female cones were cut and placed in vases of water at room temperature. When micropylar drops appeared on the cones, pollination trials were conducted with:

- viable pollen of the same species;
- not-viable pollen of the same species killed by exposure to 120 °C for 1 hour;
- silica gel dust of different sizes (10 – 15 µm, 40 – 63 µm and 63 – 200 µm).

The diameter of the micropylar drops (assumed spherical) was measured under a stereo microscope before and 30 minutes after pollination and volumes were calculated. Eight to 15 drops were used for each treatment (Table 1). Volumes of unpollinated drops were taken as control. The data was analyzed by the Wilcoxon test for paired samples using the program Statistica for Windows (release 4.5).

Results

The mean volume of the micropylar drop was 8.8 x 10^{-2} mm^3. No significant variation was observed in not pollinated drops, while significant decrease in volume was observed when viable pollen, not-viable pollen, silica gel 10 – 15 µm and silica gel 40 – 63 µm were applied (Tab. 1 and Tab 2). In the case of viable and not-viable pollen the volume of the micropylar drop decreased to 0 (total reabsorption) while there is only a partial reabsorption in the case of silica gel 10 – 15 µm and 40 – 63 µm.

Table 1. Statistical significance of changes in volume in relation to treatment according to the Wilcoxon test

	n° of drops	**Mean volume mm^3 ***	**Z**	**P**
Not-pollinated	8	1.39 x 10^{-2}	N. S.	N. S.
Viable pollen	10	3.17 x 10^{-3}	2.803	< 0,05
Dead pollen	13	2.20 x 10^{-4}	3.059	< 0,05
Silica gel 10-15µm	12	7.75 x 10^{-2}	2.201	< 0,02
Silica gel 40-63µm	8	4.01 x 10^{-2}	2.201	< 0,02
Silica gel 63-200µm	15	9.62 x 10^{-3}	N. S.	N. S.

Z = statistics of the test; P = probability

* mean volume of micropylar drop after each treatment

Table 2. Summary of the effects of the different treatments on reabsorption of the micropylar drops

	viable pollen 19-22µm	**not-viable pollen 19-22µm**	**silica gel 10-15µm**	**silica gel 40-63 µm**	**silica gel 63-200µm**	**nothing added**
No reabsorption					x	x
Partial reabsorption			x	x		
Total reabsorption	x	x				

Fig. 1 A-C. A female cone of *Juniperus oxycedrus*. A. Before pollination. B. 10 min. after pollination. C. 20 min. after pollination

A

B

C

Discussion

The results of this study suggest that the pollination mechanism in *Juniperus oxycedrus* subsp. *oxycedrus* is highly non-specific: the micropylar drop is reabsorbed into the ovule even in response to not-viable pollen and inorganic particles of similar size to *Juniperus* pollen. Since deposition of not-viable pollen induced total reabsorption of the micropylar drop, it led to complete failure of fertilization. Since silica gel particles caused partial resorption of the drop, they decreased the probability of pollen capture. The combination of these two effects may explain the poor seed production observed in different populations of juniper plants (Oritz et al., 1998).

This lack of specific capacity to recognize its own pollen is a weak point in the pollination process of *Juniperus*, especially if the plants are exposed to sources of particulate, dust, etc. The ecological implications are more evident if we consider that many airborne inorganic particles arise from human activities, such as vehicles travelling on unpaved roads, and crushing or grinding operations (CEPA, 1998; APAT, 2002). The knowledge of the existence of this obstacle can, however, be helpful to overcome the problem itself.

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Seed germination of *Halimium* and *Helianthemum* species

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Summary

The aim of this study was to understand the seed germination characteristics of four *Halimium* species *(H. halimifolium, H. atriplicifolium, H. ocymoides* and *H. umbellatum)* and five *Helianthemum* species *(H. alme-riense, H. appeninum, H. cinereum, H. hirtum* and *H. squamatum)*, under different incubation temperatures and mechanical scarification for germination enhancement. Constant 15, 20 or 25 °C and alternating 15/25 °C temperature regimes and 16/8 hours light/dark photoperiod conditions were used. Germination values recorded were: final germination percentage and germination rate expressed as days to reach 50 % of the final germination percentage (T_{50}). Incubation temperatures had no significant effect on final germination percentage for untreated seeds of the five *Helianthemum* species. However, variation due to temperature was significant for scarified seeds, with the lowest germination percentage attained at 25 °C. For the four *Halimium* species, incubation temperatures had only a significant effect for untreated seeds of *H. halimifolium*. In all *Halimium* and *Helianthemum* species studied, the highest germination percentages were obtained by manual scarification of seeds. Therefore, seed coat dormancy is clearly present in *Halimium* and *Helianthemum* species. The high germination among most species studied following mechanical rupture of the seed coat shows that the mechanism of dormancy lies in the seed coat. The physical dormancy caused by impermeable hard seed-coats appears to be the main reason for the poor germination of untreated seeds of all *Halimium* and most *Helianthemum* species studied.

Introduction

The family Cistaceae consists of eight genera and about 200 species with a circum-Mediterranean distribution (Thanos et al., 1992). Five genera of this family *(Cistus, Fumana, Halimium, Helianthemum* and *Xolantha)* form an important part of the Mediterranean shrub (Talavera et al., 1997). All the species of the genus *Halimium* and almost half of the *Helianthemum* species are found in the Mediterranean rim (Thanos et al., 1992). In the Iberian Peninsula, the genus *Halimium* (Dunal) Spach (Nogueira et al., 1993) and *Helianthemum* Mill. (López González, 1993) are represented by six and 24 species, respectively. In the present work, four *Halimium* species [*H. halimifolium* (L.) Willk., *H. atriplicifolium* (Lam.) Spach, *H. ocymoides* (Lam.) Willk. and *H. umbellatum* (L.) Spach ssp. *viscosum* (Willk.) O. Bolňs & Viso] and five *Helianthemum* species [*H. almeriense* Pau, *H. appeninum* (L.) Mill., *H. cinereum* (Cav.) Pers., *H. hirtum* (L.) Mill., and *H. squamatum* (L.) Dum. Cours.] were tested for germination. Three of the nine species studied *(Halimium atriplicifolium, Helianthemum almeriense* and *Helianthemum squamatum)* are Iberian endemic shrubs.

Seed coat hardness and impermeability to water might be the most important causes of seed dormancy in several species of Cistaceae (Thanos et al., 1992). Therefore, the promotion of seed germination of different Cistaceae species can be obtained, in the laboratory, by mechanical scarification and thermal pretreatments (Thanos et al., 1992). Heat and scarification release these seeds from dormancy by breaking the impermeable seed coat allowing imbibition and germination

Table 1. Seed collection site and altitude, and ecological preference of *Halimium* and *Helianthemum* species studied. All seeds were collected in July 2002

Species	Collection site (Province)	Altitude (m)	Distribution and ecological preference
Halimium halimifolium	El Saler (Valencia, E Spain)	10	W Mediterranean shrub preferably on sandy soils
H. atriplicifolium	Arganda (Madrid, central Spain)	618	Endemic shrub of central and S of Spain
H. ocymoides	Badajoz (Badajoz, SW Spain)	249	Shrub on siliceous soils of W and central Iberian peninsula and N of Africa
H. umbellatum	Robledillo de Trujillo (Cáceres, SW Spain)	497	Central and S Iberian Peninsula and N of Africa, preferably on soil acids
Helianthemum almeriense	Sorbas (Almería, SE Spain)	409	Endemic gypsophyte shrub of arid SE Spain
H. appeninum	El Arenal (Ävila, central Spain)	1350	Mediterranean shrubby vegetation on rocky soils
H. cinereum	Albacete (Albacete, SE Spain)	650	Central and W Mediterranean basin, on calcareous soils
H. hirtum	Pinilla del Valle (Madrid, central, Spain)	1100	SW Europe and N Africa, preferably on calcareous or gypsum soils
H. squamatum	Rivas (Madrid, central Spain)	560	Iberian endemic shrub widely distributed on gypsum soils

to proceed. Mechanical scarification has proved beneficial in increasing the germination in hard seed coat species by making possible radicle emergence and water uptake by the embryo (Bewley and Black, 1994; Baskin and Baskin, 1998; Bhatt et al., 2000). Relatively few studies exist on the reproductive biology of *Halimium* (Peña et al., 1988; Talavera et al., 1997; Thanos et al., 1992) and *Helianthemum* species (Gutterman and Agami, 1987; Thanos et al., 1992; Martin et al., 1995; Pérez-García et al., 1995; Escudero et al., 1997, 1999, 2000; Mota et al., 2003).

In this work, some aspects of germination behaviour of four *Halimium* and five *Helianthemum* species have been studied under controlled laboratory conditions. The objectives of this research were to evaluate: (i) the effect of constant or alternating temperatures on germination of these species, and (ii) the effect of mechanical scarification to enhance seed germination.

Materials and methods

Plant material

In each species studied, ripe fruits (capsules) containing mature seeds were collected in the field in July, 2002. Table 1 shows the collection site, plus the altitude of the seeds of the species studied. The criterion followed to select the capsules was that they were completely ripe. The seeds from all selected capsules, likewise, showed a similar degree of ripeness, as it was indicated by their colour and hardness. For each species, germination trials were carried out with seeds originating from fruits randomly chosen from 20 different plants. Seeds were cleaned, kept in hermetic-sealed glass containers and stored in the laboratory at room temperature (approximately 23 °C) until the start of the trials. The seeds were stored for at least 6 months.

Seed germination tests

In all species studied, mechanical scarification of seed-coat was done in one group of seeds to enhance seed germination. In this pretreatment, the seeds were mechanically scarified by abrasion between two sheets of fine-grained sandpaper. In all trials, four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 ml distilled water) in 7-cm diameter glass Petri dishes. The Petri dishes were randomly placed in seed germinators set with a 16-h light/8-h dark photoperiod under either one of the constant temperature regimes (15, 20, 25 °C) or alternating temperatures of 25/15°C. For the latter, the lower temperature corresponded to the 8-h period of darkness. The light was provided by cool white fluorescent tubes with an irradiance of 35 $mmol \cdot m^{-2} \cdot s^{-1}$. Distilled water was added as needed to maintain constant moisture during the experiments, and germinating seeds were counted and removed every 2 days over a 60-day incubation period. The criterion for germination was emergence of the radicle through the seed coat. Final germination percentage on the day 60 was calculated. The time necessary (in days) to reach 50 % of the final germination percentage (T_{50}) was calculated by linear interpolation from the two germination values closest to median germination (Thanos and Doussi, 1995; Thanos et al., 1995). When the final germination percentage was equal to or less than 5 %, the T_{50} value was not calculated.

Statistical analysis

For each trial, the final germination percentages were calculated and arc-sine transformed values were subjected to analysis of variance using the computing package SPSS. One-way factorial ANOVA was performed with pretreatment or temperature as the main factors and the replicates as the error

term. A comparison of means among results from the different incubation temperatures was carried out through the least significant difference test (LSD) at the 5 % level of probability.

Results

Table 2 shows the effect of incubation temperature on the germination of intact and scarified seeds of the four *Halimium* species. Very low germination percentages of unscarified seeds (control) were achieved at the assayed temperatures, ranging from 1 to 18 %. Manual scarification of the seed coat resulted in a dramatic increase in the final germination percentage at all temperatures assayed. Variation attributable to incubation temperature was only significant for intact seeds of *H. halimifolium* and scarified seeds of *H. umbellatum*. The highest germination percentages of scarified seeds in these four species were reached at the two lowest incubation temperatures.

Table 2. Effect of different incubation temperatures on the germination of intact (control) and scarified seeds from four *Halimium* species. Final germination percentages (mean values + standard error) after 60 days of incubation. For each species, mean values in a row followed by the same letters are not significantly different at the 5 % level of probability as determined by the least significant test.

Species	Treatment	15 °C	20 °C	25 °C	15/25 °C
H. halimifolium	Intact seeds Scarified seeds[a] Significance level[b]	16 ± 2.0b 94 ± 1.0a ***	18 ± 3.0b 89 ± 1.7a ***	7 ± 2.6ab 77 ± 2.2a ***	4 ± 1.4a 87 ± 5.9a ***
H. atriplicifolium	Intact seeds Scarified seeds Significance level	3 ± 0.9a 64 ± 5.1a ***	3 ± 0.9a 68 ± 5.1a ***	5 ± 0.5a 53 ± 4.8a ***	5 ± 0.9a 56 ± 4.9a ***
H. ocymoides	Intact seeds Scarified seeds Significance level	4 ± 0.7a 78 ± 3.4a ***	3 ± 2.6a 77 ± 5.2a ***	2 ± 0.7a 61 ± 3.6a ***	3 ± 1.7a 69 ± 3.6a ***
H. umbellatum	Intact seeds Scarified seeds Significance level	6 ± 1.0a 68 ± 0.6b ***	4 ± 2.0a 63 ± 4.8b ***	1 ± 0.9a 48 ± 3.8a **	1 ± 0.9a 67 ± 1.2b ***

[a] Scarified seeds: manual scarification of seeds was achieved by abrasion between two pieces of sandpaper.

[b] Significance level: for each temperature, significance of intact vs. scarified differences

*** $P < 0.001$; ** $P < 0.01$

Table 3. Days (mean values + standar error) needed to reach 50 % of the final germination percentage (T_{50} values) of seeds from four *Halimium* species at four incubation temperatures. When germination percentage was equal to or less than 5 %, the T_{50} was no calculated (nc).

Species	Treatment	15 °C	20 °C	25 °C	15/25 °C
H. halimifolium	Intact seeds Scarified seeds[a]	15.1 ± 5.1 2.6 ± 0.5	4.2 ± 0.7 2.0 ± 0.0	15.0 ± 2.9 5.6 ± 1.4	nc 4.9 ± 0.3
H. atriplicifolium	Intact seeds Scarified seeds	nc 1.5 ± 0.0	nc 3.5 ± 1.7	nc 10.9 ± 1.5	nc 9.7 ± 1.8
H. ocymoides	Intact seeds Scarified seeds	nc 4.4 ± 0.5	nc 3.7 ± 0.6	nc 13.2 ± 1.4	nc 13.2 ± 1.4
H. umbellatum	Intact seeds Scarified seeds	10.0 ± 4.8 5.0 ± 0.0	nc 5.0 ± 0.0	nc 33.2 ± 7.0	nc 21.0 ± 1.7

[a] Scarified seeds: manual scarification of seeds was achieved by abrasion between two pieces of sandpaper.

In all *Halimium* species studied, the germination rate of scarified seeds was higher than that of intact seeds (Table 3). For scarified seeds of each species, the lowest T_{50} values were obtained at 15 °C and 20 °C (Table 3).

Table 4 shows the effect of different incubation temperatures on the germination of intact and scarified seeds of the five *Helianthemum* species. Final germination percentage of the untreated seeds (control) ranged from 11 to 64 % depending upon the species. Dormancy was rare in seeds of *H. appeninum* (germination was higher than 60 % at 15 °C and 20 °C in intact seeds). For each species, no significant differences in germination percentages of intact seeds were found among the different incubation temperatures assayed. However, incubation temperatures significantly affected final germination percentages of scarified seeds in most species. Thus, in the five *Helianthemum* species, the lowest germination percentages of abraded seeds were obtained at the highest constant temperature (25 °C). In general, mechanical scarification of the seed coat resulted in a significant increase in the germination percentage. Only in *H. appeninum* (at all three constant temperatures) and *H. hirtum* (at 25 °C) final germination was not significantly enhanced.

Table 4. Effect of different incubation temperatures on the germination of intact (control) and scarified seeds from five *Helianthemum* species. Final germination percentages (mean values + standard error) after 60 days of incubation. For each species, mean values in a row followed by the same letters are not significantly different at the 5 % level of probability as determined by the least significant test.

Species	Treatment	15 °C	20 °C	25 °C	15/25 °C
H. almeriense	Intact seeds	17 ± 3.0a	13 ± 1.7a	18 ± 4.1a	15 ± 1.1a
	Scarified seeds[a]	80 ± 1.7c	59 ± 2.6b	43 ± 5.9a	54 ± 3.6ab
	Significance level[b]	***	***	*	***
H. appeninum	Intact seeds	64 ± 2.4a	61 + 8.2a	43 ± 8.6a	51 ± 3.0a
	Scarified seeds	73 ± 3.6b	73 + 3.0b	46 ± 4.3a	76 ± 4.2b
	Significance level	ns	ns	ns	**
H. cinereum	Intact seeds	24 ± 1.0a	20 ± 3.2a	11 ± 0.9a	22 ± 4.6a
	Scarified seeds	87 ± 4.6b	94 ± 1.7b	66 ± 5.0a	86 ± 2.2b
	Significance level	***	***	***	***
H. hirtum	Intact seeds	41 ± 2.6a	44 ± 3.7a	38 ± 9.1a	42 ± 5.7a
	Scarified seeds	74 ± 2.2bc	67 ± 3.0ab	58 ± 5.9a	83 ± 2.6c
	Significance level	***	**	ns	**
H. squamatum	Intact seeds	14 ± 4.1a	11 ± 0.9a	14 ± 1.1a	12 ± 3.5a
	Scarified seeds	85 ± 2.6a	88 ± 4.2a	80 ± 2.3a	81 ± 5.9a
	Significance level	***	***	***	**

[a] Scarified seeds: mechanical scarification of seeds was achieved by abrasion between two pieces of sandpaper.

[b] Significance level: for each temperature, significance of intact vs. scarified differences

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant

In most cases, the germination rate of scarified seeds was higher than that of intact seeds (Table 5). In both intact and scarified seeds of all five *Helianthemum* species, the highest T_{50} values were recorded at 25 °C (Table 5).

Table 5. Days (mean values + standard error) needed to reach 50 % of the final germination percentage (T_{50} values) of seeds from five *Helianthemum* species for four incubation temperatures

Species	Treatment	15 °C	20 °C	25 °C	15/25 °C
H. almeriense	Intact seeds	2.2 ± 0.6	3.0 ± 0.7	31.5 ± 4.6	1.9 ± 0.3
	Scarified seeds[a]	3.0 ± 0.7	1.9 ± 0.3	25.4 ± 4.6	1.9 ± 0.3
H. appeninum	Intact seeds	2.0 ± 0.0	5.5 ± 0.0	38.5 ± 1.6	2.9 ± 0.7
	Scarified seeds	2.0 ± 0.0	2.9 ± 0.7	31.2 ± 1.9	2.5 ± 0.4
H. cinereum	Intact seeds	8.7 ± 0.9	8.7 ± 3.0	11.7 ± 3.3	8.6 ± 1.6
	Scarified seeds	2.5 ± 0.0	1.6 ± 0.4	5.0 ± 0.0	3.9 ± 1.2
H. hirtum	Intact seeds	2.0 ± 0.0	5.1 ± 0.3	23.2 ± 10.0	2.0 ± 0.0
	Scarified seeds	2.0 ± 0.0	3.4 ± 0.7	22.2 ± 3.8	2.5 ± 0.4
H. squamatum	Intact seeds	8.2 ± 1.9	6.0 ± 0.0	9.7 ± 3.3	4.0 ± 0.3
	Scarified seeds	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0

[a] Scarified seeds: mechanical scarification of seeds was achieved by abrasion between two pieces of sandpaper

Discussion

All *Halimium* species and most of the *Helianthemum* species studied showed the typical adaptative germination strategies of Mediterranean plants (Keeley, 1987; Bell et al., 1993; Baskin and Baskin, 1998).

The germination behaviour of the four *Halimium* species studied is very similar. Thus, the untreated seeds of these species reached low final germination percentages at all the tested incubation temperatures. Manual scarification resulted in a dramatic enhancement of germinability under controlled conditions. Earlier studies (Peńa et al. 1988) indicated that untreated seeds of *H. halimifolium* showed very poor germination (less than 10 %) and the manual scarification of these seeds at relatively low temperatures (10 °C and 20 °C) enhanced their germination. These authors found that the highest germination percentage followed mechanical scarification (about 65 %), but this was lower than the best germination obtained in our study (94 %) for this species. Peńa et al. (1988) concluded that *H. halimifolium* shows a physical dormancy, located in the seed coat. This conclusion can be confirmed by the results showed in this research for this species and can be also extended for *H. ocymoides, H. atriplicifolium* and *H. umbellatum*. Therefore, the physical dormancy caused by hard seed coat appears to be the main reason for poor germination of the four *Halimium* species studied.

Studies have shown that scarified seeds of most *Helianthemum* species attain their highest germination percentage at relatively low constant temperatures (15 °C and 20 °C) or alternating temperatures (15/25 °C). This behaviour is a typical strategy of Mediterranean plants with optimal temperatures between 15°C and 20 °C (Vuillemin and Bulard, 1981; Corral et al., 1990; Thanos et al., 1992; Bells and Bellairs, 1992; Pérez-García et al., 1995; Thanos and Doussi, 1995; Thanos et al., 1995; Escudero et al., 1997; Pérez-García and Escudero, 1997). For these plants, soil moisture conditions would be conductive for germination in the early spring, when winter plus spring rainfall would meet the moisture requirements. However, scarified seeds of *H. squamatum*, a genuine gypsophyte, germinated at higher constant temperature (25 °C), which might reflect a higher water retention in gypsum soils during the early summer, as postulated by several authors (Meyer, 1986; Escudero et al., 1997; Escudero et al., 1999). Field data suggested a multifocus strategy for germination of *H. squamatum* (Escudero et al., 1999) based on diversification of germination behaviour (Kigel, 1995). Thus, although under natural conditions most seeds will germinate at low temperature (winter), a reduced percentage of seeds might be able to germinate with late spring rains (Escudero et al., 1999).

In previous experimental studies, seed dormancy of *Helianthemum* species has been broken by hot water, sulphuric acid and dry-heat treatments in *H. polygonoides* (Pérez-García et al., 1995), and by scarification in *H. squamatum* (Pérez-García et al., 1995; Escudero et al., 1997), *H. vesicarium* and *H. ventosum* (Gutterman and Agami, 1987). The seeds of all *Helianthemum* species studied (except *H. appeninum*) showed physical dormancy that can be broken by manual scarification at most of the incubation temperatures tested. Mechanical scarification improves seed germination by making the seed coat more permeable to water, allowing the increase of seed imbibition. Therefore, under natural conditions, only a small percentage of seeds can germinate without breaking dormancy. In these species, several factors (e.g. diurnal fluctuations in temperature, rainfall wash, wetting and drying cycles, mechanical abrasion by soil particles, microbial action) can alter seed coats in a degree similar to manual scarification (Quinlivan, 1968; Taylor, 1981; van Staden et al., 1994; Baskin and Baskin, 1998).

Final germination percentages reached by untreated seeds of *H. appeninum*, both at 15 °C and 20 °C, were higher that those obtained by Martin et al. (1995) at the same temperature ranges. This might be explained by interpopulation variation present in this species.

T_{50} values of intact and scarified seeds indicate the germination rate at different temperatures. All *Helianthemum* species germinated relatively fast at constant 15 °C, 20 °C and alternating (15/25 °C) temperatures, reaching 50 % germination within a 9 day period, while some seeds germinated as early as the second day. However, for most species studied, the germination rate decreased drastically at 25 °C. In previous studies (Pérez-García et al., 1995; Escudero et al., 1997) we noted that seed germination of several Iberian species was more rapid at low temperatures (around 15 °C) and was slow or ceases above 20 °C. Only in the case of *H. squamatum* seeds, the germination rate at 25 °C was similar to the results obtained at the other three temperatures. In contrast, slow germination has been reported for other Cistaceae species (Corral et al., 1990; Thanos et al., 1992; Pérez-García and Escudero, 1997). Thus, it seems evident that both strategies, slow and fast germinators, can be found within Cistaceae family.

In conclusion, mechanical scarification drastically improved germination in all species studied, suggesting that dormancy in these seeds might be due to the hardness and impermeability of the seed coat. The increase was significant in all *Halimium* and *Helianthemum* species, except in *Helianthemum appeninum*, which had a high seed germination (about 60 %) before the treatment. In *Halimium umbellatum* and in most *Helianthemum* species *(H. almeriense, H. appeninum, H. cinereum, H. hirtum)*, the effect of manual scarification depended on the incubation temperature. However, both in *Helianthemum squamatum* and in most *Halimium* species *(H. halimifolium, H. atriplicifolium, H. ocymoides)* the enhanced germination after scarification was significant irrespectively of the incubation temperature.

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Testing forest tree seed in Sweden

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Summary

In Sweden there is no official seed testing station that issues certificates for forest tree seed. Instead there are two laboratories (one in Säver in northern Sweden, the other in Lagan southern Sweden), which specialise in testing forest tree seed and test tree seed-lot throughout Sweden. The laboratories principally adhere to the Rules of the International Seed Testing Association (ISTA), but there are a few minor modifications. For instance, an additional interim germination result is noted for *Picea abies* at day 10. This gives a more accurate indication of what happens in the growing conditions of a greenhouse. After completion of the laboratory germination test, the remaining ungerminated seeds are examined to identify the reason for non-germination. This knowledge is used to apply different cleaning procedures and other treatments, such as Imbibition-Drying-Separation (IDS), to the seed lot in order to improve the germination capacity and rate. Furthermore, seed is tested for germination at different temperatures on a thermogradient table. The results are very important for the nursery, since sensitivity to low and/or high temperatures can be crucial for a nurseries overall economy.

Demand for high quality seeds

In Sweden 75 – 80 % of all seedlings are produced as container-seedlings. Thus the nurseries have very high demands on seed quality, both genetic and physical quality. Normally only one seed is sown in each container.

The genetic quality, high yield and good climatic adaptation, is very important for the total yield of forest production, since export of forest products is an important part of the Swedish GNP. Seed is produced in seed orchards all over the country for the different climatic zones, from the mildest maritime areas along the southern shores of the Baltic Sea up to almost arctic conditions on latitude 68° N. Beside the climatic adaptation, the seed orchards also produce seed with higher technical quality than normal stand seed. One property is that seed orchard seed often has a higher 1,000-grain-weight.

The intensive methods for producing container-seedlings not only require high quality seed (minimum germination capacity 97 %), but also sowing equipment with high precision and a controlled environment during germination in the greenhouse (Lestander, 1984). The goals are to avoid both empty containers, and containers with more than one emerging seedling. Empty containers are clearly uneconomic. While, containers with more than one seedling have to be thinned - which is very costly (Petré and Nyström, 1982); and result in lower seedling quality (Hultén and Bohlin, 1974). Nursery managers demand seed with a high germination capacity, fast germination rate and clean seed without any inert particles. Some sowing machines also require seed that is fractioned to size- and/or weight classes, in order to give a good sowing result. As an indication of the importance of high quality seed, every percent of empty containers in the Swedish nurseries means a loss of approximately 1 MSEK or 120 000 US$.

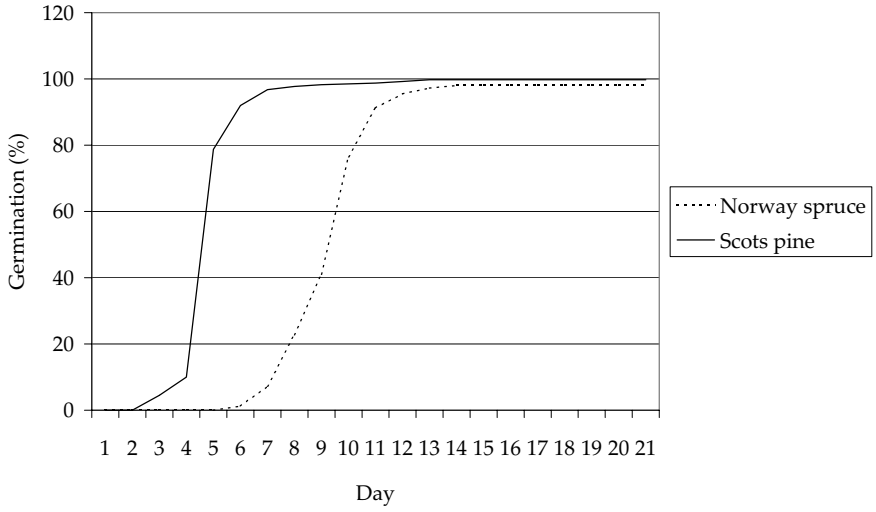
Seed testing aims to quantify different properties, such as germination percentage, purity, moisture content etc. The interesting properties, and which test methods that should be used, depends on for which purpose the test is made. For commercial seed it is important to have a measure of the quality in order to be able put the right price on the seed lot, and to decide if it is possible to buy or sell the seed. Secondly, in order to make the right decision about a cone harvest, it is important to know the expected quality of the seed. Furthermore, different tests are needed to monitor and control the extraction process of the seed, i.e. drying, dewinging, etc., which can have implications for the end result. Tests are also needed for retesting stored seed, which can be up to 40 years old – especially from northern Sweden where weather conditions lead to infrequent crops. Another reason for seed testing is to make an analysis and diagnosis of the seed lots status, in order to be able to decide which kind of seed conditioning is needed to raise the physical quality (Simak, 1982; Sahlén and Henriksson, 1985).

Laboratories in Sweden

Two laboratories in Sweden specialise in testing forest tree seed, Skogforsk laboratory in Säver in northern Sweden, and our company Svenska Skogsplantor AB in Lagan in southern Sweden. Both laboratories test seed according to the ISTA rules (ISTA, 2004). There is one exception from the rules, and that is that the germination is registered also after 10 days for *Picea abies*, Norway spruce. In Figure 1, the difference in emergence between the two main species in Sweden, *Pinus sylvestris* (Scots pine) and *Picea abies* (Norway spruce) can be seen. It can be noted that Scots pine is more rapid in germination than Norway spruce, thus the ratio "germination rate at 7 days/21 days" does not say so much about the quality of Norway spruce seed. For the nurseries the information of the ratio "10 days/21 days" for Norway spruce is far more relevant.

One has to remember that the measure of the germination at day 7 and 10 respectively could be difficult, since the slope of the accumulated curve is very steep. Even small changes in the germination environment can result in displacement of the germination curve, which affects the ratio "7d/21d" or "10d/21d" to a great extent. It could be so sensitive that the counting of germinants should be done at the same hour of the day as the germination test started. For the above mentioned reasons it is difficult to compare results of the ratio, and tests should, if comparison is necessary, therefore be made at the same laboratory under the same germination conditions (Sahlén and Henriksson, 1985).

Fig. 1 Accumulated germination of *Pinus sylvestris* and *Picea abies*. Note the difference in emergence after 7 days between spruce and pine



Germination test as a tool for further treatment of a seed lot

All non-germinating seed are cut and examined for the reason of non-germination after the ordinary germination test. The seed is classified in three classes:

- empty seed
- dead filled seed
- fresh filled seed, but not germinated

Depending on the reason for non-germination different treatments can by applied to the seed lot, in order to raise the germination percent or the vigour.

The amount of empty seed in a seed lot should be very low, 0 – 2 %. Otherwise the lot has been badly cleaned. For our customers we do not accept any empty seed at all. So if a seed lot contains any empty seeds, it will be sent back to the seed station for additional cleaning. Removal of empty seeds is done in a gravity separator. The same procedure is used if the seed lot has a purity less than 99.9 % (the goal is 100 %).

Dead filled seeds are removed by use of the IDS method, invented by Simak (1981). The method is based on the principle that after a seed-lot has been incubated (I) in water, and partially dried (D), live seeds lose their absorbed water more slowly than dead seeds. This causes a differentiation in density between viable and dead seeds during a certain period of drying at which the two types can be separated (S), e.g. by floating in water.

The third case is usual in older seed lots. The speed of germination drops more quickly over time than the germination capacity does. So even if the seed is viable, the vigour of the seed is low. The vigour can be improved in different ways and with use of different methods, i.e. water soaking, water incubation, PEG-treatment and cold-wet treatment. The most promising results have been obtained with water incubation, which also can be used as the I-step in the IDS method, resulting in a considerable improvement of vigour (Bergsten and Simak, 1985).

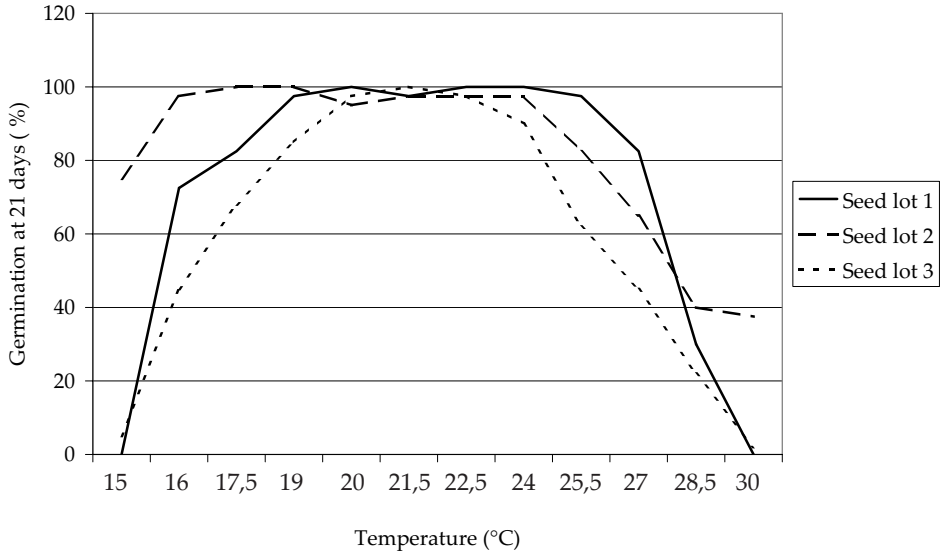
Thermogradient test

Seeds are also tested for their sensitivity to different temperatures during the germination phase. This information is very important for the nursery. Sowing in the container nursery is done three times during a year, in mid March, at the end of May and in the beginning of July. The same seed lot can be sown at all three times, so that the seed should be as adaptable to different germination temperatures as possible. In March it can be a problem to keep up the temperature during cold nights, and in May it is not unusual that we have summer conditions in southern Sweden. Adaptability is especially important for the last sowing in July, when the temperature in the greenhouse can be more or less out of control during a hot summer day.

Therefore we started with thermogradient tests some 20 years ago, and today it is a standard test for all seed lots used in container nurseries. The test is performed by studying germination at temperatures ranging from 15 °C to 30 °C. In Figure 2, three different seed lots have been tested.

Seed lot 1, has a good adaptation to temperatures between 19 - 26 °C, but outside this range, the germination capacity drops dramatically. Seed lot 2 is not so sensitive to extreme temperatures, it can tolerate temperatures down to 16 °C without losing any germination, and the drop at high temperatures is not so big as for seed lot 1. Seed lot 3 exhibits a seed lot with a very narrow adaptability to different temperatures. This seed lot has a good germination only between 20 – 23 °C. Using such a seed lot in a nursery can cause great economic losses, if it is impossible to keep the exact temperature in the greenhouse during the first weeks after sowing.

Fig. 2 Thermogradient test



During the years we have learnt that there is a big difference in sensitivity for varying temperatures not only between provenances but also within provenances between different years of ripening.

Conclusions

Seed testing in Sweden is closely related to the customers, the nurseries. Co-operation between researchers, testing stations and practical nursery management has been very important for the development of different testing procedures, and the application of the results from ordinary tests according to the ISTA rules. R&D activities are still needed to improve the interpretation of the results and to implement them into new cleaning and/or conditioning methods so that the quality of seed can be increased even more. Since empty containers and every percentage increase in germination is the equivalent of a lot of money, it is imperative to continue this work.

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Tree seed testing in the Czech Republic

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Abstract

In the Czech Republic there is only one official seed-testing laboratory that issues national and international (ISTA) certificates for forest tree seed. This laboratory is located at the Forestry and Game Management Research Institute at the Research Station Uherské Hradiště and is authorised by the Ministry of Agriculture to provide testing of forest tree seed within the country and for export. The seed quality is tested according to the Czech technical standard (ČSN 48 1211) that is (with some small exceptions) harmonised with the ISTA Rules. This paper gives a brief review of quality testing of forest tree and shrub seeds in the Czech Republic.

Accreditation of the laboratory

The quality of forest tree seeds in the Czech Republic has been officially tested in a nationally (L 1175) and internationally (CZDL02) accredited laboratory of the Forestry and Game Management Research Institute located at the Research Station Uherské Hradiště. The laboratory started its activity in the early 1950´s and first test records date back to 1952. Since 1997 the Czech Accreditation Institute has accredited the laboratory to carry out quality tests on forest tree and shrub seeds and to issue national certificates. In 1968 the laboratory became a member of the International Seed Testing Association (ISTA) and since 1998 the laboratory has been re-accredited and re-authorised by ISTA to issue international certificates. The accreditation area covers sampling and testing of purity, germination, (tetrazolium) viability, moisture content and 1,000 seed weight of 135 tree and shrub species.

At present the laboratory carries out testing on the basis of the Certificate of Accreditation accorded to it by the Accreditation Body of the Czech Republic in accordance with the CSN EN ISO/IEC 17025 and by the ISTA rules in accordance with the ISTA Accreditation Standard.

Since 1997 seed quality for national trade is tested according to the Czech technical standard ČSN 48 1211 that is consistent (with a few exceptions) with the ISTA Rules.

National testing

The testing of forest tree seed quality in the Czech Republic has not been obligatory. However, seeds processed and stored at a state-owned Tree Seed Centre at Týniště nad Orlicí have been tested every year on a regular basis. Purity and 1,000 seeds weight of these seed lots are determined only once at seed lot harvest and are not repeated at re-testing of stored seeds. Moisture content (at 103 ± 2 °C) has been determined using the Brabender apparatus that provides results within an hour. Most

Table 1 . List of genera (species) and number of seed samples tested from 1994 to 2003

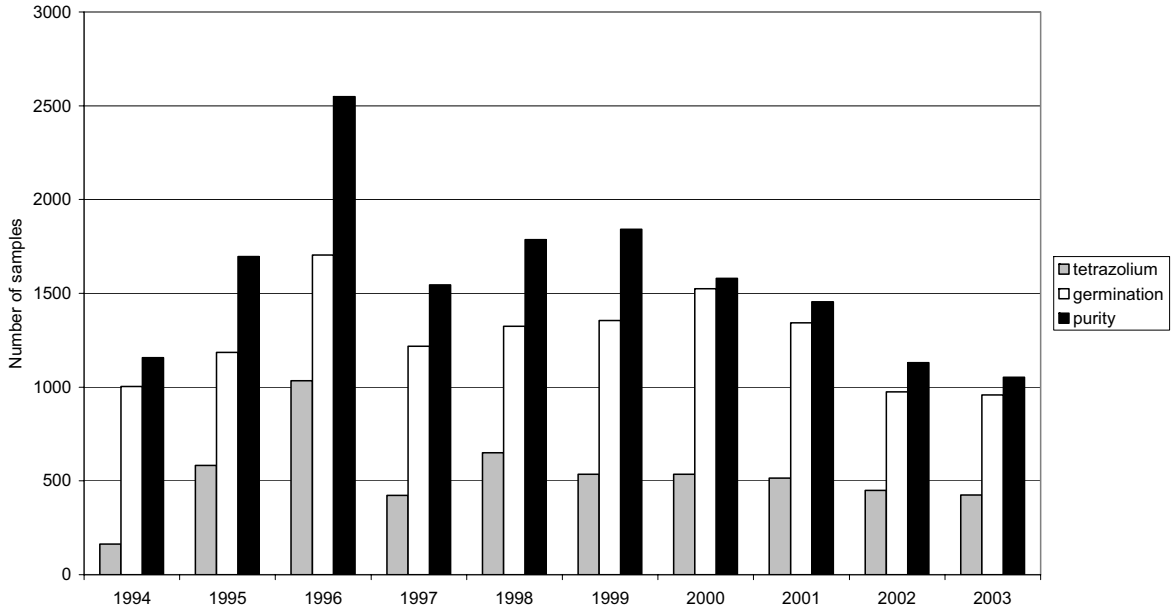
Genus (species)	**Number of samples**
Abies (alba, amabilis, balsamea, cephalonica, concolor, grandis, magnifica, nordmanniana, pinsapo, procera, veitchii)	*1,470*
Acer (campestre, platanoides, pseudoplatanus)	*120*
Alnus (glutinosa, incana, viridis)	*52*
Betula (pendula, pubescens)	*252*
Carpinus betulus	*38*
Castanea sativa	*1*
Catalpa bignoiodes.	*7*
Cedrus (atlantica, deodara , libani)	*20*
Chamaecyparis lawsoniana	*5*
Cornus (mas, sanguinea)	*25*
Corylus collurna	*14*
Crataegus monogyna	*24*
Euonymus europaeus	*32*
Fagus sylvatica	*2,816*
Fraxinus excelsior	*95*
Ginkgo biloba	*15*
Larix (decidua, kaempferi)	*1,816*
Ligustrum vulgare	*24*
Liriodendron tulipifera	*6*
Malus sylvestris	*9*
Morus spp.	*1*
Picea (abies, engelmannii, mariana, orientalis, pungens, sitchensis)	*5,634*
Pinus (albicaulis, aristata, cembra, conctorta, heldreichii, jeffreyi, mugo, nigra, parviflora, peuce, ponderosa, pumila, rigida, strobus, sylvestris, wallichiana)	*2,325*
Platanus spp.	*5*
Prunus (avium, mahaleb, padus, serodina, spinosa)	*43*
Pseudotsuga menziesii	*260*
Pyrus spp.	*28*
Quercus (petraea, robur, rubra)	*157*
Robinia pseudoacacia	*25*
Rosa (canina, rugosa)	*23*
Sequoiadendron giganteum	*8*
Sophora japonica	*9*
Sorbus (aria, aucuparia, intermedia)	*418*
Syringa spp.	*0*
Taxodium distichum	*10*
Taxus baccata	*17*
Thuja (plicata, occidentalis, orientalis)	*25*
Tilia (cordata, platyphyllos, tomentosa)	*190*
Tsuga canadensis	*9*
Ulmus (glabra, minor)	*5*
Viburnum (lantana, opulus)	*43*
Wisteria chinensis	*11*
Total: 95 species	**16,087**

germination tests are performed either on Jacobsen germination tables or in plastic boxes (used for accelerated ageing tests) in germination cabinets at 30/20 °C (8hrs light/16 hrs dark).

In the last 10 years (1994 – 2003) about 16,000 seed samples were tested, i.e. 95 species were tested annually (Table 1). The average annual number of samples is about 1,500, but this has gradually gone down (Figure 1). The most frequent species tested are Norway spruce (*Picea abies* (L.) H. Karsten), European beech (*Fagus sylvatica* L.), Scots pine (*Pinus sylvestris* L.), Europen larch (*Larix decidua* Mill.) and white fir (*Abies alba* Mill.) (Figure 2, 2a). A decline in spruce and an increase in beech samples is attributable to several factors, e.g. by the increase in natural regeneration, an ongoing change of species composition in Czech forests with the goal of increasing broadleaved species, namely beech, by beech masts nearly every year from 1992, and poor seed crops of larch, pine and spruce in the last 10 years. Scots pine seeds have been characterised by the highest energy of germination and germination capacity of pure filled seeds compared to Norway spruce seeds with a low germination in 7 days and mean germination of about 80 % (Figures 3 and 4). Filled, larch seeds have a high germination as well, but on average the seed lots contain about 65 % empty seeds. A similar situation exists for white fir seeds with mean viability of filled seeds being 76 %, while viability of pure seeds is only 37 % with about 45 % of empty seeds in a seed lot (Figure 5). In contrast to the ISTA Rules, the seeds with a radicle as long as the seed are considered to be normal seedlings as we assumed that in seeds with epigeal germination it is possible to recognise abnormal seedlings at this early stage.

In 1998, germination tests on beechnuts were introduced because results of viability (tetrazolium) tests had not given sufficient information on beechnut quality and dormancy as requested by clients. The beechnuts, mixed into a peat and sand substrate, are incubated at 3 °C and germinating seed with a protruded radicle are counted each week until germination is over. Thus, we have extensive data on viability and germination of beechnut seed lots of different crops before seed processing, fresh seeds (after processing) and seeds stored for different periods. The germination data are important since they provide feedback to laboratory staff to help them improve the evaluation of beechnut viability and to get a better relationship between viability and germination results. Also, the laboratory can provide a better advisory service concerning the determination of pre-chilling requirements of different beechnut seed lots of for breaking dormancy (Figure 6).

Fig. 1 Number of seed samples tested for purity, germination and viability tests from 1994 to 2003.



ISTA certificates

In European forestry there has not been high a demand for introduced tree species and foreign seed provenances and that accounts for the low international trade in forest tree and shrub seeds. In the Czech Republic the number of the ISTA certificates issued has declined in the last 10 years from 34 Orange Certificates to six Blue Certificates (Table 2).

Health tests

Health testing (non-accredited test) of tree seeds has been done occasionally at the request of customers. Such tests have focused on imported conifer seeds, followed by beechnuts and recently on acorns (*Quercus robur* L. and *Quercus petraea* (Mattusch.) Liebl. seeds) infected with the serious pathogen *Ciboria batschiana* (Zopf.) Rehm. This pathogen can seriously damage and even destroy stored acorns thus only pathogen free seeds should be stored. Since 1992 there have been facilities and equipment for processing and long-term storage of broadleaved seeds (mainly beechnuts and acorns) at the Tree Seed Centre. The equipment also includes a thermotherapy apparatus that provides for efficient treatment and eradication of the pathogen from infected acorns. The laboratory has been asked to determine *Ciboria batschiana* infection in different seed lots before and after thermotherapy. Pathogen determination has been done using a wet chamber method.

Other tests

The laboratory also carries out the evaluation of seed ripeness and embryo development before cone collection of white fir, Norway spruce and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Based on test results the time of seed collection has been recommended. Excised embryo tests are done only occasionally and in the most cases this test has been used to confirm the tetrazolium viability results.

Fig. 2 Number of seed samples tested for purity, germination and viability test from 1994 to 2003

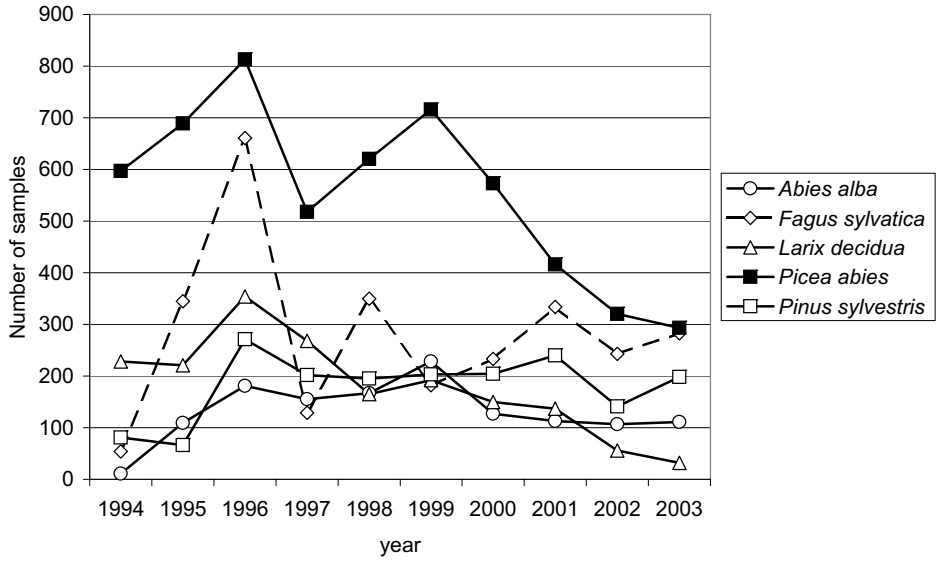


Fig. 2a Proportion of seed samples of the most frequents species tested from 1994 to 2003

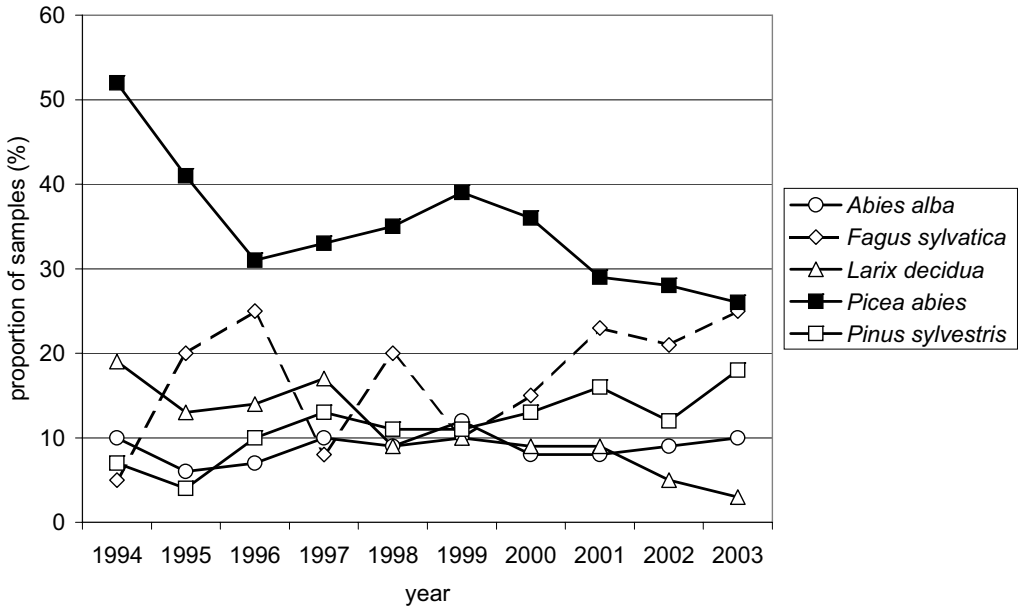
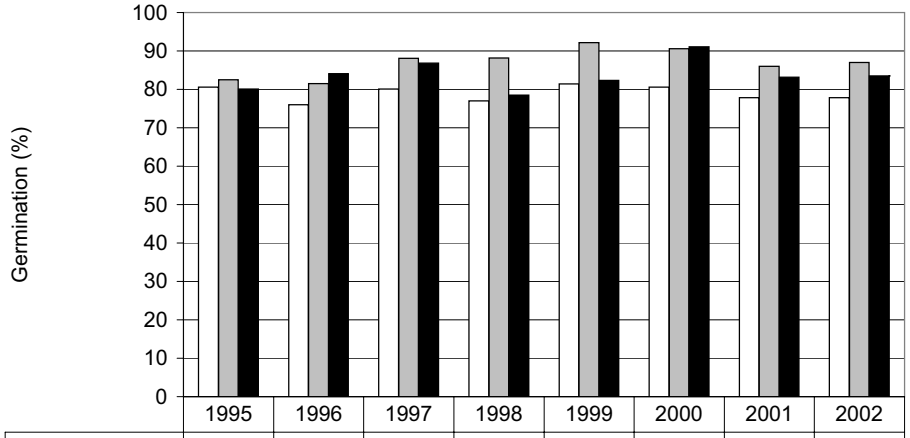


Table 2. Number of ISTA Orange and Blue Certificates issued from 1996 to 2003

	Year							
Species	*1996*	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	*2003*
Abies alba		*4*						
Acer campestre	*2*							
Acer platanoides	*1*		*1*					*1*
Acer pseudoplatanus	*1*							
Carpinus betulus	*4*		*3*	*1*				
Crataegus monogyna								*1*
Euonymus europeus			*2*	*1*		*1*	*2*	
Fagus sylvatica	*2*							
Fraxinus excelsior	*1*		*2*	*1*		*1*		
Larix decidua	*7*		*3+1*	*4*	*8*	*2*	*2*	
Ligustrum vulgare				*1*	*1*	*1*		
Lupinus polyphyllus	*2*	*1*	*1+1*	*1*		*2*		
Picea abies	*9*	*8*	*3*	*2*	*2*			*1*
Pinus mugo				*3*	*1*	*3*	*2*	*2*
Pinus sylvestris					*1*			
Pinus strobus							*1*	
Robinia pseudoacacia			*2*	*1*	*1*			
Rosa canina						*1*		
Sorbus aria	*1*	*1*	*4*					
Sorbus aucuparia	*2*		*1+1*	*1*		*1*	*4*	*1*
Sorbus intermedia	*1*		*3*	*2*	*2*	*1*	*2*	
Taxus baccata				*1*	*1*	*1*	*3*	
Tilia cordata			*1*					
Tilia platyphyllos	*1*			*1*			*1*	
Orange certificates in total	34	14	18	0	0	0	0	0
Blue Certificates in total	0	0	3	20	17	14	17	6
Total	***34***	***14***	***21***	***20***	***17***	***14***	***17***	***6***

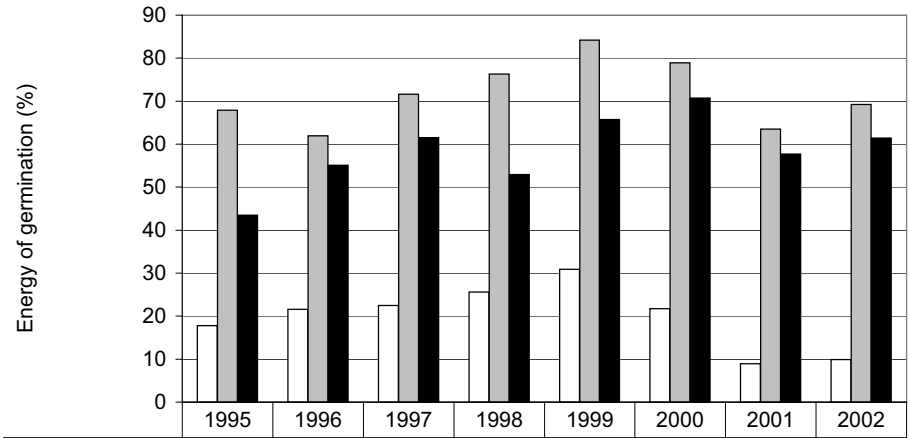
Fig. 3 Germination of pure filled seeds of *Picea abies*, *Pinus sylvestris* and *Larix decidua* tested from 1995 to 2002



	1995	1996	1997	1998	1999	2000	2001	2002
□*Picea abies*	81	76	80	77	81	81	78	78
▒*Pinus sylvestris*	83	82	88	88	92	91	86	87
■*Larix decidua*	80	84	87	79	82	91	83	84

year

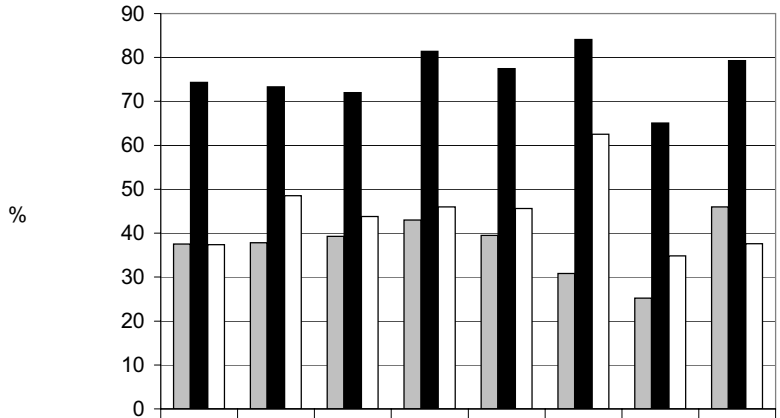
Fig. 4 Energy of germination of pure filled seeds of Picea abies, Pinus sylvestris and Larix decidua tested from 1995 to 2002



	1995	1996	1997	1998	1999	2000	2001	2002
□*Picea abies*	18	22	23	26	31	22	9	10
▒*Pinus sylvestris*	68	62	72	76	84	79	64	69
■*Larix decidua*	44	55	62	53	66	71	58	61

year

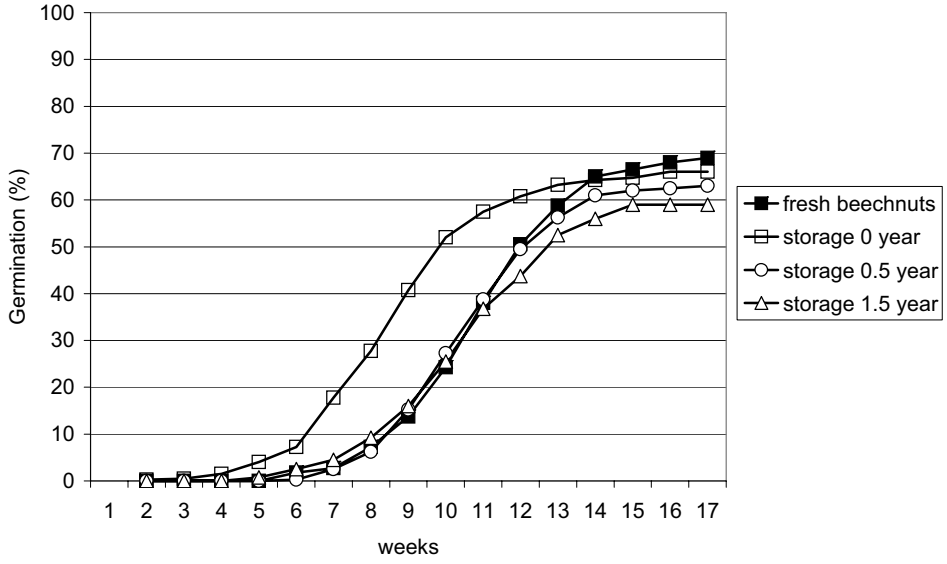
Fig. 5 Quality (viability and a proportion of empty seeds) of Abies alba seeds tested from 1995 to 2002



	1995	1996	1997	1998	1999	2000	2001	2002
Viability of pure seeds	38	38	39	43	40	31	25	46
Viability of pure filled seeds	74	73	72	81	78	84	65	79
Empty seeds	37	49	44	46	46	63	35	38

year

Fig. 6 Process of germination of fresh and stored beechnuts from the same seed lot



Acknowledgement

I would like to thank to the laboratory staff who do the real work that I have briefly summarised.

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International Rules for Seed Testing International Seed Testing Association: Seed Science and Technology.

Compararing of viability and germination of stored and fresh European beech (*Fagus sylvatica* L.) seeds

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Abstract

Beechnuts from 132 seedlots collected in the fall of 1995 and stored, with a moisture content of 8 to 12 % in sealed polyethylene bags, at –5 °C were tested for tetrazolium viability (ISTA Rules 1996) and germination (in a moist, sand-peat substrate mixture at 3 °C) in 1998. The relationship between tetrazolium viability and germination was very low. In a second set of experiments the viability of fresh beechnuts from the 2000 and 2001 crops was evaluated. Germination was done by the same method as before. Rigid evaluation of both the surface and inner side of beechnuts decreased the overestimation of the tetrazolium test compared to germination even when the biggest differences between viability and germination occurred in beechnuts with the highest viability.

Introduction

Since the 1995 harvest, 405 seedlots (99,832 kg) of beechnuts have been stored at –7 °C in the Czech Republic's State Tree Seed Centre in Týniště nad Orlicí. In spring of 1998, after 3 years of storage, beechnut viability was determined by tetrazolium tests (using the method in the Czech technical standard ČSN 48 1211, 1997 that is the same as the procedure in the ISTA Rules, 1996). The results showed that beechnuts from 66 seedlots (8,072 kg in total) had 2,800 viable seeds per kg on average while only 331 seedlings per 1 kg were produced in nurseries (Procházková and Bezděčková, 1999). As the result of poor seedling emergence in 1998, both germination tests and tetrazolium viability tests of beechnuts were introduced in laboratory practice. The evaluation of viable beechnuts became stricter and germination results have provided suitable feedback for improving the viability classification.

In the work reported here we compared the results of viability and germination of stored beechnuts collected in 1995 and tested in 1998 and fresh beechnuts collected in 2000 and 2001.

Materials and methods

Materials

Viability and germination of beechnuts collected in 1995 (132 seed lots), in 2000 (209 samples) and 2001 (317 samples) were tested at the Seed Testing Laboratory of the Forestry and Game Management Institute's Research Station in Uherské Hradiště. Seeds with a moisture content of 8 to 12 % were stored in sealed polyethylene bags at –7 °C at the Tree Seed Centre in Týniště n. O. until they were brought to the laboratory in insulated boxes. There the seeds were stored at –4 °C until tested. The beechnuts from the 1995 crop were stored for 3 years, while those from the 2000 and 2001 crops were stored for 0.5 year before testing.

Viability test

Viability (4 replicates of 100 seeds each) of beechnuts was evaluated according the Czech technical standard ČSN 48 1211 (1997), the same procedure as in the ISTA Rules (1996).

Germination test

Germination of beechnuts was done as described by Procházková et al. (2002). Four hundred seeds of each sample were mixed with a peat-sand (1 : 1 vol. /vol.) substrate (one volume of seeds and two volumes of substrate) for germination in 17 x 12 cm boxes at 4 °C. The boxes were kept sealed. The moisture content of the substrate was 28 to 32 %. Each week the seeds were checked and those with a visible radicle were considered to have germinated. These germinated seeds were counted and removed. The germination test was finished when no germination was observed for the next 2 weeks. Then, all ungerminated seeds were cut and the dead (rotten), empty and 'fresh' seeds were evaluated (ISTA Rules 1996).

Evaluation of results

Based on the viability test results the samples were divided into 'viability classes' (Table 1). Then the germination and viability of samples within the different 'viability classes' was compared.

Results and discussion

1995 crop

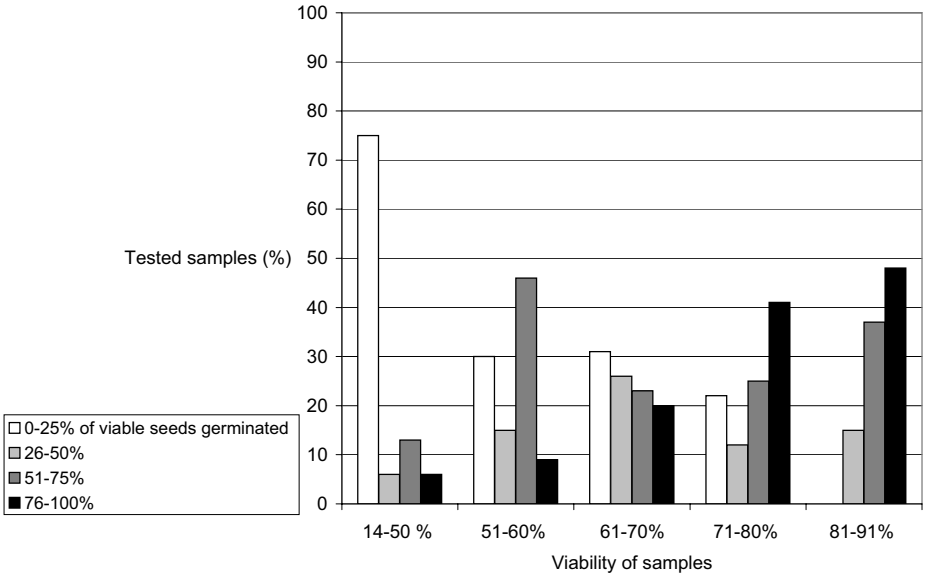
The mean viability of beechnuts from all 132 seed lots after 3 years of storage was 68 % and germination 39 %, the lowest and the highest viability was 14 % and 91 %, respectively the difference between mean viability and germination in each "viability class" ranged from 25 % for the highest quality seeds to 36 % for middle quality seeds (Table 1).

The percentage of samples with germinating seeds differs in each "viability class" (Figure 1). Erom beechnut samples with viability ranging from 14 to 50 % in less than 10 % of the samples more than 75 % viable seeds germinated and in 75 % of the samples not more than 25 % of the viable seeds germinated. In contrast, in nearly half of the samples with viability between 81 and 91 % more than 75 % of viable seeds germinated. This indicates that there was only a "50 % probability" that the difference between the tetrazolium tests results and the germination results of beechnuts with the highest viability (above 81 %) was less than 25 %. For beechnuts with viability below 70 % there was less than a 20 % chance that the difference between viability and germination was below 25 %. Overall, in only 30 % of all the samples the viability deviated from germination by about 0 to 25 %.

Table 1. Mean viability and germination of beechnuts collected in 1995 and stored for 3 years

Viability class	Number of samples	Mean viability (%)	Mean germination (%)	Difference (Viability – Germination) (%)
14-50 %	16	36	9	28
51-60 %	13	57	26	31
61-70 %	35	66	31	36
71-80 %	41	75	47	28
81-91 %	27	85	60	25

Fig. 1 Germination of viable beechnuts in different "viability classes" (crop 1995, beechnuts stored for 3 years)



2000 crop

The mean viability of beechnuts from all 209 seed lots after 6 months of storage was 75 % and germination 64 %, the lowest and the highest viability was 14 % and 91 %.

For beechnut samples with viability ranging from 56 to 69 % (mean viability of these samples was 64 % and germination 56 %) germination was higher than viability in 18 % of the samples, while viability was higher than germination in 79 % of the samples (Figure 2a). Similar results were obtained for beechnuts with viability over 70 % (Figures 2b, 2c). In beechnuts from the 2000 crop the "overvaluation" of viability compared to germination was the highest in samples with the highest viability.

2001 crop

The mean viability of beechnuts from all 317 samples after 6 months of storage was 75 % and germination 71 %, the lowest and the highest viability was 36 % and 96 %, respectively.

For beechnut samples with viability ranging from 36 to 69 % (mean viability of these samples was 63 % and germination 59 %) germination was higher than viability in nearly the same number of samples (Figure 3a). Similar results were obtained in beechnuts with viability ranging from 70 to 79 % (Figure 3b) while in beechnuts with the highest viable germination reached higher values compared viability in 33 % of the samples (Figure 3c). In beechnuts from the 2001 crop the "overestimation" of viability compared germination was similar to that for beechnuts collected in 2000 and stored for the same period (6 months) - the largest difference between viability and germination was in the samples with the highest viability.

Feedback (from germination test results) over the past several years indicates that the results of germination tests (and increasing experience of technicians in viability evaluation) and the viability results started to approach to germination test results, especially for fresh and short-term stored beechnuts. The classification of viable beechnuts was even more strict than that stated in Table 6A

Part II (ISTA Rules 2004). Normally all seeds with a completely stained embryo and those with unstained or necrotic parts as noted in columns 6 are viable. For beechnuts the maximum area of unstained, flaccid or necrotic tissue permitted is: "Radical tip, 1/3 distal area of cotyledons if superficial". However, our experience shows that even superficial necrosis indicates possible future problems during long germination tests and pre-chilling (stratification), or both, since beechnuts require 5 – 6 months to germinate. Consequently, beechnuts with such a large (1/3) necrotic area is now being classified as dead.

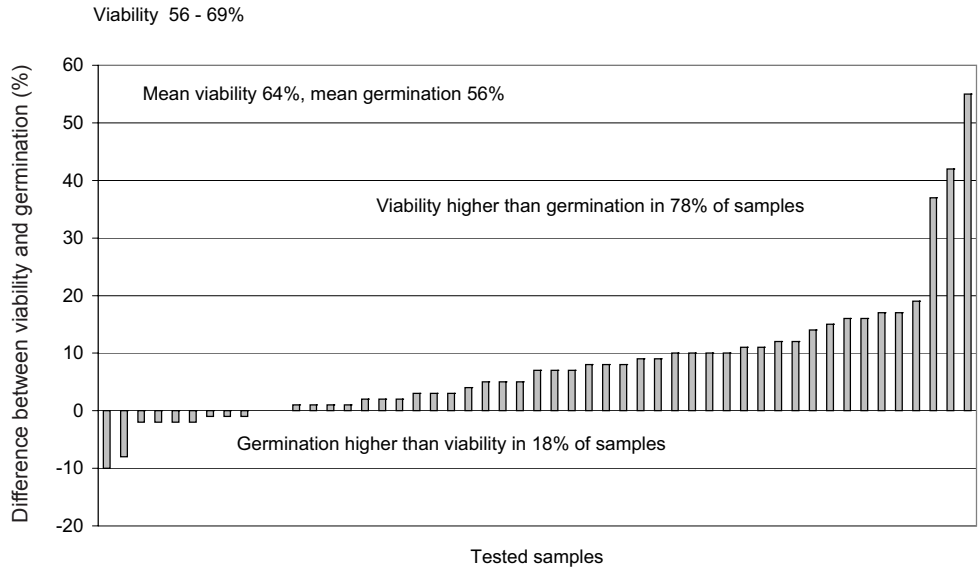
Conclusion

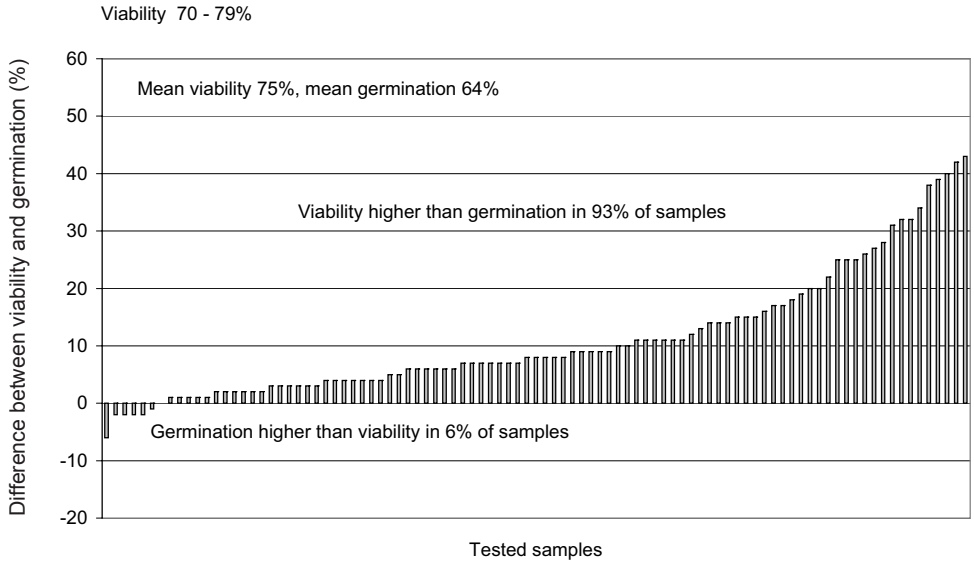
Strict evaluation of both surface and the inner surface of cotyledons of beechnuts decreases overestimation of tetrazolium test results compared to germination test results.

Acknowledgement

I would like to thank to the laboratory staff who do the real work that I have briefly summarised here. Special thanks go to Marie Ottová, a very experienced technician who helped ensure that our laboratory results more closely relate to what we see in nurseries.

Fig. 2a – c Difference between viability and germination of fresh beechnuts (crop 2000)





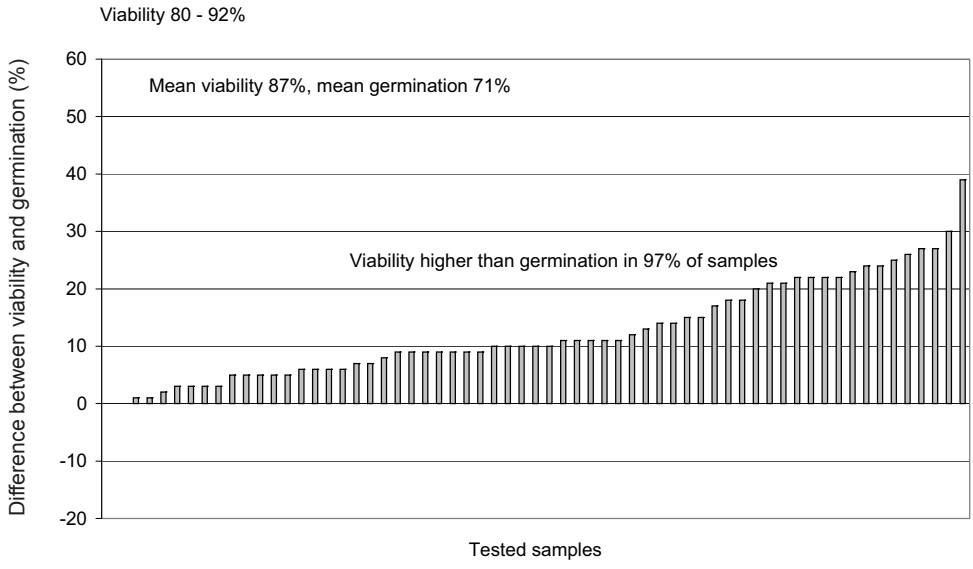
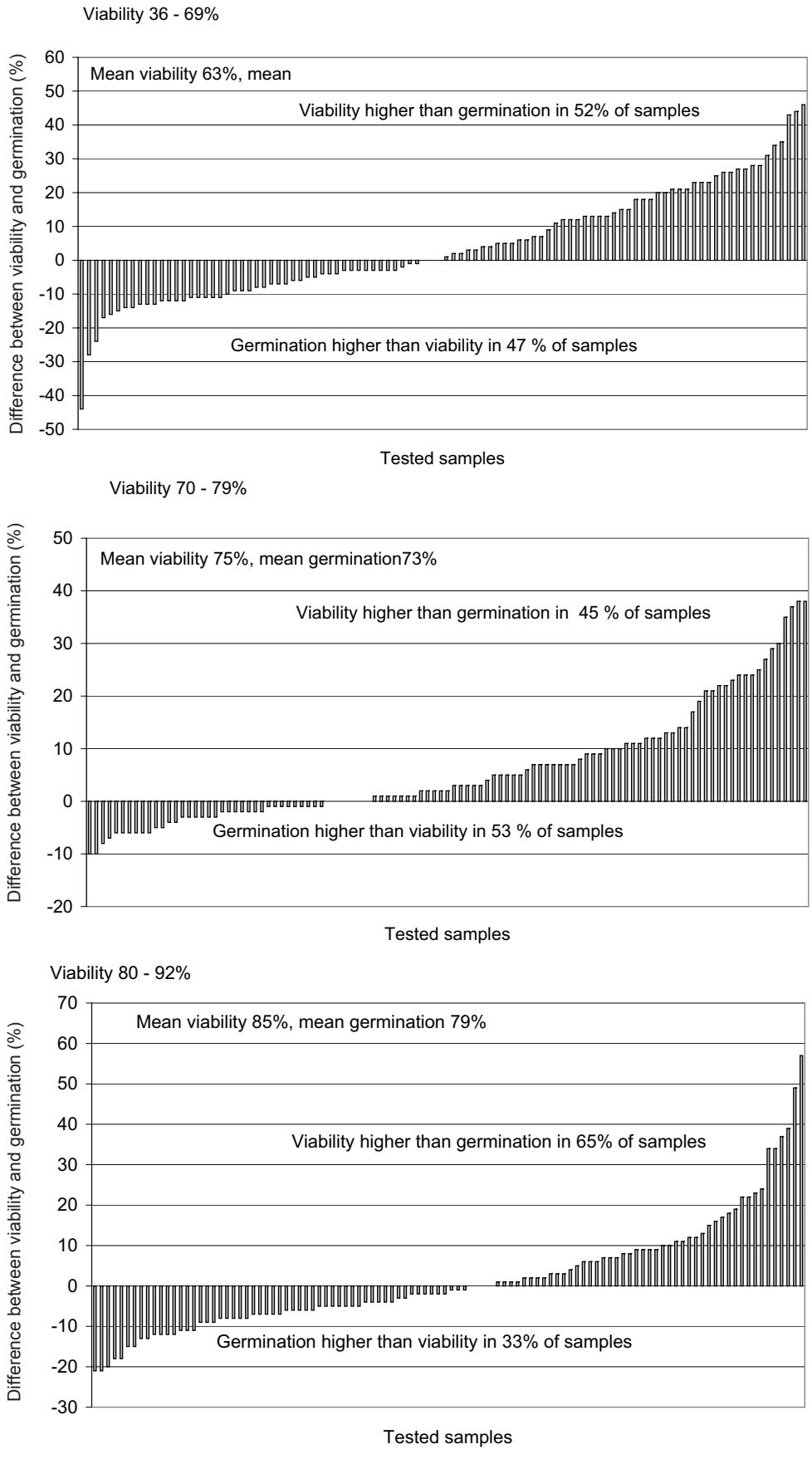


Fig. 3a – c Difference between viability and germination of fresh beechnuts (crop 2001)



Germination of silver fir seeds (*Abies alba* Mill.) from different seed stands in Croatia

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Abstract

Fir forests in Croatia are spread on the territory of selection forests on the area of 220,136 ha. Silver fir composes pure and mixed stands with spruce and beech. In Croatia, it is spread in the Dinaric region and in mountainous regions between the rivers Sava and Drava. Ecological constitution of silver fir, despite its narrow ecological valency regarding water and temperature, has indigenously high competitive capacity in comparison with beech and spruce.

The aim of this research was to determine the level and variability of laboratory seed germination. Silver fir acorn samples were gathered in autumn 1995. Acorns were manually processed, seed was homogenized and average samples were taken for carrying out this research. The size of average seed samples, the manner of preliminary treatment and germination method were carried out according to ISTA methodology.

Average values of germination energy and germination capacity were low. Average values of germination rate and germination capacity were 14.4 %, and 23.4 %, respectively. Average share of empty seed was 58.4 %. Increased share of empty seed directly influences diminished seed germination. The percentage of sick and rotten seed has significant impact on diminished seed germination and it is 9.7 % on average, for all plots, so the protection of seed from pathogenic fungi has to be intensified.

Key words: Silver fir, seeds, germination energy and germination capacity, empty seeds, rotten seeds

Paired germination tests in three pine species

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Abstract

Several of the southeastern yellow pines – *Pinus elliottii, P. palustris* and *P. taeda* – exhibit shallow, variable dormancy. Nursery and testing experience and research demonstrate that most seed lots benefit from some prechilling, but some seed lots are harmed by prechilling. Testing both with prechilling and without prechilling is recommended for these species.

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Programme

Monday, October 20, 2003

8:00 – 9:00	Registration	
9:00 – 9:20	Official opening Bohumír Lomský, vice director of FGMRI	
9:20 – 9:40	Zdeňka Procházková	Activities of the ISTA FTS Committee 2001-2002
Session 1		
9:40 – 10:10	Peter G. Gosling	Double the trouble and no current benefit - How to obtain more from 'double' germination tests
10:10 – 10:30	Gary Johnson	Paired germination tests in three pine species
10:30 – 10:50	COFFEE BREAK	
Session 2		
10:50 – 11:10	Gary Johnson and Ellen Chirco	Excised embryo tests of peach, apple and pear
11:10 – 11:30	S. Mugnaini, M. Nepi, E. Pacini, B. Piotto	Poor seeding in Junipers may depend upon non-specific pollination mechanisms
11:30 – 11:50	F. Pérez-García and M. E. González-Benito	Effect of temperature and various pretreatmens on seed germination of some *Halimium* and *Helianthemum* species
11:50 – 12:10	Zdeňka Procházková	Handbook for Seedling Evaluation
12:10 – 13:40	LUNCH	
Session 3		
13:40 – 14:10	D.G.W. Edwards	Breaking dormancy in tree seeds with special reference to firs *(Abies species)*
14:10 – 14:30	Zdeňka Procházková	Ring test of *Abies* spp.: information about the progress
14:20 – 14:40	Zdeňka Procházková	Germination test: Evaluation of normal and abnormal seeds
14:40 – 15:00	COFFEE BREAK	
Session 4		
15:00 – 15:30	Peter G. Gosling	What are the differences between a 'germination' test and a 'viability' test?
15:30 – 16:00	D.G.W. Edwards	Kinetics of water absorption in fir seeds, and its implications for tetrazolium and excised embryo tests
16:00 – 16:20	Zdeňka Procházková	Comparing viability and germination of European beech (*Fagus sylvatica* L.) seeds
Practical part I		
16:20 – 18:00	Stefanie Krämer	Presentation of Tetrazolium Handbook TZ method presentation Practical training – TZ method (preparation of *Abies* seeds)

Tuesday, October 21, 2003

8:00 – 13:00	Excursion	ÚKZUZ –ISTA seed testing laboratory for agriculture crops (CZDL03)
13:00 – 14:30	LUNCH	
Practical part II		
14:30 – 16:00	Stefanie Krämer	Tetrazolium test: Evaluation of viability of *Abies* seed
	Lena Bezděčková	Tetrazolium test: Evaluation of viability of *Fagus sylvatica, Prunus, Tilia, Acer* seeds
16:00 – 16:20	COFFEE BREAK	
Practical part III		
16:20 – 18:00	Gary Johnson	Excised embryo test
	Lena Bezděčková	Purity of conifer seeds
	Lena Bezděčková	Germination of *Pinus sylvestris* and *Picea abies* (evaluation)
19:00	OFFICIAL DINNER	

Wednesday, October 23, 2003

8:30 – 12:00	Excursion to dendrological garden	
12:00 – 13:30	LUNCH	
Session 5		
13:30 – 13:50	Finnvid Prescher and Elisabeth Prescher	Testing forest tree seed in Sweden
13:50 – 14:10	Abdalla Abdelmonem	Survey of seed-borne diseases of some woody trees in Egyptian gardens
14:10 – 14:30	Elena Foffová	Evaluation of the ISTA comparative test for laboratories testing forest tree seeds (2001).
14:30 – 15:00	Zdeňka Procházková	Proficiency testing programme
15:00 – 15:20	COFFEE BREAK	
Session 6		
15:20 – 16:00	Zdeňka Procházková	EU Legislation on Marketing of Forest Tree Seeds: future needs Future aims of activities of the FTS Committee Tree seed testing in the Czech Republic
16:00 – 18:00	Miscellaneous (Discussion) CLOSING of the workshop	

Alternative programme

1day-long post meeting tour

Thursday 23.10.2003 Visit to the State Tree Seed Centre in Týniště nad Orlicí

3day-long post meeting tour

Thursday 23.10.2003

	Uherské Hradiště	Excursion to the Research Station (ISTA - SKDL02 laboratory)

Friday 24.10.2003

13:00-15:00	Liptovský Hrádok, Republic of Slovakia	Visit the Tree Seed Centre
15:00-17:00	Liptovský Hrádok	Visit the Research Station (ISTA lab CZDL02)

Saturday 25.10.2003

8:30	Liptovský Hrádok	Departure
19:00	Prague	Arrival