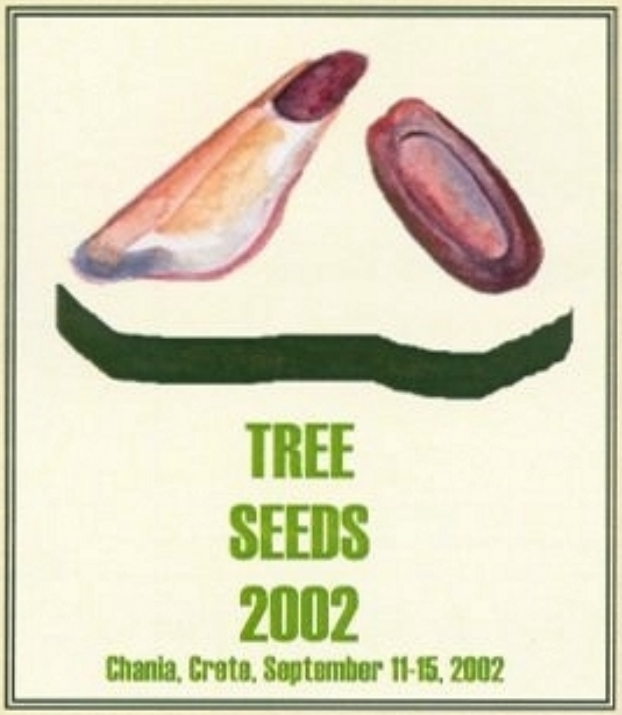
ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS



PROGRAMME
&
BOOK OF PROCEEDINGS

2002 Annual Meeting of IUFRO 2.09.00
'Research Group for Seed Physiology and Technology'

TREE SEEDS 2002

Chania, Crete, Sep. 11-15, 2002

Co-organized by IUFRO, the University of Athens, MAICh
and the Hellenic Ministry of Agriculture
Sponsored by ISSS, the International Society for Seed Science

Athens, September 2002

ΕΘΝΙΚΟΝ & ΚΑΠΟΔΙΣΤΡΙΑΚΟΝ ΠΑΝΕΠΙΣΤΗΜΙΟΝ ΑΘΗΝΩΝ

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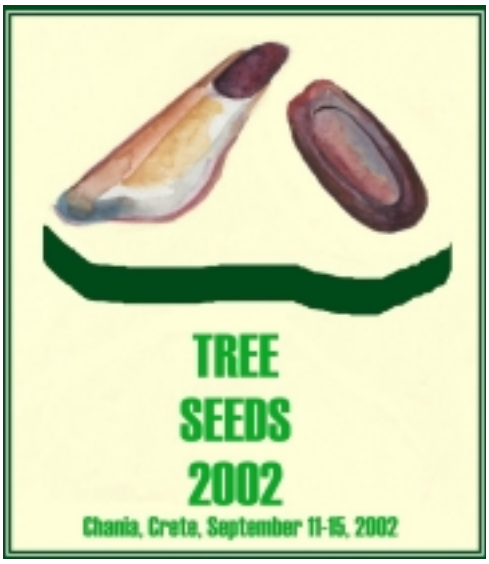

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hellenic ministry of agriculture

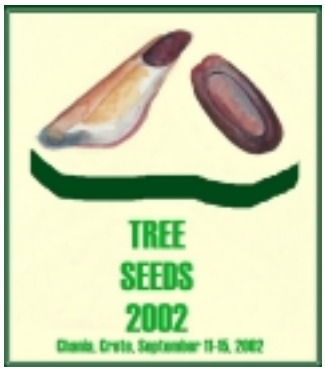
MAICh

Athens, September 2002

Acknowledgements

I express my sincere gratitude to the co-organisers of TREE SEEDS 2002: the Hellenic Ministry of Agriculture, Greece (Mr Georgios Dris, Minister & Mr Fotis Chatzimichalis, Vice Minister), the University of Athens (and especially Prof. Michalis Dermitzakis, Vice Rector), the IUFRO 2.09.00 *'Research Group for Seed Physiology and Technology'* (Dr Jack Vozzo, chair) and the Mediterranean Agronomic Institute at Chania (Mr Alkinoos Nikolaidis, Director). I also thank the International Society for Seed Science (ISSS), the Ministry of Culture, Greece, the Printing Department of the University of Athens, the Civil Protection Service of Chania (Mr Manolis Bouzakis), the Directorate of Forests, Chania (Mr Vasilios Kasiotakis and Dr Haroula Kargiolaki), the Archaeological Museum of Chania (Ms Maria Vlazaki) and the Conference Center of MAICh (Ms Roulie Zervou, Ms Katerina Karapataki, Ms Christina Protopapadaki, Ms Athanasia Mavrommati, Ms Maria Giannakaki). I extend my appreciation and thanks to the co-editors of the Book of Proceedings (Dr Tannis Beardmore, Dr Kris Connor and Dr Ike Tolentino). Finally, I thank all the colleagues of the International Organizing Committee for their help in shaping TREE SEEDS 2002 as well as all my collaborators (Dr Maria Doussi & Mr Petros Panayiotopoulos at the Univ. of Athens, Dr Evangelia Daskalakou at the Mediterranean Forest Institute, Athens and Ms Christina Fournaraki at MAICh). A special word of thanks to our artist, Ms Ioanna Daskalakou (painter, Athens School of Fine Arts) who has drawn the logo of the Conference.

Costas A. Thanos
September 2002





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Organizing Committee

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Asc Prof Enrique L. Tolentino, Univ. Los Baños, the Philippines
Dr Gary Johnson, National Tree Seed Laboratory, USA
Ass Prof Kyriacos Georghiou, Univ. Athens, Greece
Ass Prof Antonis Skordilis, Univ. Thrace, Greece
Dr Maria Doussi, Univ. Athens, Greece
Ms Christina Fournaraki, MAICh, Greece
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PROGRAMME

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PROGRAMME

Wednesday 11 September

19:00-21:00 **Registration TREE SEEDS 2002**

21:00 **Gala Dinner**

Thursday 12 September

09:00-09:30 **Registration TREE SEEDS 2002**

09:30-11:00 OFFICIAL OPENING & SESSION 1

09:30-09:50 Official Opening

Chair: **The Organising Committee**

Alkinoos Nikolaidis
(Director, Mediterranean Agronomic Institute of Chania)
Welcome address

Official Opening of the Conference

09:50-11.00 SESSION 1

Chair: **Kyriacos Georghiou (Greece) - Christina Fournaraki (Greece)**

09:50-10.00
J.A. Vozzo (delivered by Gary Johnson)
(Chair of IUFRO RG 2.09.00 / USDA Forest Service, Southern Research Station, Starkville, Mississippi - USA)
Symbiosis – What can you offer to strengthen our RG?

10:00-10.20

Y.A. El-Kassaby[1], A. Benowicz[1] and D.G.W. Edwards[2]
([1]The University of British Columbia, [2]FTB Forest Tree Beginnings - Canada)

Genetic control of germination and aging: lessons for practice and conservation

10:20-10:40

A. Mosseler[1], J.E. Major[1] and O.P. Rajora[2]
[1]Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, [2]Dept Biology, Life Sciences Centre, Dalhousie University, Halifax - Canada)

Seeds as indicators of genetic and reproductive status in red spruce and eastern white pine

10:40-11:00

P. Panayiotopoulos and C.A. Thanos
(Dept Botany, University of Athens - Greece)

Cone - seed biometry and germination ecophysiology in *Pinus nigra* from several Greek provenances

11:00-11:30 Coffee Break

Thursday 12 September

11:30-13:30 COMMON SESSION MEDPINE 2 & TREE SEEDS 2002

***Chair:* Louis Trabaud (France) - Haroula Kargiolaki (Greece)**

11:30-12:10 **(MEDPINE 2 – Review Lecture)**

R. Nathan
(Ben-Gurion University of the Negev - Israel)

Spatiotemporal dynamics of recruitment in Aleppo pine

12:10-12:30 (MEDPINE 2)

F. Krouchi[1], K. Tahir[1], R. Aba[1], A. Raffin[2] and M. Rezzig[3]
([1]Université M. Mammeri de Tizi-ouzou - Algeria, [2]INRA - France, [3]Institut National de recherche forestière - Algeria)

Variation in cone and seed characteristics of *Pinus pinaster* Ait. in Algeria

12:30-12:40

C. Fournaraki
(Mediterranean Agronomic Institute of Chania - Greece)

The Plant Conservation Unit at MAICh – Herbarium, Seed Bank, Botanical Garden

12:40-12:50

K. Thompson
(Treasurer of ISSS / University of Sheffield - UK)

A brief presentation of the International Society for Seed Science (ISSS)

12:50-13:20

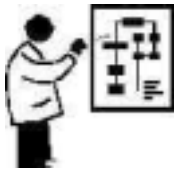
Manolis Bouzakis
(Civil Protection Service, Prefecture of Hania)
Hania – the place, the history, the people

13:30-15:00 Lunch

Thursday 12 September

15:00-16:30

POSTER VIEWING SESSION A



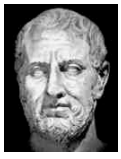
16:30-17:00 Coffee Break

17:00-22:00 **Chania City Tour**

Friday 13 September

6:30-21:00 **Excursion to the National Park of Samaria Gorge**

Saturday 14 September



8:30-11:00

Visit to the Archaeological Museum of Chania

11:00-11:30 Coffee Break

Saturday 14 September
11:30-13:10 SESSION 2

Chair: **Gary Johnson (USA) - Ioannis Takos (Greece)**

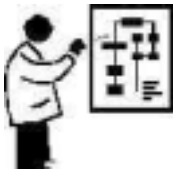
11:30-11:50
B. Varghese and S.C. Naithani
(Pt. Ravishankar Shukla University - India)
Biochemical basis of desiccation–sensitivity in tropical forest tree seeds

11:50-12:10
M.I. Daws[1], C.S. Gaméné[2], H.W. Pritchard[1], C Harris[1] and S Glidewell[3]
([1]Seed Conservation Department, RBG Kew – UK, [2]Centre National de Semences Forestières - Burkina Faso, [3]Scottish Crop Research Institute, Dundee – UK)
The importance of intra- and inter-seed variation in water content for assessing the desiccation tolerance of recalcitrant tree seeds

12:10-12:30
E.L. Tolentino Jr.
(University of the Philippines Los Baños - Philippines)
Seed and germination characteristics of *Swietenia mahogani* (L.) Jacq.

12:30-12:50
N.V. Obroucheva and O.V. Antipova
(Institute of Plant Physiology, Moscow - Russia)
Physiological characteristics of dormant and germinating horse chestnut seeds

13:10-14:30 Lunch



Saturday 14 September
14:30-16:30
POSTER VIEWING SESSION B

16:30-16.55 Coffee Break

16:55 **Group Photo**

Saturday 14 September

17:00-19:20 SESSION 3

Chair: **Yousry A. El-Kassaby (Canada) - Yitzchak Gutterman (Israel)**

17:00-17:20
M. Jensen
(Department of Horticulture, Danish Institute of Agricultural Sciences – Denmark)
Changes in percentage and speed of root and shoot germination in *Quercus robur* seeds after a controlled moisture content chilling treatment

17:20-17:40
P.G. Gosling, Y. Samuel and A. Peace
(Forestry Commission Research Agency - UK)
Optimum moisture content and prechill duration for dormancy breakage of Douglas fir seeds (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco)

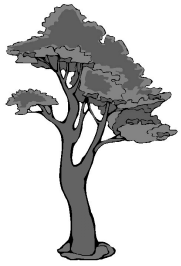
17:40-18:00
G. Johnson
(National Tree Seed Laboratory, United States Forest Service - USA)
Resin removal effect on Bald Cypress [*Taxodium distichum* (L.) Rich. var. *distichum*] germination

18:00-18:20
A. Derridj and F. Krouchi
(Université Mouloud Mammeri de Tizi-Ouzou - Algeria)
Effects of various factors on germination of *Cedrus atlantica* M. seeds

18:20-18:40
E. Tillman-Sutela[1], A. Kauppi[2], J. Kaitera[3] and A. Hilli[1]
([1]Finnish Forest Research Institute, Muhos, [2]Department of Biology, University of Oulu, [3]Finnish Forest Research Institute, Rovaniemi – Finland)
Damages caused by fungi in Norway spruce, *Picea abie*s (L.) Karst. seeds

18:40-19:00
L. Mtwisha, W. Brandt, J. Farrant and G. Lindsey
(University of Cape Town - South Africa)
ASP52: A large LEA-like protein in the cell walls of the seeds of the Camel Thorn tree, *Acacia erioloba*, protects proteins against stress-induced conformational changes

Sunday 15 September



08:30-13:30 **Half-Day Excursion**
Nursery of the Chania Forest Service – Chryssopigi Monastery – Venizelos' graves – Phryganic vegetation at Akrotiri – Stavros village

13:30-15:00 Lunch

Sunday 15 September
15:00-17:00 SESSION 4

Chair: **Ken Thompson (UK) - Maria Doussi (Greece)**

15:00-15:20
D.G.W. Edwards
(FTB Forest Tree Beginnings - Canada)
Seed-to-wing attachments in important members of the Pinaceae, with additional observations on members of the Cupressaceae and Taxaceae

15:20-15:40
C.M. Culshaw[1], P. Espinosa[1], H.W. Pritchard[1] and J. Engels[2]
([1]Seed Conservation Department, RBG Kew – UK, [2]International Board for Plant Genetic Resources, Rome – Italy)
Thermal scarification of hard seeds by wet heat treatment risks accelerated seed ageing: evidence from five woody taxa

15:40-16:00
C. Fournaraki[1] and C.A. Thanos[2]
([1]Mediterranean Agronomic Institute of Chania, [2]Dept Botany, University of Athens - Greece)
Seeds of *Zelkova abelicea*, an endemic tree of Crete

16:00-16:20
C.A. Thanos, M. Saeed and P. Panayiotopoulos
(Dept Botany, University of Athens - Greece)
Pine seed architecture

16:20-16:40
K. Thompson
(University of Sheffield - UK)
Do trees and shrubs have persistent seed banks?

16:40-17:10 Coffee Break

Sunday 15 September
17:10-18:30 SESSION 5

chair: **George Edwards (Canada) - Enrique L. Tolentino, Jr (Philippines)**

G. Johnson
(National Tree Seed Laboratory, United States Forest Service - USA)
The 2003 Annual Meeting of IUFRO RG 2.09.00

J.A. Vozzo (delivered by Gary Johnson)
(Chair of IUFRO RG 2.09.00 / USDA Forest Service, Southern Research Station, Starkville, Mississippi - USA)
Closing Remarks

General Discussion
Concluding Comments and Remarks
Thanks and goodbyes

19:30-20:30 **Farewell Reception**

POSTERS

L.F. Beníto-Matías, N. Herrero Sierra, A. Muñoz, J.L. Nicolás and J.L. Peñuelas
(Centro Nacional de Mejora Forestal "El Serranillo" - Spain)
Efecto de las temperaturas de helada en bellotas de *Quercus ilex* L.

C. Blade[1,2], R. Guardia[3] and V.R. Vallejo[1,2].
([1]Centro de Estudios Ambientales del Mediterráneo (CEAM) Valencia [2]Universitat de Barcelona, [3]Centre de Documentació de Biodiversitat Vegetal del Parc Científic de Barcelona - Spain)
Response of *Pinus halepensis* to different seeding techniques in a climatic gradient

L. Calvo, L. Valbuena, E. Marcos, R. Tárrega and E. Luis-Calabuig
(Universidad de León - Spain)
Effects of light/darkness and inhibitory components on the germination of *Pinus pinaster*

M.I. Daws[1,2], C.S. M. Houlihan[1] and D.F.R.P.Burslem[1]
([1]Department of Plant & Soil Science, University of Aberdeen – UK, [2]Current address: Conservation Department, RBG Kew - UK)
Effects of pre-treatments on the germination of seeds of four Panamanian pioneer tree species

E. Estrelles, J. Prieto, N. Fuentes, D. Ballesteros, L. de Fez and A.M. Ibars
(Universitat de València - Spain)
Germinative behavior of *Leguminosae* seeds under different temperature treatments

J.M.R. Faria[1,2], A.C. Davide[2], H.W.M. Hilhorst[1]
([1]Laboratory of Plant Physiology - Wageningen University and Research Centre - The Netherlands, [2]Departamento de Ciencias Florestais – Universidade Federal de Lavras - Brazil)
Desiccation sensitivity in *Inga vera* subsp. *affinis* seeds

N. Herrero Sierra, L.F. Beníto-Matías, S. Domínguez Lerena, J.L. Nicolás Peragón and J. Peñuelas Rubira
(Centro Nacional de Mejora Forestal "El Serranillo" - Spain)
Effect of low temperature on the conservation of pregerminated seeds of *Pinus halepensis* and *Pinus pinaster*

A. Hilli[1], E. Tillman-Sutela[1] and A. Kauppi[2]
([1]Finnish Forest Research Institute, Muhos, [2]Department of Biology, University of Oulu - Finland)
Germination of pretreated Scots pine (*Pinus sylvestris* l.) seeds after long-term storage

T. Merou[1], I. Takos[1] and E. Konstantinidou[2]
([1]Institute of Technological Education of Kavala, [2]Forestry Service of Drama - Greece)
Effect of treatments and seed collection time on seed germination of *Albizia julibrissin* Durazz. seeds

G.S Pamuk. and U. Bergsten
(Swedish University of Agricultural Sciences - Sweden)
Coated Scots pine seeds makes autumn direct seeding possible

B. Piotto
(Italian Environment Protection Agency - Italy)
Knowing propagation of the Mediterranean trees and shrubs from seed to preserve their biodiversity

Z. Procházková
(Forestry and Game Management Research Institute - Czech Republic)
Seasonal and chilling effects on germination of Norway spruce (*Picea abies* (L.) H. Karsten) and Scots pine (*Pinus sylvestris* L.) seeds

O. Reyes and M. Casal
(Universidad de Santiago de Compostela - Spain)
***Quercus rotundifolia* germination related to forest fires: the effect of high temperatures, ash, smoke and charcoal**

E.-M. Savonen
(The Finnish Forest Research Institute - Finland)
Germination behaviour of *Picea abies*: effects of incubation temperature, light, collection time and tree on germination capacity and rate

J.D. Simpson
(Canadian Forest Service - Canada)
Canada's National Tree Seed Centre

I. Takos[1], E. Konstantinidou[2] and T. Merou[1]
([1]Institute of Technological Education of Kavala, [2]Forestry Service of Drama - Greece)
The effect of desiccation on the seed germination of *Laurus nobilis* L.

T. Tylkowski
(Institute of Dendrology, Polish Academy of Sciences, Kórnik – Poland)

Thermal conditions for dormancy release of *Cornus sanguinea* L. seeds

S. Wetzel and L.C. Duchesne
(Canadian Forest Service - Canada)
Food preferences of mice and voles may be a critical determinant of forest succession in white pine ecosystems of Canada

C.B. Wood[1], S. Hodges[1], W. Bin[2] and H.W. Pritchard[1]
([1]Seed Conservation Department, RBG Kew – UK, [2]Xishuangbanna Tropical Botanic Garden, Yunnan - PR China)
Semina Palmarum: the seed conservation biology of palms

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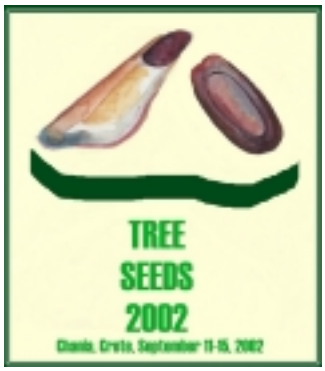

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An *ex situ* Conservation Strategy for Butternut (*Juglans cinerea*): Cryopreservation of Embryonic Axes

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Abstract

Butternut (*Juglans cinerea* L.) survival is threatened in North American by the fungus *Sirococcus clavigignenti-juglandacearum.* To date, there is no control for this fungal disease and long-term seed storage, to ensure survival of the species, is not a viable option. Embryonic axes (embryo with cotyledonary material removed) will tolerate storage at low temperatures, but tolerance is dependent on the duration that the nuts have been stored. Axes isolated from nuts stored at +4°C for 0, 5, 12 and 18 months were exposed to 0, –5, -10, -15, -40 and –196°C. Only axes isolated from 5 and 12 month-stored nuts tolerated storage at 0 and –5 °C, while axes isolated from 12 month-stored nuts also tolerated -10, -15, -40 and –196°C. The highest % germination was obtained from axes isolated from 12 month stored axes; germination ranged between 52 –98 % in axes stored at all temperatures. Axes stored at –196°C for 2 years maintained high % of germination, suggesting that this may be a feasible means of preserving butternut in the long-term.

Introduction

Butternut (*Juglans cinerea*) trees are native to North American and predominantly occur in forests ranging from eastern Ontario to New Brunswick, throughout the New England States (excluding northern Maine), south to Georgia and west to Missouri (Millikan and Stefan, 1989). Throughout the range of butternut, trees are being killed by a fungus (*Sirococcus clavigignenti-Juglandacearum*) (Nair et al. 1979). This fungus induces multiple cankers at the base of the trunk (and on branches) that coalesce and girdling the tree, resulting in partial or complete death (reviewed in Ostry et al. 1994). To date, control for this disease does not exist. Overall butternut mortality as a result of this disease exceeds 77% in American forests (Ostry et al. 1994), while in Canada, butternut mortality has been estimated in Ontario to be 80 % (Fleguel, 1996). For this reason butternut is considered an endangered and threatened plant species under the Endangered Species act in the United States (listed under Category 2). However, in Canada, butternut has not yet received status on the Committee on the Status of Endangered Wildlife Canada (COSEWIC) Species at Risk List.

Butternut produces a seed, which is classified as a recalcitrant seed (Barton, 1936, Forest Service, 1948). Recalcitrant seeds are intolerant of drying (below 5% water content on a fresh weight basis) and low temperatures (below -18°C) and are therefore difficult to store in the long-term using conventional storage techniques (Roberts, 1973). This raises considerable problems for the conservation of species, which produces recalcitrant seeds. Research has shown that some recalcitrant tree seeds can be stored at ultra low temperature storage (at -196°C) (Grout et al. 1983, Pence, 1990, de Boucaud et al. 1991, Poulsen, 1992) or low temperatures (i.e., -20 °C, -70°C) (Pritchard et al. 1995; Vertucci et al. 1991). Cryopreservation methods for various Juglans ssp. zygotic (Pence, 1990; de Boucaud et al. 1991: Beardmore and Vong, 1998) and somatic embryos (Lee, 1989; de Boucaud et al. 1994) have met with variable success. The purpose of this work was to determine whether low temperature storage, in particular cryopreservation is a feasible method of *ex situ* conservation for this species.

Materials and Methods

Seed collection and handling

Butternut fruits (Figure 1A) collected in October 1998 were harvested from 19 trees in one population in southern New Brunswick. Fruits were dehusked, cleaned and the nuts were stored in Gortex (DuPont, Boston, MA) membrane invigoration tubes (Downie et al. 1995) at 4°C until required. Seed from tree 6 were used for microscopy.

Embryonic axes excision and sterilization

Embryonic axes (referred to as axes) were excised from the nut (Figure 1B) and approximately 3 mm of cotyledonary tissue was left attached to the hypocotyl (Figure 1C). Axes were surface sterilized in 5% sodium hypochlorite solution for 5 min., rinsed 2 times in sterile water and subsequently blotted on sterile filter paper for

approximately 10 seconds to dry axes. For the control, 0 month storage duration, 20 sterile axes were placed on Woody Plant Medium (WPM) (Lloyd and McCown, 1981) in 10 cm Petri dishes (10 mL/petri dish) placed in Convirons growth cabinets (26^{o} C with a 12/12 h day/night photoperiod).

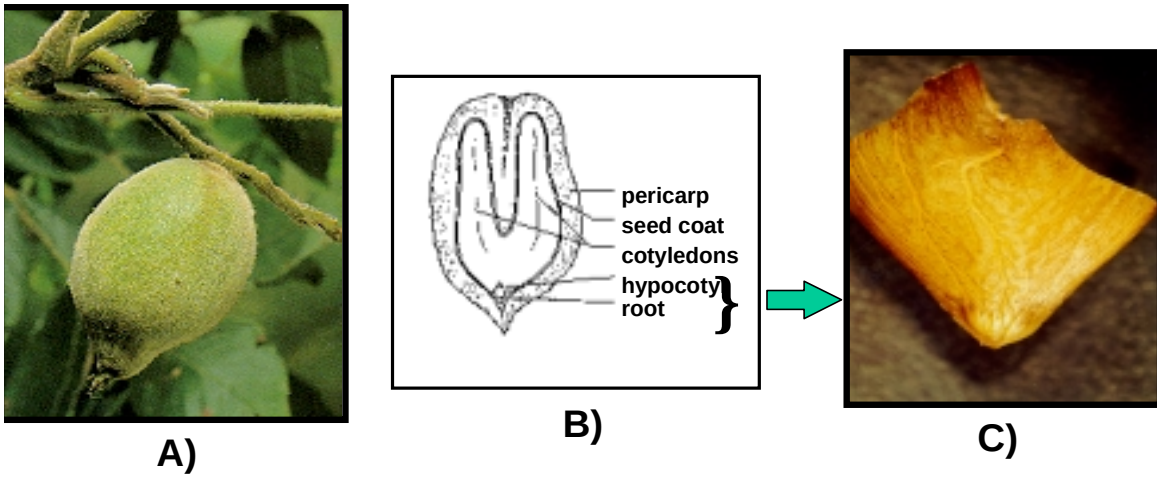

Figure 1. Butternut fruit (A), longitudinal section of the nut (B) and embryonic axes surrounded by testa (C).

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Low and ultra low temperature exposure

For low temperature exposure, the sterile axes were transferred into sterile polyproplyene cryo-vials (10 axes/2.5 mL vial) (Nalgene, Rochester, NY) and placed in a programmable freezer (Kryo 10, Planar Products Ltd. Sunbury-on-Thames, UK). Air temperature in the programmable freezer decreased from room temperature (approximately 20^{o}C) to -40^{o}C as described in Table 1. Embryonic axes were exposed to 6 low temperature treatments, 0^{o}C, -5^{o}C, -10^{o}C, -15^{o}C, -40^{o}C, and, -196^{o}C (Table 1).

Table 1. Low and ultra low temperature treatments for butternut embryonic axes.

Low and ultra low temperature treatment: final temperature of exposure for embryonic axes	**Rate of decline to the temperature**	**Hold duration at final temperature**
0^{o}C	1) room temperature to 0^{o}C at a rate of 5^{o}C/min.	4 h at 0^{o}C
-5^{o}C	1) room temperature to 0^{o}C at a rate of 5^{o}C/min. 2) 0^{o}C to -5^{o}C at a rate of –0.33^{o}C/min.	4 h at -5^{o}C
-10^{o}C	1) room temperature to 0^{o}C at a rate of 5^{o}C/min. 2) 0oC to -10^{o}C at a rate of -0.33^{o}C/min.	4 h at -10^{o}C
-15^{o}C	1) room temperature to 0^{o}C at a rate of 5^{o}C/min. 2) 0^{o}C to -15^{o}C at a rate of -0.33^{o}C/min.	4 h at -15^{o}C
-40^{o}C	1) room temperature to 0^{o}C at a rate of 5^{o}C/min. 2) 0^{o}C to -40^{o}C at a rate of -0.33^{o}C/min.	4 h at -40^{o}C
-196^{o}C	1) room temperature to 0^{o}C at a rate of 5^{o}C/min. 2) 0^{o}C to -40^{o}C at a rate of 0.33^{o}C/min. 3) -40^{o}C to -196^{o}C: vials were removed from the programmable freezer after reaching -40^{o}C and then immersed directly in liquid nitrogen for 24 h.	24 h at -196^{o}C

Determination of axes survival after low temperature exposure

For thawing axes after low temperature exposure, cryo-vials (containing the axes) were placed in a water bath at 40^{o} C for 5 min. Axes were then transferred from the cryo-vials onto WPM in 10 cm Petri dishes and placed in Convirons growth cabinets (26^{o} C with a 12/12 h day/night photoperiod). Root and shoot growth was monitored daily. Axes were scored as exhibiting shoot growth when the apical meristem had elongated and was green in colour. Root growth was scored when elongation was exhibited in the root area. Axes were considered germinated when both root and shoot growth was evident. There were three replicates, 20 axes/ replicate for each combination of tree, temperature and storage duration.

Effect of storage duration of axes viability

Axes isolated from nuts collected from 4 trees (tree # 2, 8, 11 and 12) were stored either in the vapour phase of liquid nitrogen (-196^{o}C) or in a –5^{o}C cooler for either 6, 12 and 24 months. After each storage duration, axes were germinated by placed axes on Woody Plant Medium (Lloyd and McCown, 1981) in 10 cm Petri dishes (10 mL/petri dish) placed in Convirons growth cabinets (26^{o} C with a 12/12 h day/night photoperiod). There were 3 replicates, 20 axes/replicate for each treatment combination of tree, temperature and storage duration.

Measurement of water content

Water content was measured using the Karl Fisher titrimetric method according to the manufacturer's instructions (Mettler-Toledo Inc. Hightstown, New Jersey). Measurements were done on embryonic axes collected from each of the 19 trees. For each tree, 3 replicates were done with 15 axes/replicate.

Results

Water contents of the axes isolated from control, 0 month nuts (referred to as 0 months axes) ranged between 5-32 %, with a mean water content of 11.4% (Figure 2A). During storage of the nuts, axes water contents declined up until 12 months of nut storage (referred to as 12 month axes) and at this time water contents ranged between 5-12%, with mean water content of 8.2%. Axes isolated from 18 months stored nuts (referred to as 18 month axes) had water contents between 5-13%, with mean water content of 7.8%.

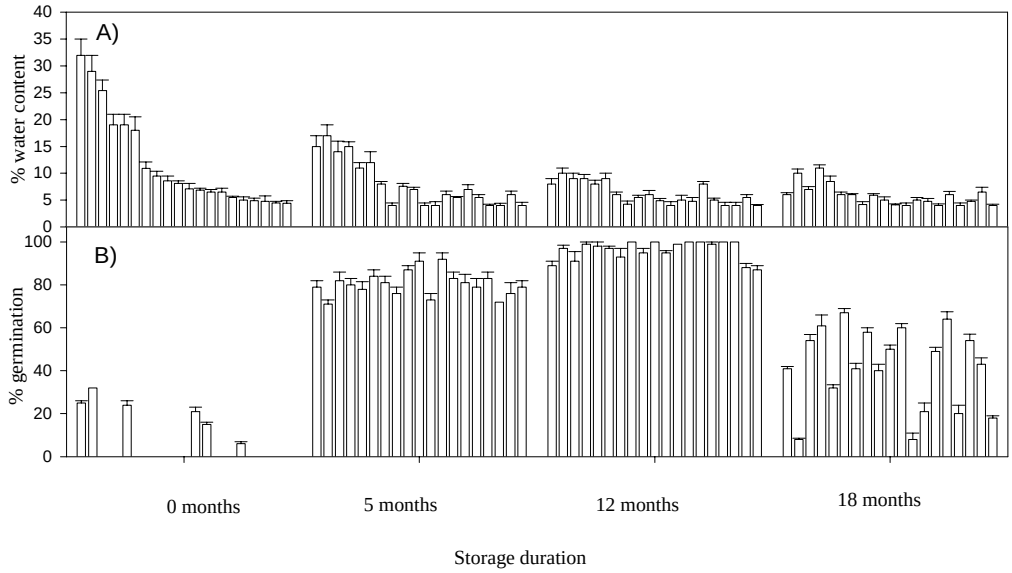

Figure 2. Butternut axes A) % water content and B) % germination after 0, 5, 12 and 18 months of storage at +5^{o}C.
Each bar +/- Standard Error at each storage duration represents the % water content (A) or % germination (B) of axes isiolated from the nuts of one tree. Data for all 19 trees is presented consecutively for each storage duration, starting with tree #1 and ending with tree #19.

Only 6 of the 19 trees had control 0 months axes, which germinated (germination range: 5-36%, with mean germination of 17%), and after 5 months of storage, axes isolated from all 19 trees germinated (germination

range: 72-92%, with a mean germination of 78%) (Figure 2B). A further increase in axes germination occurred in the 12 month axes (germination range: 92-100%, with a mean germination of 96%), and germination declined in the 18 month stored axes (germination range: 8-67%, with mean germination of 41%). None of the 24 month stored axes germinated (results not shown).

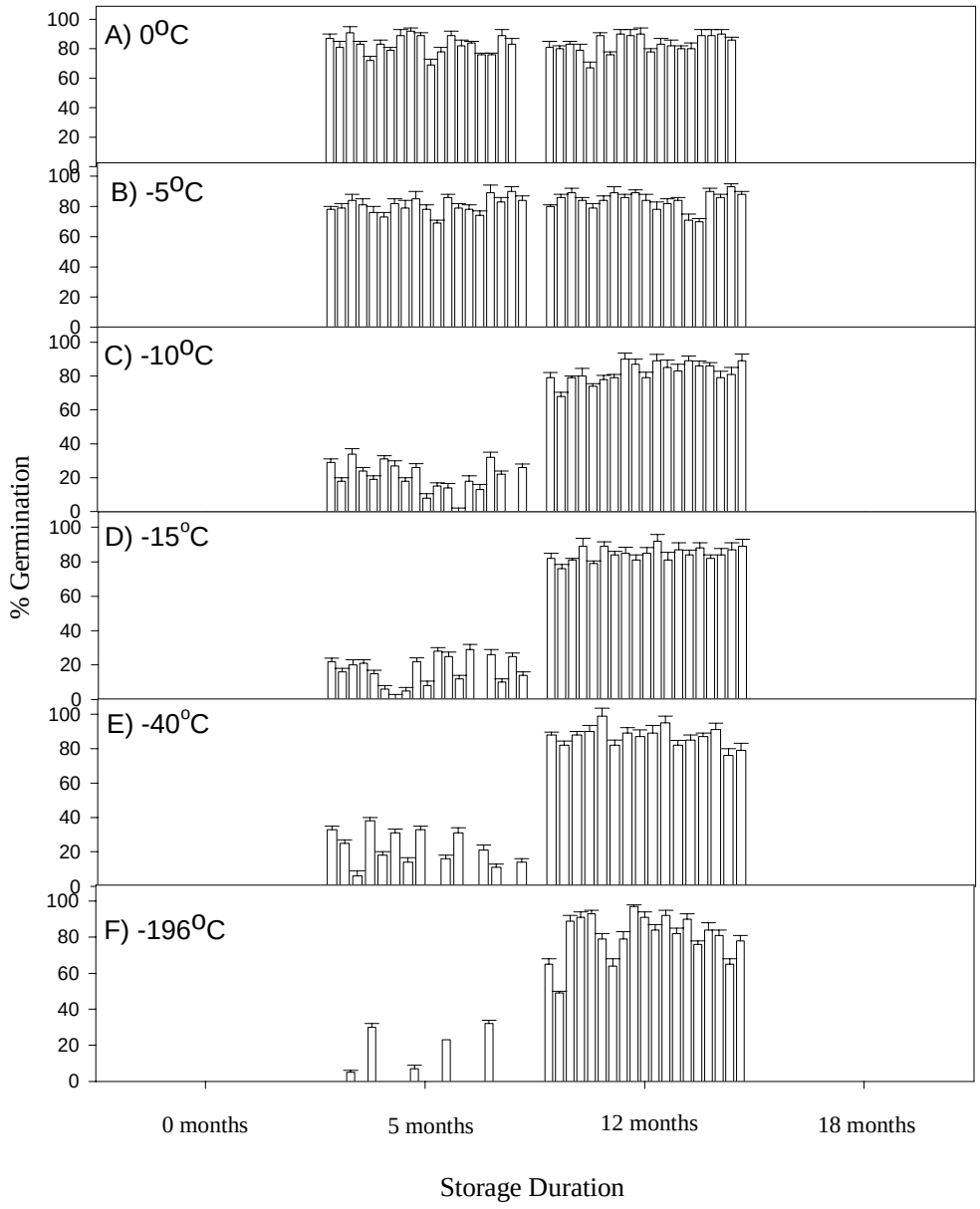

Figure 3. Germintion of butternut axes after exposure to A) 0°C, B) -5°C, C) -10°C, D) -15°C, -40°C and F) -196°C. Each bar +/- Standard Error at each storage duration represents the % germination of the axes isolated from the nuts of one tree. Data for all 19 trees is presented consecutively for each storage duration, starting with tree #1 and ending with tree #19.

All 19 trees produced 5 months axes, which germinated after exposure to 0 and –5°C (germination ranged between 69-92%, with a mean germination of 78%) (Figure 3A,B). However, not all trees produced nuts with axes which germinated after exposure to temperatures below –5°C (Figure 3C,D,E,F). The number of germinants declined with decreasing temperatures below –5°C. Exposure of the 5 month axes to –10, -15, -40 and –196°C resulted in a large decline in germination, as compared to axes exposed to 0 and –5°C. In addition, after exposure

to –196°C only 5 out of the 19 trees had embryonic axes, which germinated. Axes isolated from all nuts stored for 12 months had a high % germination after exposure to 0 to –196°C (germination ranged between 52 –98 %, with a mean germination of 83%) and after 18 months of storage none of the axes were tolerant of these low temperatures (Figure 3A-F).

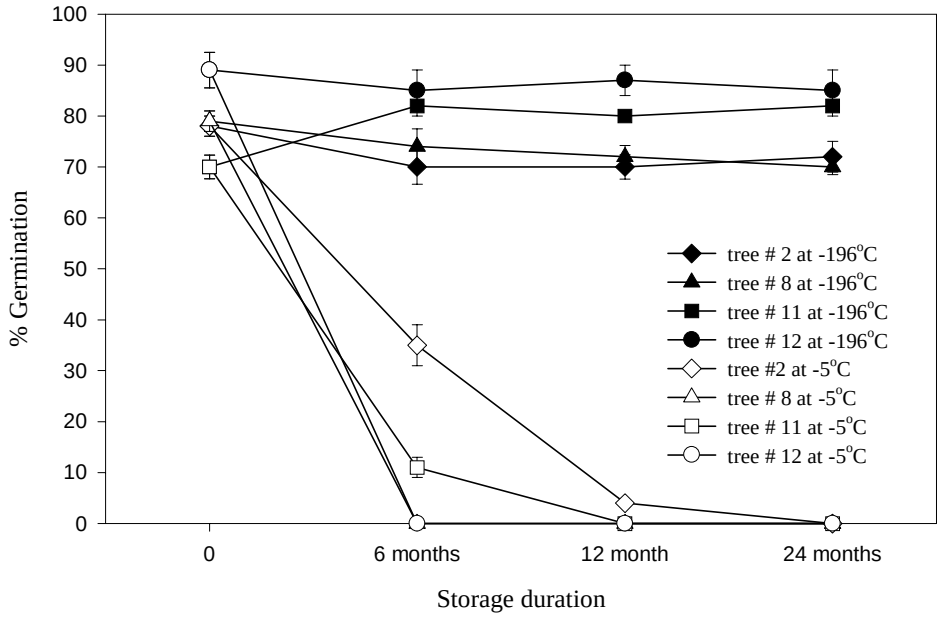

Figure 4. Percent germination of butternut axes after 0, 6, 12 and 24 months of storage at –5 and –196°C. Data is presented for axes isolated from 4 trees, tree-2, tree-8, tree-11 and tree-12.

Axes isolated from the nuts of 4 trees (tree #2, 8, 11 and 12) did not tolerate storage at –5°C for 6, 12 or 24 months (Figure 4). These axes exhibited 72-89 % germination after storage at –196°C for durations up to 24 months (Figure 4) and these germinated axes formed healthy plantlets (Figure 5).

Figure 5. Germinating butternut embryonic axes after 24 months of storage at –196°C.

Conclusion

The duration of nut storage prior to excision of the axes is a critical factor when considering axis' tolerance to low temperatures. Axes isolated from nuts stored for 12 months exhibit the highest percent germination after exposure to -10, -15, -40 and –196°C. This increase in low temperature tolerance may be related to a decline in water content, since water content of the 12 month stored axes decreased to a range of 5-12%, with mean water content of 8.2%. However, the change in water content is not the only reason for increased tolerance, since 0

month axes at low water contents did not tolerate low temperature exposure. Possibly, over the 12-month storage duration a gradual loss in water content allows the tissue to adapt to this desiccation stress, and the biochemical changes that occurred increasing desiccation tolerance also increased low temperature tolerance.

Cryopreservation is proposed to be the only method currently available, which will ensure that the material will be preserved in the long-term (Sakai, 2000). Axes were still viable after 24 months of storage at –196^{o}C, suggesting that cryopreserving axes may be a means for the long-term preservation of butternut, which could serve as a reserve of noninfected butternut for future use.

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Efecto de las Temperaturas de Helada en Bellotas de *Quercus ilex* L.

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Abstract

The effect of two freezing temperatures (-7 and -12 ºC) and the duration of freezing (1, 3 and 7 days) on the germination and viability of *Quercus ilex* acorns was studied. Acorns from five provenances were used. Germination tests were performed following the ISTA rules for *Quercus* species. Viability was evaluated by two methods: the tetrazolium and the electroconductivity tests. Duration and intensity of freezing reduced germination in all provenances, but those from colder sites were less affected than those from warmer ones. Resistance to freezing was not influenced by the size or the water content of the acorns. No correlation was found between germination and either the tetrazolium or the electroconductivity test. It is concluded that the climate of the provenances is related to the freezing resistance of *Q. ilex* acorns. The tetrazolium and electroconductivity tests are shown to be poor predictors of seed viability. More studies are needed to develop new methods in order to estimate the viability of *Q. ilex* acorns.

Key words: *Quercus ilex*, provenances, freezing temperatures, tetrazolium test, electronconductivity test

Introducción

La dispersión de la semilla desde el árbol madre y su posterior germinación y crecimiento como plántula son etapas importantes para comprender cómo ocurre la sustitución de unos individuos por otros en los ecosistemas forestales mediterráneos. Aún se desconoce muchos de los mecanismos que permiten la regeneración de las especies leñosas, y que sin duda serían de mucho interés en nuestros esfuerzos de reforestación. La semilla una vez formada necesita llegar a un ambiente propicio para que su crecimiento tenga lugar. Tras su dispersión, las semillas deben permanecer en estado de quiescencia o de letargo hasta que se den las condiciones ideales en cuanto a humedad, temperatura y, en muchas ocasiones, luminosidad, para iniciar los procesos germinativos que conlleven el asentamiento de una plántula y el posterior crecimiento de un nuevo árbol. Durante este período de espera, que en ocasiones suele ser de varios meses, el medio ambiente produce cambios en la cubierta de las semillas, lo que facilitará la germinación en algunas especies, mientras que en otras provocará una disminuición de la viabilidad.

La encina (*Quercus ilex*) es una de las especies más importantes de la cuenca mediterránea, y define muchas de las formaciones forestales más representativas que se pueden encontrar. El fruto de la encina, la bellota, se forma a finales de otoño y su diseminación tiene lugar durante el invierno. La bellota en ocasiones padece inviernos extremadamente secos y muy fríos, que desconocemos como afectan a su viabilidad. Desde el Centro de Mejora Forestal "El Serranillo" se distribuye semilla de esta especie a todo el estado español, la cual es recogida en el campo por personal especializado, y que, según hayan sido las condiciones ambientales del año, puede haber sufrido o no daños en su viabilidad.

Por otra parte, no se dispone de protocolos auspiciados por ninguna asociación internacional de análisis de semillas que evalúen el estado de la bellota de *Quercus ilex*. En el laboratorio de semillas del CMF "El Serranillo" se siguen las recomendaciones de la ISTA. El problema existente es que muchas de las especies mediterráneas, entre ellas la encina, no tienen un protocolo adaptado para la evaluación de la viabilidad.

Por todo ello, nos planteamos la realización de este trabajo, en el que dos preguntas de tipo biológico: ¿qué temperaturas de frío son capaces de resistir las bellotas en el campo? y ¿existe relación entre la resistencia y el origen geográfico de la semilla?, nos dan pie a responder otras de tipo práctico: ¿se pueden utilizar el test de tetrazolio y el de electroconductividad? y, en su caso, ¿cuál es el mejor método para evaluar la viabilidad de las bellotas de *Quercus ilex*?

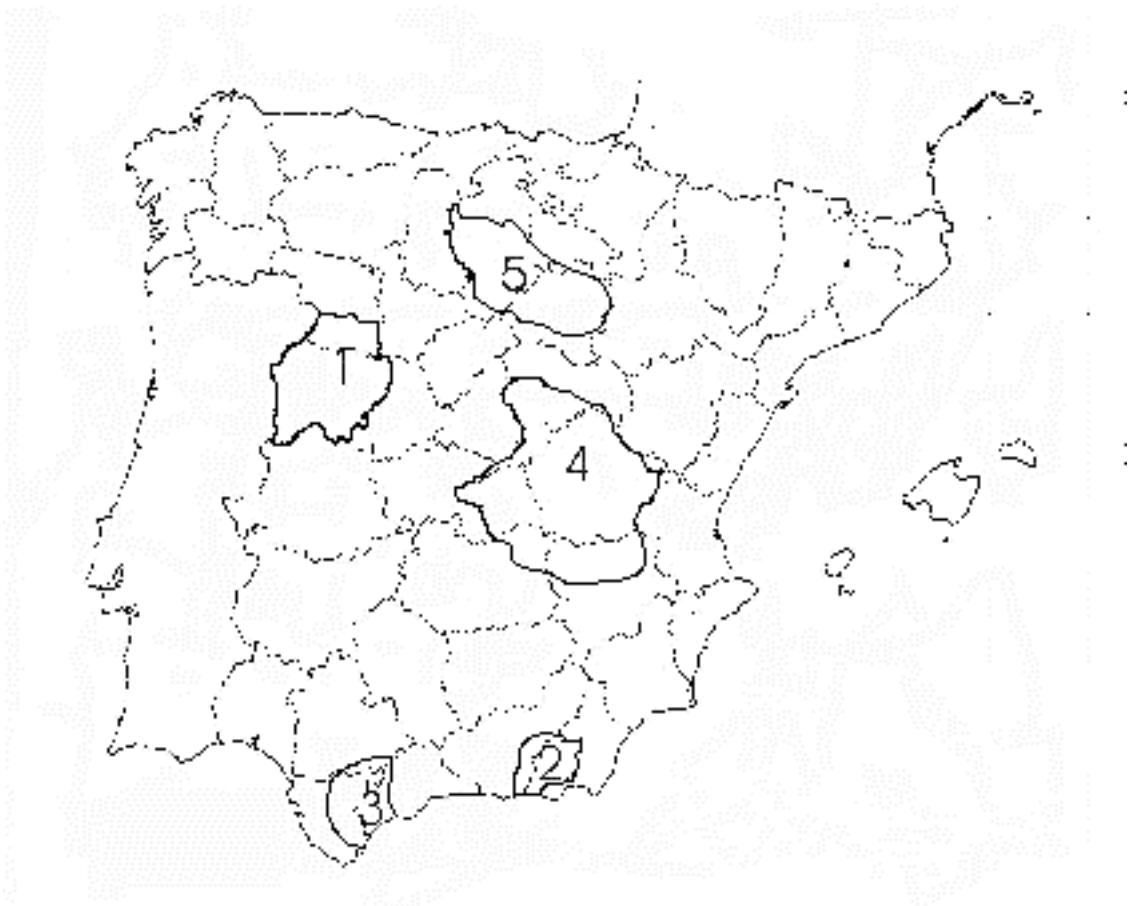
Material y Métodos

Se seleccionaron 5 procedencias atendiendo a las condiciones de pluviosidad, temperatura y heladas (seguras o probables) anuales. Se trató de seleccionar lugares con condiciones climáticas muy diferentes, para que el

análisis de la influencia del origen de la semilla resultará lo más contrastado posible. Las procedencias seleccionadas se indican en la tabla 1 y en el mapa 1.

Tabla 1. Características climáticas de las procedencias seleccionadas. P (mm): precipitación media en milimetros, tm (ºC): temperatura media en grados centígrados, hs: meses de helada segura, hp: meses de helada probable.

Procedencia	**P (mm)**	**tm (°C)**	**hs**	**hp**
1: Región Extremadurense/Noriental	670	15.9	0	4
2: Sierra de Gador	605	13.2	1	3
3: Sierra de Cádiz-Ronda	1209	16.5	0	2
4: La Mancha	525	13.7	2	5
5: Páramos Castellanos-Orientales	434	11.4	4	6



Mapa 1. Procedencia de los lotes seleccionados. 1: Región Extremadurense/Noriental, 2: Sierra de Gador, 3: Sierra de Cádiz-Ronda, 4: La Mancha, 5: Páramos Castellanos-Orientales.

Para cada uno de los lotes se realizaron las siguientes pruebas:

- Caracterización morfológica de la bellota. De cada una de las procedencias se tomaron 10 semillas en las que se efectuaron las siguientes mediciones: peso seco, longitud y diámetro mayor.

- Ensayo de congelación. La idea de este ensayo era evaluar el efecto sobre la bellota de unas condiciones de helada similares a las que se podría encontrar en el campo. Para ello se utilizó un arcón

congelador, con rangos de trabajo de entre 20 ºC a -25 ºC, carente de luz interior, con ventilador para permitir el movimiento del aire, y con un programador que permitía el diseño de la helada. Se consideraron períodos consecutivos de calor de 10 horas y de frío de 14 horas. La temperatura máxima a alcanzar durante la fase de calor se fijó en 7 ºC. Para los períodos de frío se distinguieron dos diferentes ciclos de helada, cuya temperatura mínima fue -7 ºC y -12 ºC respectivamente. Las temperaturas citadas se alcanzaban de una forma progresiva a lo largo del programa diseñado, manteniendose la temperatura máxima durante 2 horas y la mínima durante 3 horas. A su vez, las bellotas fueron sometidas a dichos ciclos térmicos durante 1, 3 y 7 días.

Se sometieron a estos ciclos de helada 4 repeticiones por lote y tratamiento (temperatura y duración) de 150 bellotas, introducidas en recipientes de plástico y mezcladas con un poco de turba para evitar pérdidas de humedad durante el ensayo. Se introducía una sonda en los recipientes para asegurar que en el núcleo se alcanzaba la temperatura diana.

Para corroborar que no había habido pérdidas de humedad durante el ensayo de congelación, y sabiendo que el contenido hídrico está intimamente relacionado con la germinación de las semillas de *Quercus* (Domínguez et al. 1995, Finch-Savage and Blake 1994, .Finch-Savage and Clay 1994, Finch-Savage and Farrant 1997, Bonner 1984), se evaluó el porcentaje de humedad antes y después de cada ciclo de helada.

El contenido de humedad se determinó sobre 4 repeticiones de 10 bellotas por cada procedencia y por tratamiento. Se utilizó el método descrito por la ISTA (ISTA 1999) para la medición de humedad mediante la técnica de la estufa, adaptándolo a la especie en cuestión. Antes y después del período de congelación, las 10 semillas eran troceadas por la mitad, tomándose la parte que contenía al embrión y desechando lo demás. Luego se troceaba en finas lonchas, se introducía en el pesafiltros una cantidad de 5 g y se dejaba 17 horas en una estufa a 105 ºC. Posteriormente se pesaba la muestra y se calculaba el contenido de humedad en función de la pérdida de peso.

Para evaluar el efecto de la helada sobre las bellotas se realizó un ensayo de germinación o de facultad germinativa. En este ensayo se utilizaron 4 repeticiones de 25 semillas provenientes del ensayo de resistencia a la helada. Se colocaban en bandejas de plástico, en sustrato de arena mezclada con agua y una pequeña proporción de fungicida (criptonol al 0.1%). Se mantenían en cámara a 20 ºC, en ciclos de 8 horas de luz y 16 horas de oscuridad, condiciones en las que se conoce que ocurre la germinación de la bellota. El umbral germinativo se sitúa a los veinte días de iniciado el ensayo, realizándose posteriormente conteos una vez a la semana hasta la finalización del ensayo. Se distinguieron hasta 4 categorías para clasificar las bellotas: normales, si daban lugar a una plántula con todos sus organos perfectamente desarrollados; anormales, si algunas de las partes aparecen mal desarrolladas, siguiendo la nomenclatura de la ISTA; duras, semillas aparentemente en buen estado pero que tras finalizado el ensayo no se aprecia que hayan iniciado los procesos germinativos; y muertas.

- Ensayo de viabilidad. El test de tetrazolio es una de los más utilizados para evaluar el estado de la semilla según las normas ISTA. En el caso de las bellotas de encina hemos modificado, de acuerdo con la experiencia propia al respecto, las recomendaciones dadas por la ISTA, que aconsejan humedecer las semillas previamente al análisis. En nuestro ensayo se preparó la semilla en PSM (pretratamiento sin medio), para lo cual se colocaron las bellotas entre papel húmedo a 5 ºC durante 24 horas, tras eliminar la cubierta exterior dura y haber cortado la parte final de la bellota. Transcurrido este período se eliminó el endospermo y se sumergieron las bellotas en una solución de sal de tetrazolio al 1 % durante 22 horas. Después se sometieron 2 horas a una temperatura de 40 ºC en una estufa para acelerar la reacción. La evaluación de los embriones se hacía abriendo los dos cotiledones y exponiendo a examen visual el embrión. Las bellotas así valoradas se dividían en vivas, cuando aparecen totalmente coloreadas de rojo, o bien, las zonas no coloreadas no afectan al embrión; muertas, si alguna parte del embrión aparecía sin colorear; y de vitalidad limitada, cuando era difícil distinguir entre las dos categorías anteriores. Cada ensayo comprendía 4 repeticiones con 5 bellotas cada una.

- Ensayo de electroconductividad. Este tipo de test está actualmente evaluándose como complemento a otros tests de viabilidad, aunque aún se discute su utilidad como predictor de la germinación. Con este método se estudia el daño existente en las membranas celulares de los tejidos de la semilla mediante la medición y cuantificación de los compuestos con carga eléctrica, aniones y cationes, liberados en agua destilada transcurrido un tiempo prefijado. Para este ensayo se diseñó el protocolo que se expone a continuación. Se tomaron 12 bellotas por tratamiento, repartidas en 4 repeticiones de 3. Previamente a

su limpieza, mediante inmersión en agua destilada durante 15 minutos, fueron pesadas. Transcurrido este tiempo, se lavaban repetidas veces con agua destilada, y se introducían en tubos de ensayo con 30 ml de agua destilada. Al cabo de 24 horas se medía la concentración iónica con un electroconductivimetro módelo CRISON microCM 2201, cuya constante de electroconductividad era 0.99 mS/cm. Los resultados quedaron expresados en mS/cm/g.

Los resultados se trataron mediante el paquete estadístico STATISTICS, realizando una ANOVA.

Resultados

- Caracterización de la bellota por lotes. Los resultados se expresan en la tabla 2. Existen diferencias estadísticas entre lotes, en cuanto a peso, diámetro y longitud, como se indica en la tabla 3. Tal vez lo más interesante es ver como se pueden agrupar en dos grandes grupos según su peso, que aglutinaría por un lado a los lotes 1, 2 y 3, y por el otro a los lotes 4 y 5.

Tabla 2. Media de los caracteres morfológicos obtenidos en la caracterización de la bellota según procedencias.

Procedencia	Diámetro (mm)	Longitud (mm)	Peso (g)
1	15.47	35.04	5.11
2	16.54	32.69	5.30
3	15.14	34.68	4.79
4	14.25	28.81	3.45
5	14.37	26.01	2.89

Tabla 3. Resultados estadísticos obtenidos en el análisis de ANOVA, para significaciones menores a p=0.05. Lote indica el origen de la semilla (tabla 1), Temp la temperatura a la que se sometió en el ensayo de congelación, Días el número de días que duró la helada, SqrtFG es la raíz cuadrada de la facultad germinativa, TT es el resultado obtenido en % en el Test de Tetrazolio, HUM es la humedad del lote analizado, Peso es el peso de las semillas, Diam la anchura y Long la longitud. Log indica el logaritmo. 1= Lote, 2= Temperatura, 3=Días.

Interacción	Valor de F	Valor de P
Lote*SqrtFG	5.17	0.0009
Temp*SqrtFG	150.9	0.0000
Dias*SqrtFG	9.13	0.0002
12*SqrtFG	2.77	0.0320
23*SqrtFG	9.56	0.0002
Lote*Log(TT+1000)	11.612	0.0000
Temp*Log(TT+1000)	56.209	0.0000
Dias*Log(TT+1000)	8.241	0.0005
Lote*Diam	3.7713	0.0099
Lote*10(1/Long)	8.4068	0.0000
Lote*log10Peso	67.072	0.0000
Temp*log10Peso	54.672	0.0000
12*log10Peso	5.113	0.0009
23*log10Peso	11.717	0.0000
123*log10Peso	2.449	0.0191

- Ensayo de congelación de la bellota. Los resultados obtenidos en el análisis de contenido de humedad quedan representados en la tabla 4. No se aprecian diferencias significativas entre lotes ni entre tratamientos.

  Los resultados de germinación se expresan en la tabla 4. Las diferencias en cuanto a germinación o humedad entre los ensayos control correspondientes a cada lote obedecen a que los análisis se realizaron diferidos en el tiempo. Se observa que las temperaturas más bajas y la mayor duración del ensayo afectan a los resultados obtenidos en la germinación.

Tabla 4. Resultados obtenidos tras someter las bellotas a cada uno de los tratamientos. Se indican las medias de germinación, humedad y test del tetrazolio (todos expresados en porcentaje).

Procedencia	Tratamiento	% Germinación	Humedad	Test de Tetrazolio
1	Control -7 °C	62	33.62	52
	Control -12 °C	79	33.34	32
	-7 °C 1 día	63	33.86	57
	-7 °C 3 días	67	34.55	52
	-7 °C 7 días	68	33.45	50
	-12 °C 1 día	34	38.26	30
	-12 °C 3 días	25	38.39	32
	-12 °C 7 días	26	38.45	45
2	Control -7 °C	61	33.25	65
	Control -12 °C	72	37.41	27
	-7 °C 1 día	77	35.40	62
	-7 °C 3 días	81	37.27	70
	-7 °C 7 días	67	35.18	77
	-12 °C 1 día	57	36.32	37
	-12 °C 3 días	30	37.97	17
	-12 °C 7 días	29	38.04	55
3	Control -7 °C	63	34.48	32
	Control -12 °C	54	36.54	12
	-7 °C 1 día	57	34.37	42
	-7 °C 3 días	63	34.83	40
	-7 °C 7 días	68	34.64	65
	-12 °C 1 día	46	36.03	10
	-12 °C 3 días	33	36.67	17
	-12 °C 7 días	26	39.46	47
4	Control -7 °C	73	42.82	17
	Control -12 °C	34	39.39	15
	-7 °C 1 día	51	41.19	45
	-7 °C 3 días	51	42.05	17
	-7 °C 7 días	46	37.77	30
	-12 °C 1 día	40	39.33	2
	-12 °C 3 días	37	39.25	0
	-12 °C 7 días	16	39.59	22
5	Control -7 °C	61	39.60	52
	Control -12 °C	61	38.77	40
	-7 °C 1 día	49	35.11	80
	-7 °C 3 días	57	38.50	52
	-7 °C 7 días	61	39.36	65
	-12 °C 1 día	58	38.41	30
	-12 °C 3 días	39	38.90	37
	-12 °C 7 días	30	40.15	27

El análisis estadístico (tabla 3) nos indica cómo el origen del lote (variable Lote) afecta en mayor o menor medida a nuestros resultados en terminos de porcentaje final de germinación (FG), al igual que la temperatura diana de la helada (variable Temp) y la duración de la misma (variable Dias). Además,

existe una interacción entre el lote y la resistencia al frío (interacción Lote y Temp) y entre la duración y temperatura diana (tabla 4).

- Ensayo de viabilidad. Los resultados obtenidos, expresados en porcentaje, figuran en la tabla 4. En el gráfico 1 se reflejan los resultados alcanzados en los diferentes lotes mediante el test del tetrazolio y el ensayo de facultad germinativa. Las correlaciones obtenidas entre la raíz cuadrada del porcentaje de facultad germinativa y el logaritmo del porcentaje del test del tetrazolio son bajos (r^2= 0.07811, p=0.000348). Se aprecian diferencias estadísticas en cuanto a lote, temperatura y duración de la helada, pero no encontramos ninguna interacción de estos factores en nuestros análisis.

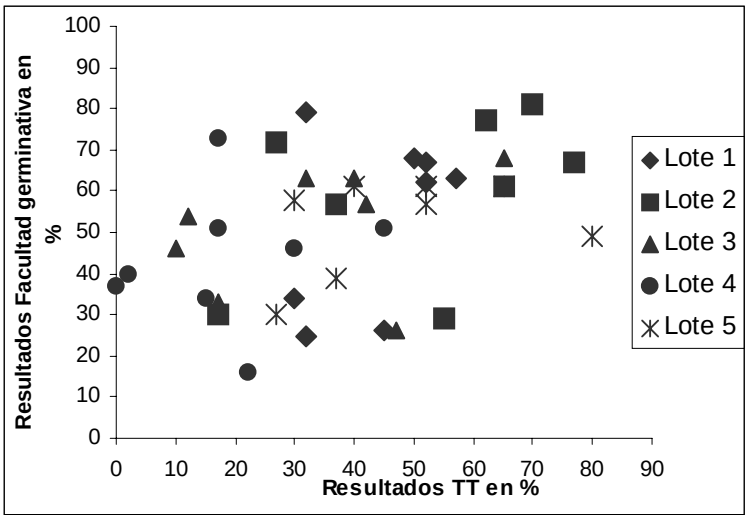


Gráfico 1. Resultados obtenidos en el test de tetrazolio (TT en el gráfico, en porcentaje) y en el ensayo de facultad germinativa (en porcentaje) por lotes.

- Ensayo de electroconductividad. Los resultados alcanzados con este test están pobremente correlacionados con la facultad germinativa (r^2= 0.05409, p=0.0030).

Discusión

En primer lugar, hay que reseñar que existen diferencias entre las procedencias seleccionadas en cuanto a mantenimiento de la facultad germinativa. Es decir, la respuesta que hemos obtenido en los lotes considerados ante una situación de estrés como es la helada depende del origen de la semilla. Por norma, la facultad germinativa se vió reducida de forma importante cuando las bellotas fueron sometidas a temperaturas de -12 ºC, independientemente de su procedencia, algo que no sucedió con la temperatura diana de -7 ºC. Ello nos induce a pensar que esta temperatura puede ser soportada por la especie sin verse prácticamente afectada en su germinación. Hay que resaltar que el lote 4, procedente de La Mancha (tabla 1), sí que vió mermada su facultad germinativa a -7 ºC. Resulta llamativo, ya que su zona de origen, La Mancha, curiosamente es la procedencia de condiciones climáticas más continentales de todas las seleccionadas, como aparece indicado en la tabla resumen 1. Podriamos suponer que la bellota previamente a su análisis había sufrido algún tipo de contratiempo, a modo de envejecimiento, que la predisponía a una pérdida más acelerada de su viabilidad.

Dado que el contenido de humedad de la bellota sólo ha experimentado unas variaciones muy ligeras, cabe decir que las diferencias habidas en cuanto a germinación han resultado independientes de la humedad de la bellota, y por lo tanto producidas por características inherentes al origen de la semilla.

A pesar de no haber encontrado relaciones estadísticas entre el número de heladas probables y/o seguras (tabla 1) correpondientes al origen y la facultad germinativa, suponemos que deben de existir caracteres genotípicos o fenotípicos que influyen en la respuesta que hemos obtenido en este ensayo. Es díficil cuantificar o determinar de alguna manera qué responsabilidad genética hay en estos resultados, ya que no es un objetivo de este trabajo. Sin duda el origen de la semilla influye en la respuesta ante determinados factores (Tripathi and Khan 1990, Milberg et al. 1996, Andersson and Milberg 1998). Y la definición de procedencia guarda relación con las características morfológicas (tablas 2 y 4) y de comportamiento, como queda reflejado en los análisis estadísticos.

Teniendo en cuenta que ante un estrés determinado, la semilla sufre un deterioro que puede estar relacionado con el envejecimiento, es difícil interpretar los resultados obtenidos en nuestros ensayos de electroconductividad y tetrazolio. El envejecimiento de la semilla produce un deterioro de las estructuras y funciones de la semilla con el tiempo (Mohamed-Yassen et al. 1994). No necesariamente tiene que producir la muerte de la semilla este envejecimiento, pero ante determinados tipos de estrés, la semilla puede morir, siendo ésta una posible explicación a lo acontecido con el lote 4. El envejecimiento de la semilla produciría una merma en la calidad germinativa del lote.

La liberación de electrolitos en semillas deterioradas es un indice de daños de la membrana (Parrish et al. 1982), que está relacionado con un envejecimiento de la semilla. La liberación de estos electrolitos al medio sin ninguna duda dependerá de las condiciones de dilución del medio y de la capilaridad de la cubierta de las semillas. Los resultados que hemos obtenido nos indican que la electroconductividad no está relacionada con el lote, ni con los daños producidos por la duración y la temperatura de la helada. En trabajos realizados con otras especies se han obtenido buenas correlaciones entre la facultad germinativa y la electroconductividad (Bonner 1988, Bonner 1991, Bonner and Agmata-Paliwal 1991). Este autor indica (Bonner 1998) que la citada técnica presenta un grave problema, que es la variación que existe entre lotes que provienen de diferentes poblaciones. Recomienda su buen uso para predecir el vigor de lotes almacenados, pero no lo preconiza como predictor de la germinación, y no está presente como técnica recomendada para el análisis de semillas de *Quercus* en EEUU (Bonner 1984). Nuestros resultados avalan esta hipótesis, ya que hemos tenido una gran variabilidad de datos que han estado pobremente correlacionados con la facultad germinativa. Podemos decir que esta técnica no ha sido válida para *Quercus ilex*, mientras que sí hemos encontrado buenas correlaciones para otros ensayos realizados con especies del género *Pinus* (Moreno-Álvarez et al. 2001).

En cuanto al test del tetrazolio, los resultados obtenidos tampoco apoyan su utilización como predictor de la germinación. Podemos apreciar las diferencias producidas por los diferentes tratamientos (tabla 4), pero no correlacionar con fiabilidad la facultad germinativa con el test de tetrazolio (gráfico 1). En ocasiones se recomienda este test como buen predictor de la germinación, tanto para semillas de *Quercus* como de otras especies, aunque a veces no presenta buenos resultados (Bonner 1984, ISTA 1999, Moreno-Álvarez et al. 2001). Posiblemente la mayor dificultad que presenta es encontrar un protocolo adaptado, de forma que las tinciones sean lo suficientemente homogeneas para asegurar una buena interpretación de los resultados, que en ocasiones se ven afectados por la cantidad de lipidos, tiempo de tinción, condiciones de temperatura en las que se lleva a cabo la reacción, o incluso experiencia del analizador en dicha especie y en el método.

Conclusiones

1. La morfología de la bellota es diferente según el origen geográfico del lote muestreado.
2. La resistencia a la helada se ha mostrado diferente atendiendo al origen de las bellotas, pero es imposible caracterizar la respuesta ante el frío como genotípica o ambiental. Serían necesarios más estudios.
3. Las bellotas de *Quercus ilex* mantienen su facultad germinativa, sin pérdidas apreciables, cuando son sometidas a una temperatura que alcanza los -7 ºC, aún en el transcurso de varios ciclos consecutivos, pero se reduce seriamente si tal temperatura diana baja a -12 ºC, produciéndose tal disminución de la viabilidad de forma progresiva en función de la permanencia en el tiempo del período de helada.
4. El mejor método para la evaluación de la viabilidad de las bellotas de *Quercus ilex* es el ensayo de facultad germinativa. Ni el test de tetrazolio ni el de electroconductividad permiten una buena predicción de la germinación en bellotas de *Quercus ilex* sometidas a períodos de frío.

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Response of *Pinus halepensis* to Different Seeding Techniques in a Climatic Gradient

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Abstract

A nursery experiment was conducted to determine the influence of seed pre-treatment and seeding technique on *Pinus halepensis* seedling emergence. The experiment was performed in three localities distributed in a gradient from sub-humid to semi-arid Mediterranean climate, under two irrigation regimes (moderately and non irrigated). Pre-treatments tested consisted of seed priming on sand (Primed) and seed priming on sand with gibberellic acid 10^{-3} M solution (Primed + GA). Seeding was performed in early October and included broadcast seeding, with and without chopped charred wood mulching, and seed dibbling at 1cm depth. Although autumn and winter were rainier than average, irrigated plots showed higher seedling emergence (75 %) than non irrigated (55 %). Broadcast seeding plus mulching showed excellent results, with percent germination around 80% under all climates and irrigation conditions. The results of the other techniques were dependent on water availability: dibbling seeding showed 80 % germination on irrigated plots and 54 % on non irrigated plots, whereas broadcast seeding without mulching showed 61 % germination on irrigated plots and only 25 % on non irrigated. Pre-treated seeds germinated first. The first month after seeding, germination percentage was higher for pre-treated than for control seeds (51% for primed + GA , 40% for primed, and 13% for control). Nevertheless these differences disappeared during the following months. Under Mediterranean climates, seeding techniques offer promising potential to enhance seedling emergence.

Keywords: *Pinus halepensis*, seeding techniques, mulching, seed pre-treatment, restoration

Introduction

During recent years, the direct seeding of forestry species, especially with coniferous, is being introduced as an alternative to the plantation technique to restore large areas affected by wild fire. Direct seeding offers mainly economic advantages by eliminating nursery and transport costs. The technique is particularly of interest in the case of many *Pinus halepensis* forests in the East of Spain where high fire recurrence has severely limited natural regeneration of woody species.

Direct seeding usually requires additional action that may contribute to favouring seed germination, such as seed pre-treatment or improving seeding technique, since this is a critical period that determines the success of the seeding. Under dry Mediterranean climatic conditions, periods of time with adequate environmental conditions for germination in the field are very short or unpredictable. In these conditions, the germination rate determines whether a seed can make use of the time when the combination of environmental factors favours germination (Adams 1999). Therefore the use of seed pre-treatments which shorten the mean germination time could contribute to enhancing seedling germination.

Although *Pinus halepensis* seeds are not affected by a real dormancy syndrome, they show a characteristic slow germination rate. *Pinus halepensis* seeds in optimal conditions can require more than three weeks to complete seed germination. (Thanos and Skordilis 1987).

Controlled seed pre-hydration treatments which involve seed osmopriming, seed hydropriming or seed priming in a solid matrix have been used to enhance germination rate of many horticultural and forest species (Bradford 1986, Gray et al. 1991, Winsa and Bergsten 1994), even under stressful environmental conditions (Bradford 1986, Carpenter and Boucher 1991). The effect of the treatments upon seed germination must endure after seed re-drying as a requirement for their suitability to be applied in direct seeding.

The seeding technique is also very important to facilitate seed germination. Covering the seeds with a layer of soil or mulching material could contribute to enhancing seedling emergence under field conditions by protecting the seeds from temperature fluctuations and increasing water availability around the seed. Organic mulching application has been shown to be a very efficient technique to preserve soil moisture content in the upper soil layer (Bautista et al 1996), nevertheless this technique is expensive and cumbersome.

The objective of this work was to test the effect of seed pre-treatment and seeding technique on seedling emergence in different climatic conditions to find a suitable *Pinus halepensis* direct seeding protocol for dry Mediterranean conditions.

Materials and Methods

Seeds

Pinus halepensis seeds were provided by the seed bank of the Regional Government of Valencia. This seed lot was collected from Sistema Iberic (E-SE of Spain) in December of 1999. Seeds were dry stored at 5 ºC until use.

Seed pre-treatments

Three treatments were tested: a) Seeds primed during 6 days on 15% moistened sand at 20ºC of temperature and darkness (primed) b) Seeds primed in the same conditions as above but the water used to moisten the sand was replaced by a solution of Acid gibberelic 10^{-3}. Molar (primed + GA). Seeds after treatment were removed from the sand and allowed to dry out at ambient temperature during 48 h. These two treatments were selected from previous laboratory experiments, in which different pre-hydration techniques and growth regulators were tested (Bladé et al. 2002) and c) Control (without any treatment).

Germination tests in the laboratory consisted of: four replicates of 50 seeds per Petri dish germinated on two layers of moistened filter paper Watman nº 2 in a germination cabinet at 20 ºC and darkness. Final germination percentage (GT) and days to 50% GT (T_{50} an inverse measure of germination rate) were calculated. All data were subjected to a 1-way of analysis of variance.

A nursery experiment was conducted on three different localities distributed in a climatic gradient: Barcelona (Sub-humid), Valencia (Dry) and Alicante (Semi-arid), located in the East of Spain. The experiment was realised under two irrigation regimes: non-irrigated and moderately irrigated. We tested the effect of seed pre-treatment on seedling emergence in combination with three different seeding techniques: a) broadcast seeding, b) broadcast seeding plus chopped charred wood mulching, and c) seed dibbling at 1cm depth. Four replicates of fifty seeds each per treatment combination (seed pre-treatment, seeding technique) were installed per each locality and the two irrigation regimes. The sowing was performed on a 30 cm diameter and 30 cm depth container filled with mineral soil. The soil type was a *Calcic Luvisol* (FAO). Containers were 30 mm irrigated prior to seeding. The pots were sowed between 12 and 18 of October. Seedling emergence was monitored three times: on 11/13/01 (a month after seeding), on 11/30/01 and on 01/15/02. Emergence data were subjected to an analysis of variance where seeding technique, seed pre-treatment and irrigation regime were considered as fixed factors, and locality as aleatory factor.

Results and Discussion

The *Pinus halepensis* seed lot used for these assays presents a germination capacity higher than 80% under laboratory conditions. In these conditions, pre-treated seeds did not result in higher germination percentage compared to the untreated ones (Table 1). Seeds of the two priming treatments germinate faster than non-treated ones, they took around 7 days less than control to achieve 50 % germination (Table 1). At optimal conditions, the addition of the growth regulator in the priming solution did not represent any advantage over priming in water. Nevertheless, we decided to test these treatments under field conditions because the gibberellic acid effect could be more relevant under sub-optimal conditions as it has been reported for horticultural species when they are early sown (Khan et al. 1995).

Table 1. Final germination percentage (GT) and days to 50% final germination (T_{50}) under laboratory conditions at 20ºC.

Seed Pre-treatment	GT (%)	T_{50} (days)
Control	82 ± 8.5 a	18.7 ± 2.7 a
Primed	76.5 ± 1.2 a	11.6 ± 0.9 b
Primed + GA	83.5 ± 5.7 a	11.9 ± 2.7 b

Means for each column followed by the same letter are not significantly different at P>0.05

In the nursery experiments, the germination took place from early November (11/13/01), one month after seeding, to middle January (01/15/02). Although the climatic conditions were favourable to spring germination, only a few seedlings (1 or 2 seedlings per pot) were recorded after January (data not shown).

Table 2. Effects of seeding technique, seed pretreatment, irrigation regime and locality on seedling emergence according to Anova.

Source of variation	E 11/13/01	E 11/30/01	E 01/15/01
Seeding technique	p<0.029	p<0.002	p<0.002
Pre-treatment	p<0.048	ns	ns
Irrigation	p<0.014	p<0.019	ns
Locality	ns	ns	ns
Seeding technique * Pre-treatment	ns	p<0.001	ns
Seeding technique * Irrigation	ns	p<0.025	ns
Seeding technique * Locality	ns	ns	ns
Pre-treatment * Irrigation	ns	ns	ns
Pre-treatment * Irrigation	ns	ns	ns
Pre-treatment * Locality	ns	ns	ns
Irrigation * Locality	ns	ns	ns
Seeding technique * Pre-treatment * Irrigation	ns	ns	ns
Seeding technique * Pre-treatment * Locality	ns	ns	ns
Seeding technique * Irrigation * Locality	p<0.025	p<0.005	ns
Pre-treatment * Irrigation * Locality	ns	ns	ns
Seeding technique * Pre-treatment * Irrigation * Locality	ns	ns	ns

In early November (11/13/01), seeding technique, seed pre-treatment and irrigation regimen significantly affected seed germination. There was also a significant interaction between seeding technique, irrigation and locality (Table 2). At this moment, seedling emergence was strongly dependent on the watering application. The best germination response has been obtained with broadcast seeding plus mulching, followed by dibbling the seeds in the soil and the worst one was observed in the broadcast seeding pots without mulch (Figure 1). The average values for each seeding technique (pooling the data of the three localities) were 31% in the broadcast seeding plus mulching pots, 5% for the dibbling seeds and 1% in the broadcast seeding without mulch on non-irrigated plots. On irrigated plots, germination percentages were 52, 41 and 20% respectively. Seed pre-treatment had enhanced the germination percentage in early November. Seed priming with the hormone solution showed the highest seedling emergence with an average of 50 % germination under irrigation conditions and 21 % under non-irrigated ones, followed by seeds primed with distilled water with 39 % and 12 % respectively. Control seeds germinated 24% and 7% in irrigated and non-irrigated pots.

Final seedling emergence (recorded on 01/15/02) was only significantly affected by the seeding technique (Table 2). Broadcast seeding plus mulch is the most reliable technique under the three Mediterranean conditions tested. It is important to note that the germination percentage achieved with this technique was greater than 80% even on non-irrigated plots (Figure 2). The results of the other techniques were dependent on water availability. Dibbling seeds at 1 cm depth in the soil showed 80 % germination on irrigated plots and 54% on non-irrigated plots, whereas broadcast seeding without mulching showed 61% germination on irrigated pots and only 25 % on non-irrigated ones. Part of the low final germination obtained in the broadcast seeding without mulching pots was due to seed predation by birds observed in the Valencia plot. Nevertheless, this is not far from reality, since seed predation is the most important cause of direct seeding failure in field conditions (Bergstern, 1985). The initial effect of the seed pre-treatment on seed germination was lost as the germination of the control seeds progressed. Nevertheless, under natural conditions these initial differences could be of relevance since seed pre-treatment decreases the time of seed exposure to soil pathogens and seed predators.

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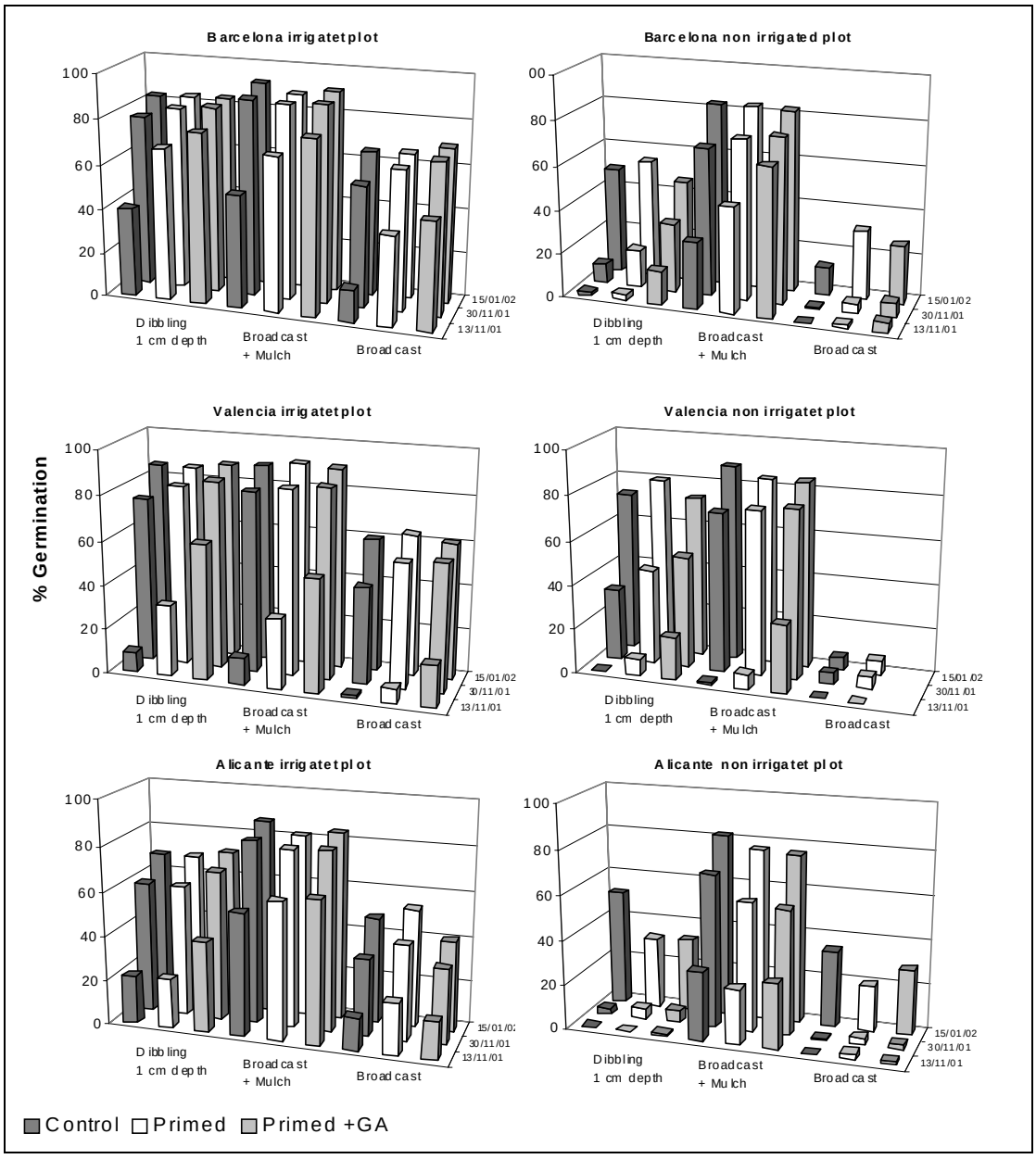

Figure 1. Cumulative percent seed germination of *Pinus halepensis* seeds as a function of seeding technique and seed pre-treatment recorded in Barcelona, Valencia and Alicante on two irrigation regimens (irrigated and non-irrigated).

Germination of Seeds and Seedling Emergence of Common Hawthorn (*Crataegus monogyna* Jacq.)

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Abstract

Seeds of common hawthorn are deep dormant and when sown without any pretretment germinate usually not earlier than after the second or third winter. In Poland, the fully ripe fruits should be picked from the shrubs in October. Stones extracted from fruits should be dried to a moisture content of about 10% (fresh weight basis). One year storage at -3°C of stones dried after harvest does not reduce the germinative capacity of seeds.
Dormancy of seeds can be overcome by stratification of stones in one of the possible thermal systems:

- 25°/3°C (16 weeks at 25°C followed by 14 -18 weeks at 3°C until the first radicles start to appear);
- 20~30°/3°C [16 weeks at 20~30°C (16+8h/day or 24h+24h in a diurual cycle) followed by 14-18 weeks at 3°C until the first radicles start to appear].

Stratified seeds germinate energetically (within 3-5 weeks) and in a high percent at 3~10°, 3~15°, 3~20°, 3~25°C (16+8h/day), the seedlings emerge at 3~20°C (16+8h/day) within 4-6 weeks.
Stones when stratified at 25°/3°C or 20~30°/3°C can be dried at room temperature to a moisture content of 10% without any negative effect on the germination capacity at 3~15°C (16+8h/day).
Scarification of stones in the concentrated sulphuric acid lasting 120 min. followed by stratification at 3°C reduces the germinative capacity of seeds in contrats to the traditional warm-followed-by-cold stratification in a moist medium.
Early sowing in the nursery into a still cool soil should be preferred, because an increased temperature induces secondary dormancy of seeds.

Keywords: storage, dormancy, stratification, scarification

Effects of Light/Darkness and Inhibitory Components on the Germination of *Pinus pinaster*

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Abstract

As with many other species of pine, the only possibility of regeneration for *Pinus pinaster* is from seed. The advantage of germination is that it increases the genetic variability and stability of populations. This species has an important natural recovery rate in the field after a wildfire. However, no germination or survival of seedlings under adult trees with a capacity for producing viable seeds has been seen to exist in the field. The hypothesis of this paper is that germination under the cover of the adult trees could be controlled by the following ecological factors: availability of light, inhibition by substances produced by the aboveground mass of the tree or inhibition produced by substances emitted by decomposing fallen litter. The effect of nutrient availability on germination was analysed. The germination percentages were high in all of the treatments carried out, with a positive effect appearing in the germination of leaf exudates and a lower percentage in the seeds left in total darkness. However, no significant differences are observed among the total germination percentages. The main difference between the effects of the different treatments appears in the mean time required to germinate: it was shortest in the seeds treated with leaf exudates and longest in the seeds left in total darkness.

Keywords: germination, *Pinus pinaster*, light/darkness, inhibitory components, nutrient effects

Introduction

Maritime pine (*Pinus pinaster* Ait.) is an important species in the Mediterranean basin and is found throughout the western range, from France to Morocco and from Portugal to Tunisia. Also, *P. pinaster* diverges significantly from the common origin in different races in the Iberian Peninsula. The Iberian populations belong to 7 of these geographical races (Salvador et al. 2000). This indicates the extremely wide variety of ecological conditions under which they can develop - soils ranging from calcareous to sandy and climates from Mediterranean to Atlantic - resulting in specific adaptations of the species in terms of growth pattern and survival (Alía et al. 1995). For this reason it is difficult to extrapolate the results from research in different areas.

The studied population is in NW Spain and has especially adapted to frequent disturbances such as fire. Amongst these adaptations the capacity to bloom and bear fruit at an early stage in addition to possessing an aerial seed bank and being capable of storing fertile seeds in the closed cones for up to 50 years need to be emphasized (Tapias et al. 1998). *Pinus pinaster* is an obligate seeder (Martínez Sánchez et al. 1995). The advantage of germination is that it increases the genetic variability and stability of the populations (Baskin and Baskin, 1998). One of the observations made in the field is that no seedlings appear under adult populations of *Pinus pinaster* as a result of germination. This has had an influence on studying the possible hypotheses which identify the factors influencing the low rate of population recruitment in the field in a species like this with a high yield of seeds of great viability over long periods of time (Velez 2000).

As occurs in other Mediterranean species (Williamson 1990; Gallet 1994; Pellissier 1994) natural regeneration can be limited by the presence of determined chemical substances which act as germination inhibitors. In many other species the inhibitory effect of exudates produced by leaves on the germination of their own seeds has been demonstrated (Chaves et al. 1997; Li-Jun et al. 1997; Peñuelas and Llusiá 1998, Robles et al. 1999). López Mosquera and Guillen (1993) recorded the presence of allelopathic substances in *Pinus pinaster* both in the bark, where it acts as an inhibitor of various herbaceous species, and in the leaves. Similarly Fernández de Simón et al. (2001) have defined the variation in the composition of the terpenes and different acids of the *Pinus pinaster* acicules. These substances could be considered responsible for seed germination inhibition in mature forests. Gallet and Pellissier (1997) also showed that determined phenol compounds, which inhibit germination, are also persistent in the humus of forests limiting growth and germination at the fallen leaf and humus layer level. Pellissier (1994) documented how the germination and growth of seedlings of some conifers like *Picea abies* are inhibited by the phenol compounds of the humus. Thus, this could partially explain the natural regeneration difficulties of these species in the absence of fire, since fire would eliminate those substances and allow germination.

Another possible effect on germination may be determined by nutrient availability, which has been shown to have a positive effect on the germination and growth of different conifers (Ferm et al. 1992). This could explain the higher

germination in the field in burned areas, since the ashes would act as a source of nutrients in a readily assimilated form, whilst the unburned areas would have lower nutrient contributions.

Our intention was to contrast the hypothesis that natural regeneration, under the canopy of mature *Pinus pinaster*, is controlled either by abiotic factors, like availability of light or by the presence of inhibitory substances from the exudates of leaves or the remains of decomposition. We also tried to determine the effect of nutrient availability on germination.

Materials and methods

The biological materials used in this study were seeds of *Pinus pinaster* Aiton. The seeds were collected in a *Pinus pinaster* stand situated in the Sierra del Teleno, SW León province (M.T.U.29TQG2984), at an approximate altitude of 1100 m. The climate is Mediterranean with 2-3 months' summer dryness and annual precipitation of 650 to 900 mm. (Ministerio de Agricultura 1980).

The study area is a natural *Pinus pinaster* stand with the total size of the original wood being 11,500 ha and its anatomical and physiological characteristics distinguish it from any other natural population of the species in the Iberian Peninsula.

Seeds were collected in July 2000, coinciding with the dispersion period of these species. The seeds were stored in open paper bags, which permitted ventilation, and at laboratory temperature in a dry place until they were used. *Pinus pinaster* leaves and litter used in the treatments of autotoxicity were collected from the *Pinus pinaster* population, located in the Sierra de Teleno.

In order to discover the effect of light/dark, inhibitory components and nutrient availability on germination, a method widely used by various authors (Trabaud and Casal 1989; Tárrega et al. 1992) was employed. A total of 10 seeds per treatment were placed on Petri dishes, thus producing 5 replicates / treatment. The treatments were:

Complete darkness, the Petri dishes were completely covered so that no light could enter and were watered with demineralized water;

Effect of leaf exudates: the seeds were watered with an aqueous extract obtained by macerating *Pinus pinaster* leaves for 48 hours.

Effect of humus exudates: the seeds were watered with an aqueous extract of humus obtained by macerating humus for 48 hours.

Effect of nutrients on germination: the seeds were watered with a solution of ashes, which represents the nutrient contribution to the seeds. Ash was obtained by burning leaves and thin branches that were colleted from the same population. The quantities of ash were (0.5 g/l; 1 g/l; 1.5 g/l) and were based on the amounts of ash/m2 collected by Soto (1993) after controlled fires.

Control: the seeds were watered with demineralized water.

The dishes were placed in a controlled environment cabinet at a temperature of 20ºC ± 1ºC with photoperiods of 15 hours' light/9 hours' dark. A temperature of 20ºC was used, as in other germination studies varying between 20ºC and 23ºC (Trabaud and Oustric 1989). The seeds were examined every day. A seed was considered to have germinated when the radicle could be seen with the naked eye (Côme 1970). The experiment was continued in this way for two months.

The average time for germination was also estimated using the expression:

$$t_m = \frac{N_1 T_1 + N_2 \cdots + N_n T_n}{N_1 + N_2 \cdots + N_n}$$

Where N_1 is the number of seeds which have germinated between time T_1 and T_2, and so on (Côme, 1970).

Before carrying out the previously mentioned treatments a viability test was also carried out on a sample of 100 seeds from the same population. The Tetrazolium test (Besnier Romero 1989) was used for the viability analysis.

Data analysis

The results of germination were statistically analysed by analysis of variance to determine the significance of the differences found between the treatments applied to the seeds. The Scheffe test (1959) was used to detect any

significant differences (α=0.05) in the comparison between the pairs of treatments. Prior to this the sampling normality was checked by the David test (David et al. 1954) and the homogeneity of the variances by the Cochran test (Cochran 1941).

Results

The viability percentages of the seeds of this *Pinus pinaster* were 100%, which means that all the seeds collected in the field are able to germinate and that the possible delays in germination may be due to the existence of factors extrinsic to the seed itself.

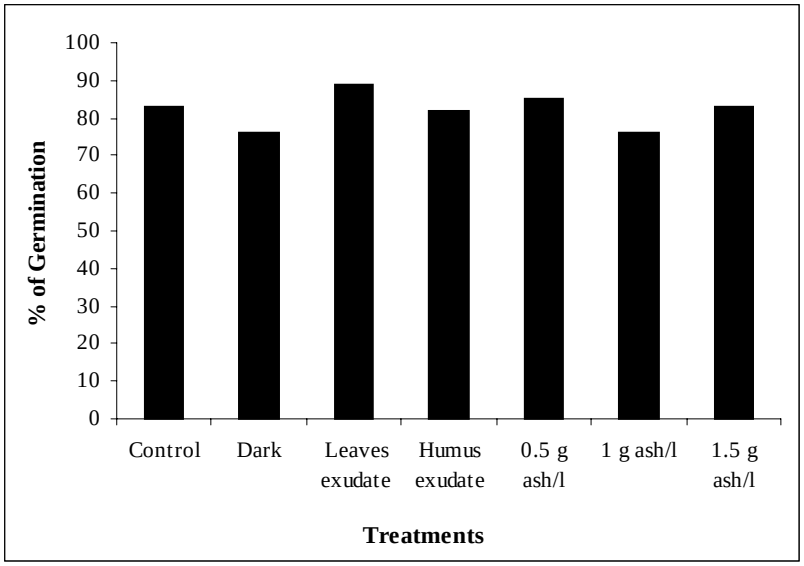

Figure 1. Germination percentages of *Pinus pinaster* seeds in the control situation and after different treatments: (Darkness, Inhibitory effects of leaf exudates; Inhibitory effects of humus exudates, and effects of three different amounts of ash (0.5 g/l; 1 g/l, 1.5 g/ l).

The total germinated *Pinus pinaster* seeds (Fig. 1) subjected to different treatments shows that there are high germination percentages in all the treatments: over 70% in all cases. The seeds watered with leaf exudate present the highest germination percentage and those watered with nutrient concentrations of 1 gram of ash per litre have the lowest germination percentages. However, no significant differences can be observed among the germination percentages in any of the treatments (Table I).

Table I. Results of analysis of variance to compare differences in percentage of germination of *Pinus pinaster* among different treatments.

Source	DF	Sum of Squares	F-test
Between groups	6	22.57	0.554
Within groups	28	190	P=0.762
Total	34	212.57	

Temporal distribution of germination (Fig. 2) shows that control, leaf exudates and humus exudates have very similar germination patterns. The first germination occurs 4 days after sowing in the leaf exudate treatment. In contrast the seeds kept in total darkness need at least 10 days to start to germinate. The control as well as those watered with humus exudates start after 7 days and those watered with different ash concentrations require 8 days. Another difference recorded among them is the maximum germination peak. This appears after 12 days in the control, those watered with leaf and humus exudates and at the opposite end are those kept in complete darkness. The seeds watered with different concentrations of ash are in an intermediate situation, as they require 15 days to reach the maximum germination peak. The most notable germination peak appears in the control situation. The germination of this species under the treatments to which they were subjected shows a certain irregularity, as different germination peaks appear in the different treatments throughout the study period (60 days). The treatments with the highest ash concentrations (1 g ash/l and 1.5 ash/l) showed the most marked peaks of germination in time.

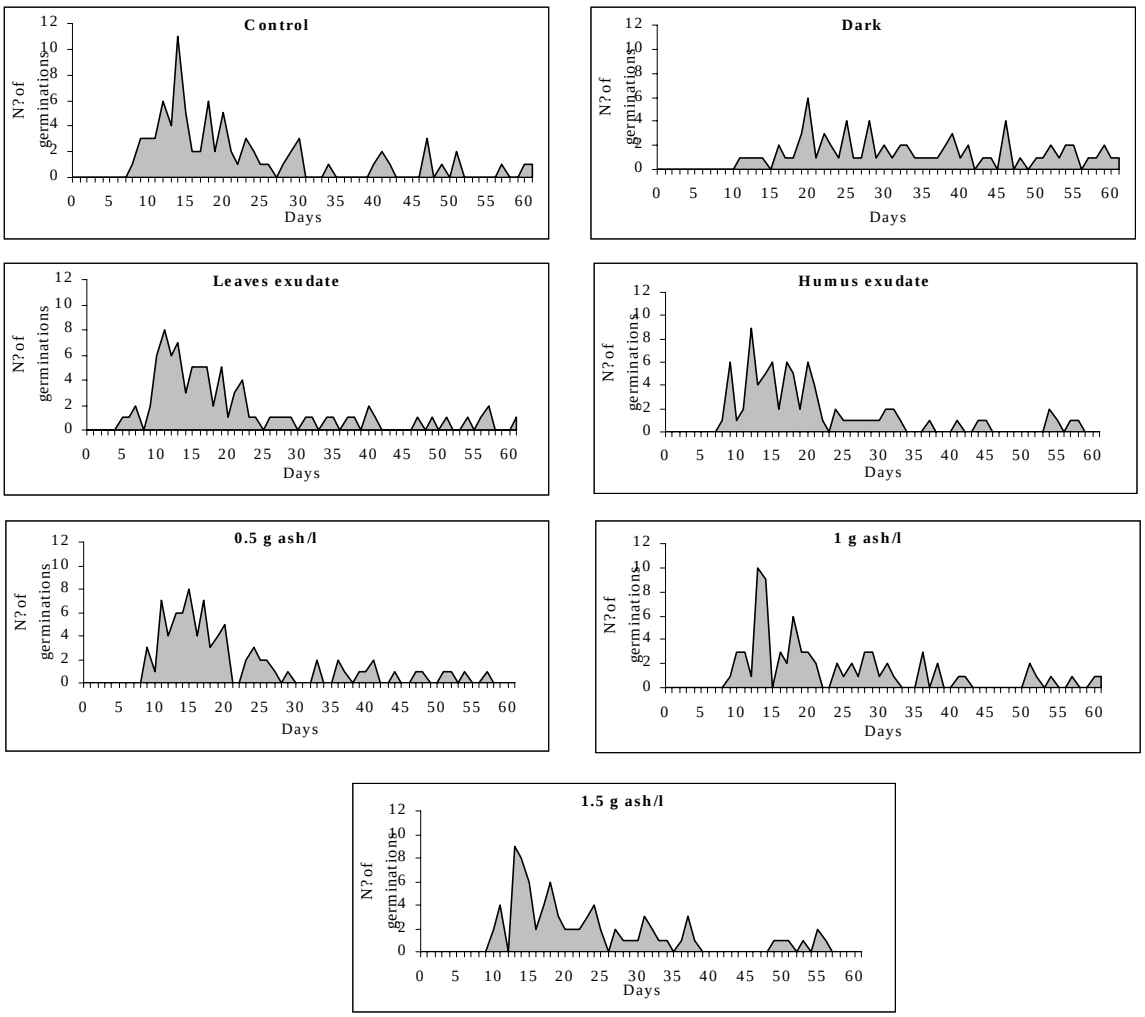

Figure 2. Distribution of germination times of *Pinus pinaster* in each treatment (Darkness, Inhibitory effects of leaf exudates; Inhibitory effects of humus exudates, and effects of three different amounts of ash (0.5 g/l; 1 g/l, 1.5 g/ l).

These results are complemented by the analysis of the mean germination times (Table II). The lowest mean germination time corresponds to the lowest concentration of ash treatment (0.5 g ash/l) (20.17 ± 4.43 days) followed by leaf exudate (20.96 ± 1.85 days). The treatment, which delayed germination most, was darkness (33.95 ± 6.02). Significant statistical differences were found among the mean germination times in the different treatments (Table III). The Scheffe test showed that the treatments responsible for these differences were darkness, with the longest germination time, and the rest of the treatments. No significant statistical differences were found among the rest of treatments.

Table II. Mean germination time (days) of the *Pinus pinaster* seeds for each of the treatments.

Treatments	Mean	Standard deviation
Control	21.8	2.25
Darkness	33.95	6.02
Leaf exudates	20.96	1.85
Humus exudates	21.17	3.56
0.5 g ash/l	20.17	4.43
1 g ash/l	22.76	3.78
1.5 g ash/l	22.20	3.38

Table III. Results of analysis of variance to compare differences in the mean germination time in the treatments.

Source	DF	Sum of Squares	F-test
Between groups	6	684.7	7.79
Within groups	28	411.3	P=0.0001
Total	34	1095.9	

Discussion

The germination percentage (83% in the control situation) of the *Pinus pinaster* population in León province is higher than the percentages given by Reyes and Casal (1995), who used *Pinus pinaster* seeds from the north of the Iberian peninsula, and recorded control situation percentages of 58%. However, in spite of the high germination percentage obtained in the laboratory and observed in the field after a fire (Luis et al. 2001), it is difficult to find pine seedlings in the undergrowth of a mature population. One possible explanation for this is that the amount of light reaching the soil surface, where the seeds are, is small due to the forest canopy effect. It has, nevertheless, been demonstrated that the seeds in this *Pinus pinaster* population are capable of germinating in the laboratory in conditions of complete darkness. Therefore, in this case light does not seem to be the abiotic factor controlling germination, in contrast to what occurs in other species (González 1993).

Various species present in the Mediterranean basin produce chemical substances which inhibit the germination of seeds of other species or their own (Reigosa et al. 1999). Many shrub species such as *Cistus albidus* (Robles et al. 1999), *Cistus ladanifer* (Chaves et al. 1997), *Thymus vulgaris* (Tarayre et al. 1995), *Lavandula stoechas* (Vokou 1992) show this effect. The inhibitory effect of arboreal species like *Quercus pubescens* (Hubert and Boglio 1989) and *Quercus ilex* (Li-Jun et al. 1997) has also been documented.

Other coniferous species have also been found capable of producing allelopathic substances, as occurs in the case of *Pinus halepensis*, which emits a considerable amount of terpenes (Peñuelas and Llusiá 1998), *Picea abies*, which emits phenol compounds from the green acicules to the soil solutions (Gallet and Pellissier 1997) and *Pinus pinaster*, which presents different amounts of terpenes and resinous acids in its acicules and bark (Fernández de Simón et al., 2001). These compounds act as powerful agents, which inhibit germination and the growth of some herbaceous species when found in the substratum (López-Mosquera 1993). Although this species is known to produce inhibitory substances in the field, it was observed that germination was not significantly inhibited in the laboratory when watered with the aqueous extract of acicules and humus. Therefore in principle it seems that, if these chemical compounds exist in the extract, they do not inhibit germination of seeds of their own species.

Likewise, the opposite effect, that is germination stimulation, could be conditioned by a high nutrient content such as that supplied by ashes. In this case of *Pinus pinaster*, germination does not seem to be affected by the different ash treatments carried out and only reaches a percentage slightly above that of the control in the case of the 0.5 g l^{-1} treatment, although the differences are not significant. Reyes and Casal (1998) recorded something similar in studies carried out on *Pinus pinaster*. Rather than a beneficial effect with high concentrations of ash, although this means a nutrient contribution, environment pH is increased considerably and this affects germination negatively.

It can be deduced from the results found that germination of the *Pinus pinaster* seeds in this population is not inhibited by factors such as light or substances emitted by their leaves or present in the humus. This induces us to think that the actions of herbivores consuming seeds of pines could be one of the causes of the reduction in the number of seeds and therefore of the non-presence of seedlings in the field. We also need to determine whether the seeds produced by adult trees penetrate the litter layer of the undergrowth and this makes epigeal growth of seedlings difficult in the field.

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Research on the Nature of Recalcitrance in Temperate Tree Seeds: GC and FT-IR Examinations of Stored and Desiccated Seeds

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Abstract

Quercus alba L., *Q. durandii* Buckl., and *Q. virginiana* Mill. acorns were collected, stored at +4°C and -2°C, and tested monthly to examine the physiological, biochemical, and moisture changes taking place during storage. *Aesculus pavia* L. seeds were similarly stored but tested only every three months, while those of *Q. nigra* L. and *Q. pagoda* Raf. were tested on a yearly basis. While all these seeds are classified as recalcitrant, not all deteriorate at the same rate nor does the lower storage temperature always enhance seed longevity. In addition, while gas chromatographic and Fourier transform infrared spectrometer analyses revealed that amounts of sucrose change dramatically in stored acorns, we found that there are interspecific differences observed in carbohydrate mobilization and also differences between cotyledon and embryonic axis tissue within a species. Also, any moisture loss prior to storage was fatal; and proper handling of seeds could be of greater significance than storage temperature. FT-IR studies have also found changes in membrane lipids and secondary protein structure in desiccating and stored acorns, emphasizing that low storage temperatures do not deter metabolic activity in hydrated, recalcitrant seeds.

Keywords: *Aesculus*, FT-IR, gas chromatography, *Quercus*, recalcitrance

Introduction

Roberts (1973) stated that seeds can be divided into two storage classes: 'Orthodox' or desiccation-resistant seeds that can be dried without damage to a moisture content (mc) of less than 12% and 'recalcitrant' or desiccation-sensitive seeds that cannot. The susceptibility of recalcitrant seeds to moisture loss and the necessity for hydrated storage makes any useful period of seed storage for some such seeds very short; others, such as *Quercus nigra* L. (Bonner 1973) acorns, can survive up to 3 years under proper storage conditions. Forest tree genera with recalcitrant seeds are abundant in the tropics but less common in the temperate zone. They do exist, however, and some important temperate tree genera, *Castanea* (Jaynes 1969, Prichard and Manger 1990), *Quercus* (Bonner and Vozzo 1987), *Aesculus*, and some *Acer* species (Bonner 1990) have seeds classified as recalcitrant.

Recent work has modified both Robert's initial definition of recalcitrance and our perspective of the nature of recalcitrance. Pammenter *et al.* (1994) and Berjak and Pammenter (1997) recognized the damage caused by aberrant metabolic processes while seeds are in hydrated storage and as water is lost. Other work has emphasized the changes in membrane and storage lipids and the physical disruption of seed membranes that take place as seeds deteriorate (Flood and Sinclair 1981; Priestly and Leopold 1983). Changes in seed proteins and carbohydrates, and the complexities of water properties in seeds have also been noted (Roberts 1973; Farrant *et al.* 1985, 1988; Pammenter *et al.* 1991; Wesley-Smith *et al.* 1992). Pritchard (1991) determined that damage began in recalcitrant seed embryos at a much higher mc than that proposed by Roberts (1973); embryos of *Quercus* exhibited damage when mc was still over 40%. Experiments by the authors with recalcitrant seeds from both temperate and tropical trees have yielded mixed results (Connor *et al.* 1996, 1998; Connor and Bonner 1998). While moisture and differential scanning calorimetry data exhibit a strong relationship with declining seed viability, results of lipid analyses have been conflicting.

This paper reports the results from three studies: (1) a one year storage study of Durand oak (*Quercus durandii* Buckley), live oak (*Quercus virginiana* Mill.), and red buckeye (*Aesculus pavia* L.) at 2 temperatures and a 120 day study of white oak (*Quercus alba* L.) at the same storage temperatures; (2) third year results of a water oak (*Quercus nigra* L.) and cherrybark oak (*Quercus pagoda* Raf.) acorn storage experiment at two temperatures and two mcs; and (3) a Fourier transform infrared (FT-IR) spectroscopy study of desiccating cherrybark oak acorns.

Materials and Methods

Durand oak, white oak, and red buckeye seeds were collected locally in Oktibbeha County, Mississippi (MS), USA. The water oak and cherrybark oak acorns were purchased from a local supplier, while the live oak acorns were collected in Washington County, MS, USA. All seeds were cleaned by floatation, soaked overnight, and then stored at 4°C until the start of the experiment. Original mc for each drying regime was determined by drying 2-4 samples of seeds at 105°C for 16-17 h. In preparation for germination tests, acorns were cut in half horizontally. The seed coat was removed from the half containing the embryo, and the half with the cup scar was discarded. Buckeye seeds were germinated intact. Germinations were conducted on moist Kimpak at an alternating temperature regime of 20°C for 16 h in the dark and 30°C for 8 h with light. Since sprouting in storage can be a common problem, counts were made of the number of seeds in a sample, which had sprouted during storage. Experiments were conducted as follows:

Experiment 1

General: This experiment examined temperate tree species with highly recalcitrant seeds. Samples of 250 fully hydrated acorns of Durand oak and live oak were stored in plastic bags at either 4°C in a Lab-Line Ambi-Hi-Low Chamber or at -2°C in a modified chest freezer. Percent germination and mc were determined for the fresh acorns and every 30 days (d) thereafter for one year, as acorn supplies and deterioration permitted. A subsample of acorns was dissected, and the embryo and cotyledon cryostored for carbohydrate analyses. Acorns were germinated as two replications of 50 seeds each per sampling period and were rehydrated overnight in tap water prior to germination testing. White oak acorns and red buckeye seeds were stored as above; however, the white oak acorns were stored in batches of 185 per bag and, since they rapidly deteriorate when stored, tested only through 120 days. Germination tests were conducted on 2 replications of 25 seeds per sampling period. Red buckeye seeds were tested only at fresh, 90-, 180-, and 360-d intervals and were stored in batches of 59 seeds per bag. Germination tests consisted of 2 replications of 15 seeds each per sampling period.

Carbohydrate analyses: At each sampling time, *Quercus* embryoic axes with immediately adjacent cotyledon tissue were dissected from surrounding tissue. Samples were immediately frozen in LN_2 and lyophillized. The cotyledons were finely-ground in a Wiley mill using a 20-mesh screen; embryonic axis tissue was ground by hand with a mortar and pestle. A 0.3-0.5g dry tissue sample was used for each carbohydrate extraction. The tissue sample was placed in 10 ml of an 80% ethanol solution and heated in a 75° C water bath for 1 h. The sample was then filtered, rinsed with more of the ethanol solution and rotoevaporated to dryness. The evaporation flask was rinsed with 10 ml of distilled water, and the sample was then filtered, rinsed, and freeze-dried overnight. The dried sample was dissolved in 1 ml of trimethylsilylimidazole, heated in a 75° C water bath for 30 min, blown to dryness and then redissolved in 1 ml chloroform and stored until analysis. Analyses were performed on a HP® 5890 gas chromatograph (gc) using a Supelco® SPB-5 capillary column (30m x 0.25 mm ID x 0.25 film thickness).

Experiment 2

High and low moisture levels for water and cherrybark oak acorns were imposed by either soaking in tap water for 16 h or by drying on a lab bench for 48 h. Lots consisting of 110-120 acorns were stored in 4-mil polyethylene bags at either 4°C or at -2°C as described above. Original percent germinations and mcs were determined for fresh acorns and thereafter at yearly intervals. Acorns were germinated as two replications of 50 seeds per sampling period and were soaked overnight in tap water prior to germination testing.

Experiment 3

Cherrybark oak acorns collected in 1999 were spread on blotter paper in a single layer on the lab bench. Cotyledon samples of fresh seeds and those that had been dried for 2,4,6, and 8d were analyzed by FT-IR spectroscopy as follows: thin slices of cotyledon tissue were placed between CaF_2 windows of a demountable transmission cell. For each spectrum, 512 scans at 2/cm resolution were collected on a Nicolet 20 DXB spectrometer using an MCT-A detector. Single beam spectra were ratioed against an open beam background to yield transmission spectra. Sampling continued until seed mc dropped below 15%, and the samples were analyzed for changes in macromolecular structure that might occur during drying and during rehydration. The experiment was replicated on acorns collected in 2000.

Results

Experiment 1

Durand oak acorns stored at -2°C had significantly higher viability than those stored at 4°C in as little as 30d (Table 1). After 210d, acorns stored at -2°C averaged 83% viability, while only 6% of those stored at 4°C survived. Red buckeye seeds also remained viable longer if stored at -2°C. The differences in viability did not occur, however, until after 90d in storage. Acorns of live oak were the only ones tested that survive longer if stored at 4°C. Storage at -2°C resulted in significant damage to the acorns. Fresh mcs were 38.1, 60.6, and 56.6% for Durand oak, red buckeye, and live oak, respectively, and did not change greatly during storage. White oak acorns began sprouting in as little as 60d when stored at 4°C and by the 90d test, 96% of all the 4°C acorns had sprouted (Table 2). Only 3% of acorns stored at -2°C had sprouted by the 120d test and viability was still 90%. Mcs remained high in the acorns.

Table 1. Germination and moisture content of Durand oak, red buckeye, and live oak seeds stored for up to one year at +4C and -2C.

Species	Days stored	Germination (%) +4C	Germination (%) -2C	Moisture Content (%) +4C	Moisture Content (%) -2C
Durand oak	0	98	98	38.1	38.1
	30	88	98	37.3	37.7
	60	87	97	39.7	39.9
	90	75	93	39.7	37.1
	120	69	93	40.3	39.1
	150	10	50	40.6	38.4
	180	--	--	---	---
	210	7	83	41.0	38.7
	360	0	13	*	39.8
Red buckeye	0	93	93	60.6	60.6
	90	100	100	63.4	61.4
	180	83	100	59.1	62.4
	360	0	44	---	*
Live oak	0	92	92	56.6	56.6
	30	84	79	52.3	57.6
	60	91	46	54.6	50.4
	90	68	20	58.9	53.4
	120	45	10	53.4	57.4
	150	30	7	61.5	59.6
	180	14	2	54.8	53.3
	210	4	6	61.3	52.6
	240	2	7	56.2	53.6

*Seeds selected for the moisture test were all dead.

Table 2. Germination, sprouting, and moisture content of white oak acorns stored for 120 days at +4C and -2C.

Days stored	Germination (%) +4C	Germination (%) -2C	Moisture Content (%) +4C	Moisture Content (%) -2C	Sprouting(%) +4C	Sprouting(%) -2C
0	96	96	51.9	51.9	0	0
30	100	100	50.1	52.1	0	0
60	42	94	52.2	50.5	4.9	0
90	0	98	51.2	50.8	95.7	1.1
120	---	90	---	50.2	---	3.2

It was obvious from the carbohydrate analyses that, even when stored at temperatures of -2°C, seed metabolism was still very active (Fig. 1). Sucrose was still being mobilized and transported despite the cool temperatures, a difficulty commonly encountered in recalcitrant seeds, which must be stored fully hydrated. In several of the species studied in this experiment, there were no oligosaccharides present. In others, quantities were very small (< 3mg/g). It is difficult to tie specific physiological events in stored seeds to fluctuations in sucrose content; however, another experiment with *Q. alba* determined that sucrose was significantly reduced in the embryo and cotyledon tissue when the radicle emerged.

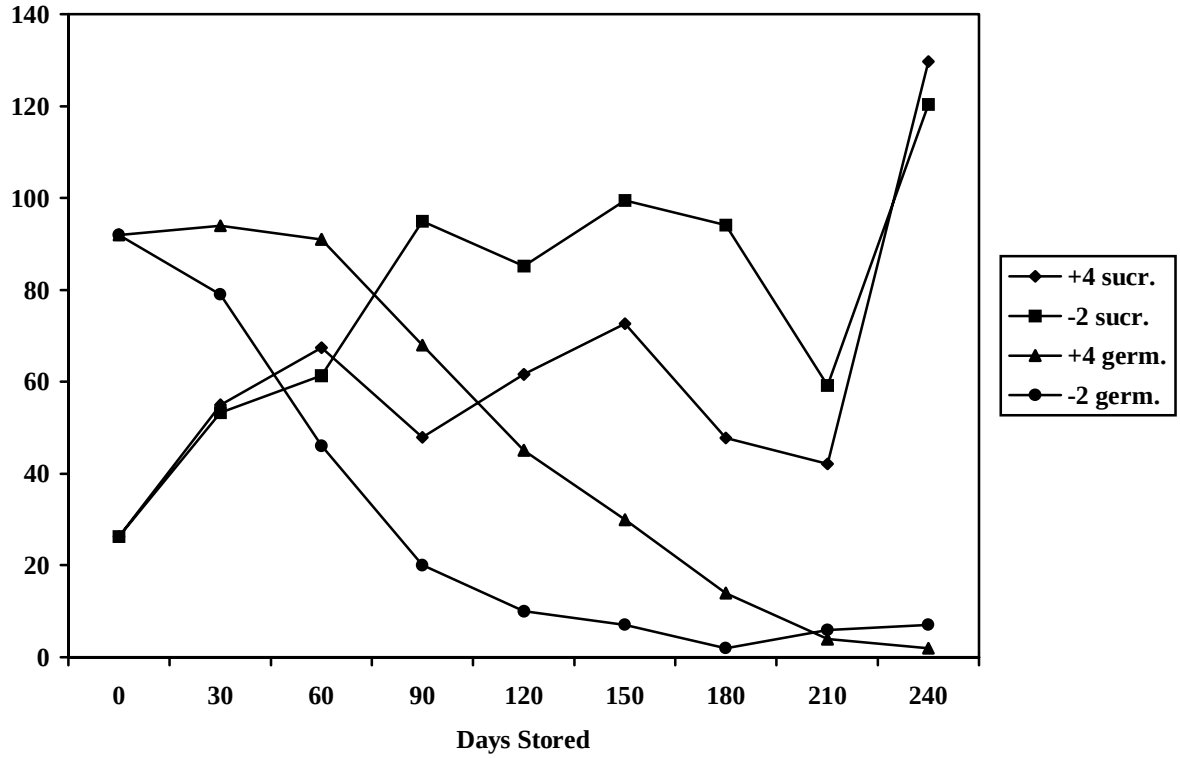

Figure 1. Germination (%) and sucrose content (mg/g dry wt.) of live oak acorn cotyledons stored at +4°C and –2°C for 240 days.

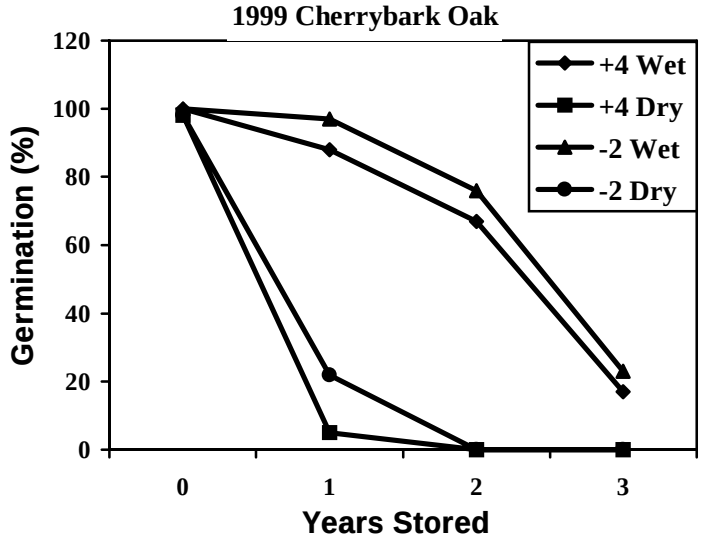

Figure 2. Germination (%) of water oak acorns and cherrybark oak acorns stored at two temperatures and two moisture contents for three years.

Experiment 2

Water oak acorn mc was 30.5% fr wt for the fresh acorns and decreased to only 25.6% after 2d of drying prior to storage. However, this slight reduction in mc reduced initial acorn viability by 9% (Fig. 2). After 1 yr, temperature of storage had a greater effect on seed viability than did initial mc. Both fully hydrated and dried acorns stored at -2°C maintained a higher viability than those stored at 4°C. This was not the case after 2 yrs of storage, when mc was the more important factor. Acorns, which had been dried prior to refrigeration, had lower viability than those stored fully hydrated. This was still the case after 3 yrs in storage, although deterioration had progressed in seeds stored fully hydrated. Mc did not change significantly from the original amount throughout the course of the experiment.

Cherrybark oak acorn mc was 29.6% for the fresh acorns and 19.9% for those dried 2d. However, drying reduced initial viability by only 2% (Fig. 2). Unlike water oak acorns, moisture content, and not temperature, was the most important factor in all test years. Only acorns stored in the fully hydrated condition retained high viability after 1 yr in storage. Those dried for 48 h prior to storage were severely affected after 1 yr of storage and were dead after 2 yrs. Changes in mc during storage were not significant.

Experiment 3

Cherrybark acorn germination was highly dependent on mc and severely declined when seed mc dropped below 17% (Table 3). Changes in molecular structure due to drying and rehydration were measured by changes in the frequency (and bandwidth) of the infrared absorbance of lipid and protein functional groups. Membrane lipid structure was measured by the frequency and bandwidth of the symmetric CH_2 stretch at 2850/cm (Sowa *et al.* 1991). An increase in vibrational frequency corresponds to increased fluidity (phase change from gel to liquid crystalline). In the liquid crystalline phase, membranes are fluid and in their normal state; when in the gel phase, membranes may leak cell solutes and cause irreparable damage to seeds. In this experiment, fresh tissues exhibited reversible shifts between gel and liquid crystalline phases upon drying and rehydration in the cotyledon tissue (Fig. 3). After drying for 8d, membrane lipids changed to gel phase and did not recover their fluidity upon rehydration.

Table 3. Cherrybark germination (%) and moisture content (fr wt) for FT-IR experiments.

Days	1999		2000	
dried	Germ.	MC	Germ.	MC
0	100	29.7	99	33.5
2	98	20.8	98	23.8
4	81	16.7	65	17.5
6	16	10.8	10	13.5
8	---	---	0	13.4

Protein secondary structure was measured using the amide I and II vibrations near 1650 and 1550/cm (Sowa *et al.* 1991). Changes in amide frequency correspond to changes in secondary structure. Alpha-helix structures absorb at higher frequencies, while beta-sheets absorb near 1630cm; denatured protein typically exhibits extended beta-sheet conformation, with infrared absorbances common at frequencies less than 1630/cm. Irreversible changes in the protein secondary structure, illustrated by shifts in the amide absorbance near 1650/cm, occurred in the cherrybark acorn cotyledon tissue (Fig. 4). Secondary structure was completely lost upon dehydration (day 8) and remained so upon rehydration of these samples (day 9).

Discussion

No one single temperature was best for storage of recalcitrant seeds. In previous experiments, chinkapin (*Q. muehlenbergii* Engelm.), northern red (*Q. rubra* L.), and Shumard (*Q. shumardii* Buckl.) oak acorns favored the lower storage temperature of -2°C (Connor and Bonner 1999). While Durand oak acorns and red buckeye seeds exhibited significantly higher viability when stored at -2°C, live oak acorns were harmed by the low temperature. Also, sprouting during storage was a problem in red buckeye (17% after 180d), live oak (18% after 120d),

Durand oak (16% after 120d), and white oak (96% after 90d) seeds stored at 4°C. Sprouting remained below 2% in seeds stored at -2°C for the same lengths of time.

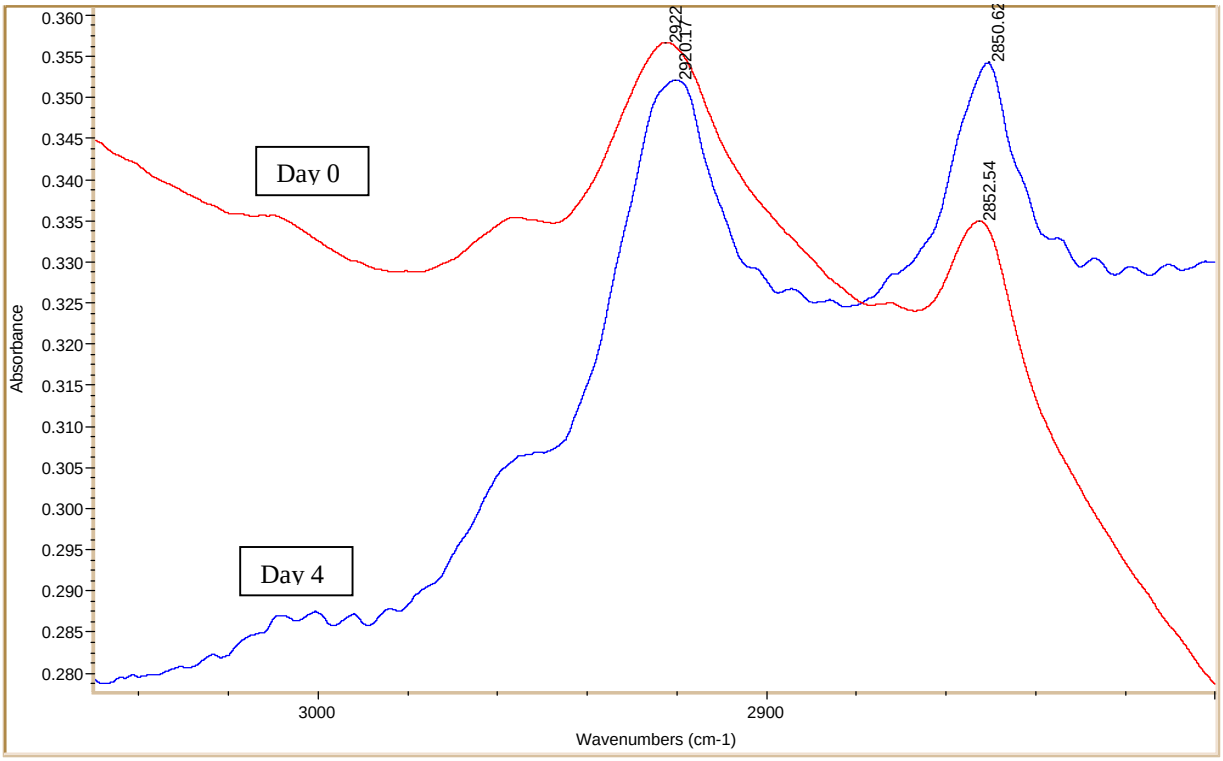

Figure 3. Membrane lipid $-CH_2-$ vibrations in cherrybark embryonic axes, symmetric (2850 cm^{-1}) and asymmetric (2920 cm^{-1}).

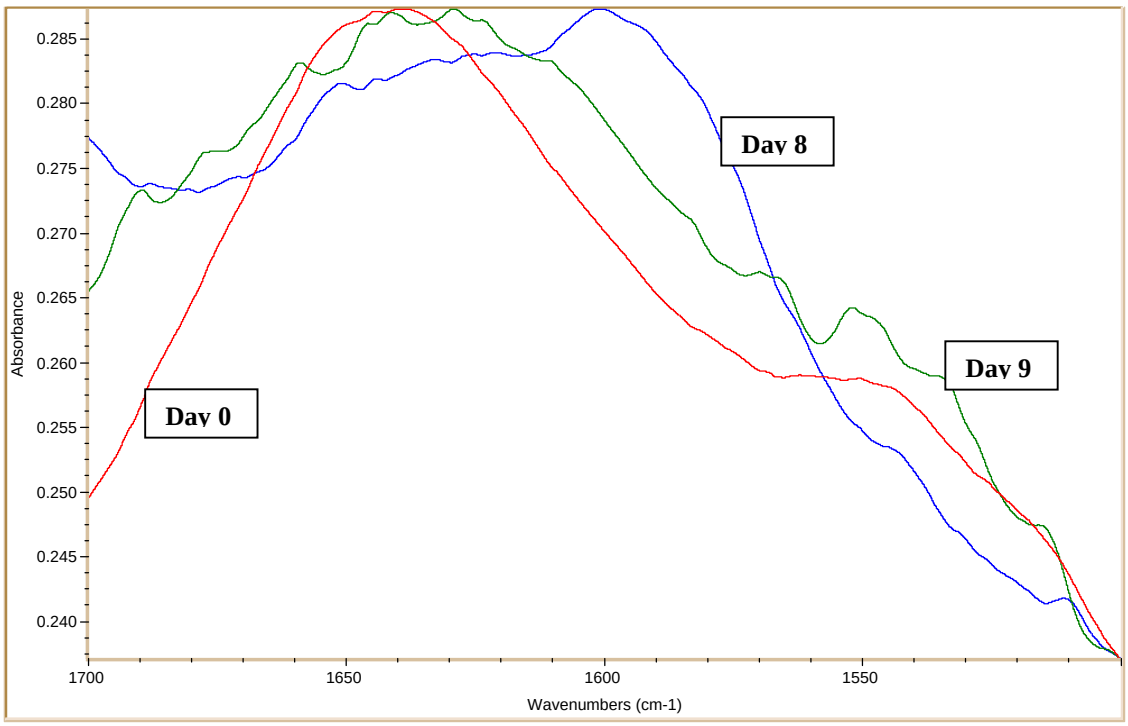

Figure 4. Protein (amide) vibrations in cherrybark oak embryonic axes. Peak frequencies at 1638.5, 1635, and 1629.9 cm^{-1}.

Both water oak and cherrybark oak acorns retained high viability after 2 yrs when stored fully hydrated. To date, sprouting and loss of moisture during storage are not factors in the successful storage of either species. While drying of water oak and cherrybark acorns for 2d before storage did not affect original viability, the damage was significant in water oak acorns stored for 1 yr at 4°C and in cherrybark oak acorns after 1 yr at either storage temperature. It is, therefore, strongly suggested that all precautions against moisture loss be taken when collecting acorns of these species that are not for immediate use. Unless the acorns are collected when fresh and maintained in a fully hydrated state, severe losses can arise when stored for only 1 yr. Orchard managers and seed processors must place emphasis on careful handling of acorns during the collection process. Also, the sooner acorns can be collected after dropping from the tree, and placed under refrigeration, the higher the probability of successful long-term (1 yr) storage.

Membrane lipids changed phase from liquid crystalline to gel upon drying and did not recover upon rehydration as viability was lost. Ions can pass indiscriminately through cell membranes in the gel phase, and this loss of selective permeability ultimately results in seed mortality. In this experiment, the change occurred first in the cotyledon tissue and then in the embryonic axes; since axes in recalcitrant seeds maintain a fairly high water content (Connor *et al.* 1996, Connor and Bonner 2001), this was not unexpected. It was interesting to note that after severe desiccation, rehydration did not restore membranes to their original fluid state.

Changes in protein secondary structure occurred in cotyledons as moisture was lost. Secondary structure was completely lost upon dehydration and remained so upon rehydration of nonviable samples. This evidence of protein denaturation occurring in the cytosol and/or cellular membranes was the most sensitive indicator of viability loss as yet encountered in these experiments. It is also contrary to behavior observed in orthodox seeds using infrared techniques (Golovina *et al.* 1997) and will be addressed in future investigations.

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Thermal Scarification of Hard Seeds by Wet Heat Treatment Risks Accelerated Seed Ageing: Evidence from Five Woody Taxa

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Abstract

A comparison was made between the effect of wet and dry heat treatments at 85 °C on the removal of hard seed impermeability for five woody species from the Fabaceae ([Leguminosae] *Acacia melanoxylon*, *A. nilotica*, *Lupinus arboreus* and *Robinia pseudoacacia*) and Anacardiaceae (*Rhus laurina*). The hard seed fraction of the seed batches ranged from 28 % in *L. arboreus* to 86 % in *A. melanoxylon*. Dry (oven) and wet (water) heat treatment for 1 and 10 min raised the germination level of *L. arboreus* seed by no more than 17 % above that of the unscarified control, whilst 100 min of either treatment reduced germination to virtually nil. Germination level in *Acacia melanoxylon*, *A. nilotica* and *R. laurina* seeds progressively increased as dry heat treatment lengthened; in contrast, *Robinia pseudoacacia* seed had highest germination after only 10 min. treatment, falling thereafter. For all species, the shortest wet heat treatment (1 min) resulted in the highest germination level, although this was not significantly better than the optimum dry heat response in *R. laurina* and *R. pseudoacacia*. In contrast, the longest wet heat treatment resulted in much lower germination than the comparable dry heat treatment, except for *A. melanoxylon*. Subsequent, additional physical scarification revealed that such seeds had lost viability. Seed moisture contents of all species increased after 100 min of wet heat treatment, suggesting that reduced viability was due to accelerated ageing. Whilst wet heat treatment is reliable at overcoming hardseededness in a range of species' seed, longer exposure times may compromise seed quality.

Introduction

Hard seed impermeability is commonly found in Fabaceae and in many other families, such as Cannaceae, Malvaceae and Anacardiaceae (Barton 1965, Rolston 1978). This trait is an important component of an ecologically safe strategy for species regeneration, especially in arid environments where it is the most common barrier to germination (Baskin and Baskin 1998). Hardseededness contributes to the establishment of a soil seed bank and its polymorphic nature means that germination will be separated in time, thereby increasing the probability of seedling establishment under favourable conditions.

Impermeability is dependent on the structure and physical properties of the seed coat. Generally, only the breaching of the seed coat fully overcomes the impermeability. This can be achieved by various means, including filing, impaction and high temperature treatment. The latter treatment is particularly pertinent in the context of the regeneration strategy of species in arid or seasonally dry environments that are prone to high, daily levels of solar gain and intermittent fires (see Thanos and Georghiou 1988). Consumption of hard seeds by animals is another means by which hardseededness is lost, as the coats are acid-scarified during digestion.

Overcoming hardseededness in the laboratory is an important initial step in the use of a range of species in restoration ecology programmes. Bulk, physical scarification - using macerators, grinders and crushers - can produce unreliable results, e.g. with *Leucaena leucocephala* (Gosling et al. 1995), as can the seed gun (Poulsen and Stubsgaard 1995). Site-directed thermal scarification using a glow-burner is highly successful but labour intensive, and the more random-location mechanical burner is generally less effective, plus there is an enhanced risk of random damage to the embryo (Poulsen and Stubsgaard 1995). Dry heat in the range c. 70 to 100°C has been shown to be effective at removing impermeability in a range of species, particularly legumes (Fabaceae) and Cistaceae (see Herranz et al. 1998, 2000). In addition, hot water treatments have been used successfully on Mediterranean ecosystem species (e.g. Doussi and Thanos 1994) and in forestry for rural development programmes (Masamba 1994), this treatment having an additional benefit of better controlling coat-borne pathogens. Seed ageing is a function of both moisture content and temperature and thus all high temperature treatments have the potential to compromise seed viability rapidly. Here we compare the effects of dry- and wet-heat treatments at 85°C on the seed germination and viability of five woody species, viz. four genera of Fabaceae (*Acacia, Lupinus, Pseudoacacia and Robinia*) and one of Anacardiaceae (*Rhus*).

Materials and Methods

Pre-cleaned seeds of five woody species were obtained from commercial sources or from the Royal Botanic Gardens Kew Seed Bank at Wakehurst Place. The species, with their taxonomic affiliations and original seed provenances, were: *Acacia melanoxylon* (Fabaceae, Mimisoideae; Australia); *Acacia nilotica* (Fabaceae, Mimisoideae; Tanzania); *Lupinus arboreus* (Fabaceae, Papilionoideae; England); *Rhus laurina* (Anacardiaceae; USA); and *Robinia pseudoacacia* (Fabaceae, Papilionoideae; USA). The Seed Bank collection of *R. laurina*, from 1987, had been desiccated to equilibrium under conditions of c. 15 % RH and c. 15 °C. The commercial seed lots (all other species), which had moisture contents ranging from 7.6 % to 10 % (fresh weight basis) on receipt were dried under the same conditions to 5.3 % to 7.7 %. Moisture contents were determined gravimetrically on five individual seeds after drying in a fan-assisted oven at 103°C for 17 h (ISTA 1999).

Fifty seeds were sown (2 x 25) for germination on 1% agar-water in 9 cm-diameter plastic Petri dishes and then incubated under a 12 h photoperiod using warm white fluorescent tubes (c. 15μmol m^{-2} d^{-1}). Germination temperatures were 21°C for *Lupinus* and *Rhus*, 26°C *for A. nilotica* and 26 / 16 °C (12 / 12 h, with light applied during the warm temperature phase) for *A. melanoxylon* and *R. pseudoacacia*. Germination was assessed as radicle emergence to about 5 mm and the test ran for 30 d for *R. laurina* and *R. pseudoacacia* and 60 d for the other species. At the end of the initial phase of the test the remaining unimbibed seeds were scarified using a metal file and returned to the germination test for a further 14 d as an assessment of seed viability after the various treatments. This technique was applied to all the treatments for four of the species, but to only a limited number of the *R. laurina* seeds as the technique was difficult to perfect because of their small size; physical injury to the embryo was difficult to avoid. Imbibed but non-germinated seed at the end of the germination test were cut open to determine whether they were empty (non-competent), or the contents were soft (inviable) or firm (viable). Percentage germination values relate to the number of full seeds per treatment. The effect of physical scarification on germination was assessed statistically using a 2-sample test for equality of proportions, carried out using S-Plus 2000 software (MathSoft Inc.).

For the dry-heat treatments, c. 25 - 30 seeds were enclosed in aluminium foil laminate bags (Barrier Foils Ltd., Manchester, UK) and exposed for up to 100 min in an oven at 85°C. For the wet-heat treatment, the same number of seeds was placed in a wire mesh basket and placed in pre-heated water in a bath at 85°C for up to 100 min. After treatment, the seeds were immediately blotted dry and their moisture contents determined (n = 6 - 10). Each treatment was repeated. Internal seed temperatures during heating were monitored every 10 s with a data logger (Grant 1200) fitted with K-type thermocouples inserted through a cavity drilled in the seed coat. Triplicate measurements were made. Thermal lag was calculated as the time taken for the seeds to reach within 10 % of the target temperature. Mean values for thermal lag and moisture content after the treatments were compared by a t-test.

Results and Discussion

Seed lot characteristics

Table 1. Characteristics of the seed lots used. Germination is based on n = 50 seeds per species, with the comparison between treatments (non-scarified and physically scarified) shown as a χ^2 value.

Species	**Seed size: L x B x D (mm)**	**Seed dry weight (mg)**	**Germination (%)**		
			control	**scarified**	**χ^2 (*P*)**
Acacia melanoxylon	4.4 x 2.8 x 1.6	15.5	10	96	70.8 (< 0.001)
Acacia nilotica	7.7 x 6.6 x 4.0	148.8	4	88	67.7 (< 0.001)
Lupinus arboreus	4.7 x 3.5 x 2.9	28.8	60	88	8.8 (0.003)
Rhus laurina	2.1 x 1.9 x 1.4	3.9	4	80	56.2 (< 0.001)
Robinia pseudoacacia	4.7 x 3.0 x 1.9	20.3	24	70	19.4 (< 0.001)

The initial level of germination observed with untreated seed varied from 4 % in *A. nilotica* and *R. laurina* to 60 % in *L. arboreus* (Table 1); the remaining seeds in the germination tests that failed to imbibe were thus considered to be hard and impermeable to water. This was confirmed by physical scarification by filing, which resulted in seed germination increasing to between 70 %, for *R. pseudoacacia*, and 96 %, for *A. melanoxylon*

(Table 1). Thus, the hard seed component varied from only 28 % for *L. arboreus* to 86 % for *A. melanoxylon* (Table 1).

Dry heat treatment

Dry heat treatment progressively raised seed germination levels in *A. melanoxylon*, *A. nilotica* and *R. laurina*, reaching 76 %, 62 % and 90 % respectively after 100 min at 85 °C (Figure 1). For both the acacias, this treatment resulted in germination levels ≥ 20 % lower than the scarified controls (Table 1). In contrast, heat treatment of *R. laurina* seeds raised the germination level (90 %) above that of the scarified control (80 %; Table 1). This was almost certainly due to damage caused to some of the seed embryos during the scarification treatment, precision of filing depth being difficult to achieve with such small seeds. *R. pseudoacacia* seed germination reached the scarified control level (both c. 70 %) after 10 min of dry heat treatment, but was reduced slightly by 100 min treatment (Figure 1, Table 1). In contrast, the longest treatment time reduced germination in *L. arboreus* virtually to nil; shorter treatment times resulted in germination levels similar to the scarified control (c. 60 %) (Figure1, Table 1).

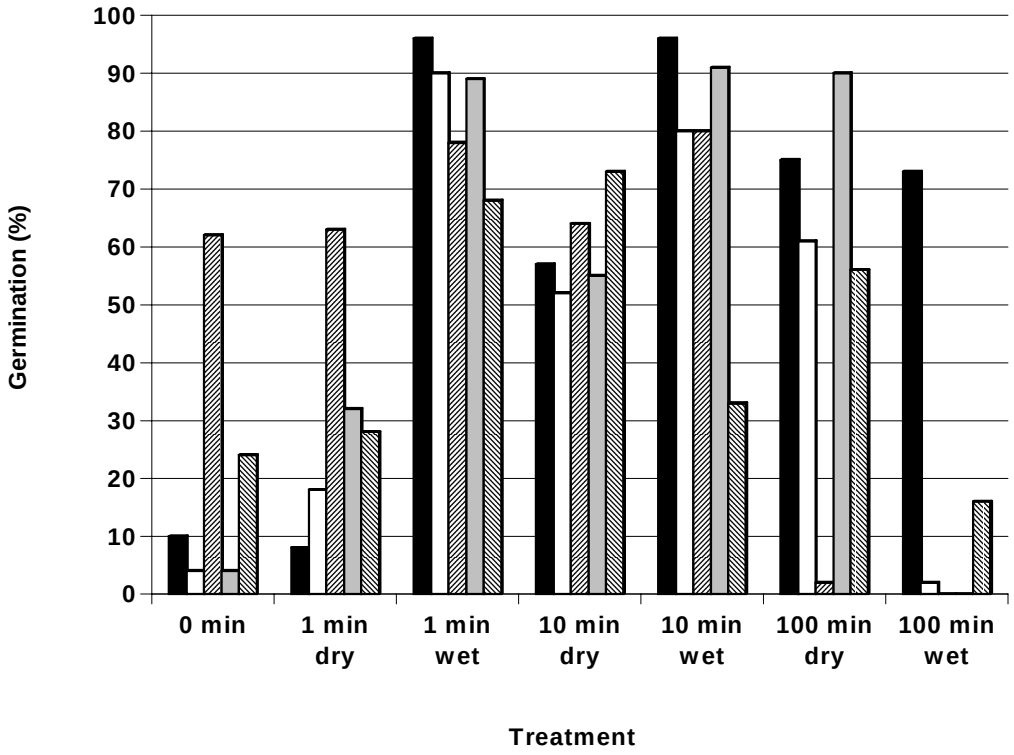

Figure 1. Effects of dry and wet heat treatment at 85°C on germination (n = 50).

A. melanoxylon (■), *A. nilotica* (□), *L. arboreus* (▨), *R. laurina* ( ), *R. pseudoacacia* (▧)

Dry heat treatment at 85°C appears to provide a balance between an appropriate level of thermal shock to remove impermeability, without too rapidly compromising seed viability. Thus it seems likely that, when shallowly-buried, the hard seeds of four species (i.e. excluding *L. arboreus*) would be stimulated to germinate after flash fires, which can raise the seedbed / soil temperature to around 90°C for a few minutes, although the maximum temperature and duration are highly dependent on the fuel layer and the soil moisture content (Valette et al. 1994). A similar conclusion was reached for two species of *Cistus* that responded well to 30 min at 80°C or 15 min at 100°C (Thanos and Georghiou 1988). However, the effectiveness of such a treatment at alleviating hardseededness is species, and probably also seed lot, dependent. Thus, few seeds of *Dichrostachys cinerea* (Fabaceae) responded to dry heat at 80°C or 100°C (van Staden et al. 1994). Also, only relatively small increases in germination percentage after dry heat at c. 85°C have been observed in *Rhus lanceolata* (3 min exposure, +5 to 15 %; Rasmussen and Wright 1988) and *Rhus ovata* (5 min exposure, +23 to 31 %; Stone and Juhren 1951).

One potential effect of such dry heat treatment is a decrease in seed moisture content, which could also contribute a physical stress (Brits et al. 1993). In an effort to reduce such an effect, the seeds were sealed in aluminium foil laminate bags. Nonetheless, during the longest period of treatment (100 min) the seeds did lose some moisture, reaching 3 - 6 % (Table 2) compared to 5 - 8 % moisture content, which was reached after the initial drying treatment (see Materials and Methods). Although the bags did not completely protect against seed dehydration, they probably did contribute to thermal lag, which was much greater for the dry heat compared to the wet heat treatment (Table 2).

Wet heat treatment

Thermal scarification using hot water more rapidly removed hardseededness than dry heat treatment (Figure 1). After the 1 min treatment, germination levels reached between 68 % (*Robinia pseudoacacia*) and 96 % (*Acacia melanoxylon*), values which were comparable to the scarified controls (Table 1). Similar successes in removing hardseededness by hot water immersion have been achieved with a range of species, including: *Rhus coriaria* (boiling water; Doussi and Thanos 1994); *Rhus lanceolata* (3 min, 94°C: Rasmussen and Wright 1988); three species of *Cassia* (Fabaceae) and numerous species of *Lespedeza* (Fabaceae) (c. 4 min, saturated atmosphere at 80°C; Cushwa et al. 1967); six acacias, *Gompholobium marginatum*, *Hovea trisperma* and *Templetonia retusa* (all Fabaceae) (0.5 to 10 min, boiling water; Bell and Williams 1998); and five species of Fabaceae, two species of Cistaceae, and one species of both Anacardiaceae and Convolvulaceae (≤ 60 s, boiling water; Doussi and Thanos 1994).

The time taken to approach the target temperature for scarification was highly dependent on the method used (Table 2). The thermal lag was < 1 min for the wet heat treatment compared to 4 to 11 min for the dry heat treatment. This explains the much higher efficiency of the shorter-exposure wet heat treatments compared to the dry heat treatment (Figure 1).

Table 2. Comparison between thermal lag and seed moisture content for dry and wet heat treatments at 85 °C. Thermal lag is the time taken for the seeds to reach within 10 % of the target temperature (triplicate determinations ± S.E.). Moisture contents (± s.e.) are based on 6 to 10 seeds after a 100 min treatment. Comparisons within species followed by a different letter are significantly different (t-test; $P < 0.05$). ND = not determined.

Species	Thermal lag (min)		Moisture content (%)	
	dry heat	wet heat	dry heat	wet heat
Acacia melanoxylon	3.6 (0.2) ^{A}	0.3 (0.1) ^{B}	3.4 (0.9) ^{A}	16.2 (7.9) ^{A}
Acacia nilotica	10.6 (0.6) ^{A}	0.5 (0.1) ^{B}	4.1 (0.5) ^{A}	5.5 (0.4) ^{B}
Lupinus arboreus	ND	ND	5.7 (0.1) ^{A}	6.6 (0.1) ^{B}
Rhus laurina	7.0 (0.9) ^{A}	0.2 (0.1) ^{B}	3.6 (0.4) ^{A}	40.6 (0.7) ^{B}
Robinia pseudoacacia	6.7 (1.2) ^{A}	0.3 (0.0) ^{B}	3.1 (0.6) ^{A}	53.0 (8.1) ^{B}

After 10 min in hot water, germination had decreased in *Acacia nilotica* and *Robinia pseudoacacia* and fell after 100 min to ≤ 16 %, for four species, or 74 %, for *Acacia melanoxylon* (Figure 1). Similarly, *Fumana thymifolia* and *Cistus creticus* (both Cistaceae) seeds showed signs of reduced germination after boiling water treatment for c.200 s (3.3 min) and c. 3000 s (50 min) respectively (Doussi and Thanos 1994). In contrast, hot water treatment of *A. nilotica* for periods of 12 to 24 h still resulted in germination levels of 16 to 50 %, depending on provenance (Masamba 1994).

Figure 2 shows the viability of the seeds after all treatments, i.e. the total germination observed in the test including those seeds that responded to physical scarification applied after thermal scarification. For both of the 1 min treatments and for the 10 min dry heat treatment, viability remained between 70 % and 100 % for all species. Compared to the dry heat treatment however, viability in *R. pseudoacacia* was lower after both the 10 and 100 min wet heat treatments. Similarly, *A. melanoxylon* and *A. nilotica* seed viabilities were lower after the 100 wet heat treatment compared to the dry heat treatment. Although the *R. laurina* seeds were not physically scarified, greater viability after the 100 min dry heat compared to the wet heat treatment was evident from the

germination response (Figure 1). Thus the decreases in germinability following both wet and dry heat treatments for 10 and 100 min were mainly due to a loss of seed viability and this loss evidently occurred sooner following the wet heat treatment.

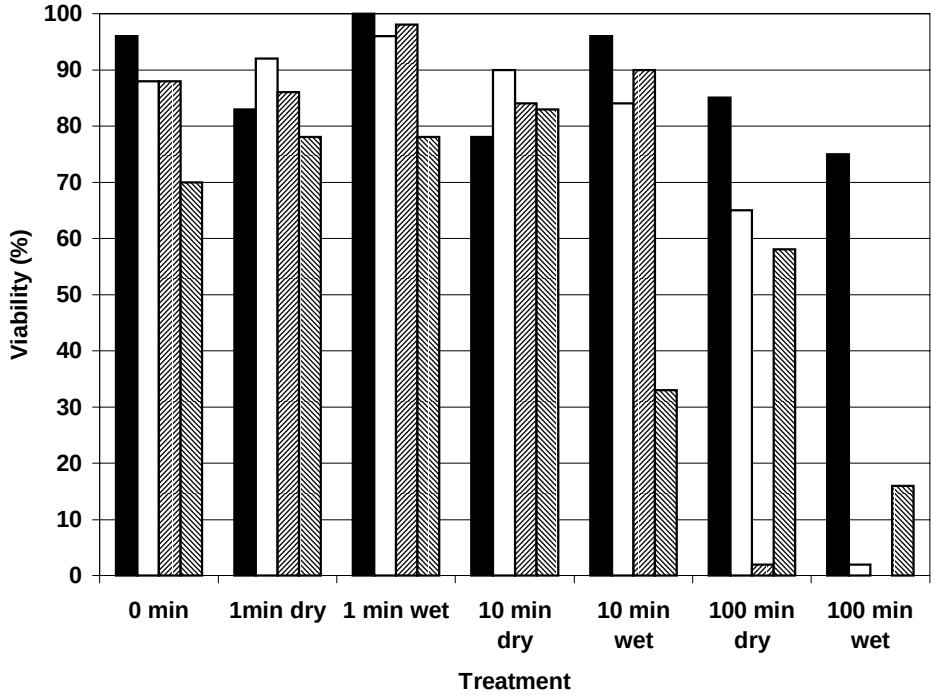

Figure 2. Viability of seeds following dry and wet heat treatment at 85°C. Viability (n = 50 seeds) was assessed after physical scarification of seeds and returning them to the germination test.

A. melanoxylon (■), *A. nilotica* (□), *L. arboreus*(▨), *R. pseudoacacia* (▧)

Wet heat treatment significantly increased seed moisture content after 100 min, to as high as 41 % *in R. laurina* and 53 % in *R. pseudoacacia* (Table 2). The s.e. values for the determined moisture contents were large for two species, suggesting considerable heterogeneity in moisture between individual seeds and presumably reflecting variable hardseededness within the seed populations. Such increases in moisture content though, probably contributed to the greater accelerated ageing of the seeds under the wet heat treatment, possibly as a result of the denaturation of rehydrated proteins at high temperature.

In conclusion, hard seeds readily responded to both wet and dry heat treatment at c. 85°C but had different optimal times for heating, suggesting that there are critical time-temperature limits for the response that are species-specific. These limits presumably relate to interspecific variation in both the properties of the seed coat and in the inherent longevity of the seeds. The consequence of such variation is likely to be reduced interspecific competition following fire.

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Seed Viability of Three *Salix* Species after 24 Months Storage at Two Moisture Contents and Four Temperatures

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Abstract

Seed of *Salix bebbiana*, *S. discolor*, and *S. eriocephala* was stored at two moisture contents (5.1-7.3% and 8.5-9.8%) and four temperatures (4, -20, -80, and –196°C) for 24 months. Seed stored at 4°C did not maintain viability. Storage at sub-freezing temperatures maintained seed viability for 24 months with little variation among treatments. Results demonstrate that cryopreservation of *Salix* seed is possible but does not offer any significant advantage over traditional storage at -20°C.

Introduction

The National Tree Seed Centre, created in 1967, is the only Seed Centre in Canada with a national mandate to obtain and store tree and shrub seed of native species for conservation and to provide seed for research purposes. Seed is collected and stored from across the geographic range of as many native species as possible. Almost all seed with orthodox storage requirements is stored in sealed containers at -20°C.

The *Salicaceae* family, which includes the genera *Salix* and *Populus*, comprises about 460 species worldwide, of which about 400 are willow species (Farrar, 1995). The three willow species used in this study: *Salix bebbiana*, *S. discolor*, and *S. eriocephala*, have a trans-Canada range and are all shrubs or shrubby trees ranging in height from 6–8 m. Willow and poplar seed is short-lived in nature and must germinate soon after dispersal. Wind-dispersed seed must find a suitable germination medium within a few days of release (Farrar, 1995; Young and Young, 1992). *Populus* and *Salix* seed will germinate while being transported by water and is ready to begin growing when it contacts soil (Farmer, 1997). Black willow (*S. nigra*) seed dispersed by water will remain viable for a longer period of time than if dispersed by wind (Pitcher and McKnight, 1990). Willow seed usually germinates within 12-24 hours upon landing on moist sand or alluvium (Young and Young, 1992).

Storage temperature and seed moisture content have long been known to affect the storage life of seed. Maroder *et al.* (2000) determined that seed moisture content of *S. alba* and *S. matsudana* seed was 40% immediately after release but quickly dropped to 11–12% within 3 hours under ambient conditions. Buch (1960) reported optimal storage moisture content for *Salix* and *Populus* seed to be between 4.0 and 7.5%. Tauer (1979) recommended drying the seed to a moisture content of 6–10%. Pence (1996) concluded that *P. deltoides* seed will survive storage in liquid nitrogen (LN) if the moisture content is below 15%. Seed of *Salicaceae*, consisting of an embryo and seed coat with almost no endosperm, does not store well unless frozen. Germination of *S. setchelliana* seed stored at room temperature in Petri dishes for 0, 5, 10, 15, and 20 days decreased from 100% at day 0 to 0% at day 20 (Douglas, 1995). Viability of *S. discolor* seed stored at 20°C decreased from 96 to 31% after 28 days, and seed of *S. bebbiana* dropped from 99 to 39% over the same period (Daigle and Simpson, 1998). *Populus balsamifera* seed stored at 7°C remained viable for only 8 months (Hellum, 1972). Viability of *S. alaxensis* seed stored at 3–5°C decreased from approximately 95% to below 10% after 10 months in storage, but the same seed stored at -10°C did not suffer any significant loss (Zasada and Densmore, 1977). Zasada and Densmore (1980) reported that willow and poplar seed could be successfully stored for up to 3 years at -10°C. Storage of *S. caprea* seed at -20°C provided better results than seed stored at -5 and 0°C (Simak, 1982). *Populus grandidentata* seed stored at -20°C at the National Tree Seed Centre germinated at 62% after 32 years. Although no record of cryopreservation of *Salix* seed could be found, cryopreservation of *Populus* seed has been successful. *Populus deltoides* stored in LN for 68 days germinated at the same level (100%) as seed stored at -20°C (Pence, 1996).

Seed storage objectives are primarily either short term, for forestry operations, or long term, for germplasm conservation (Bonner, 1990). Although the genus *Salix* is common in North America, there exist many instances where species are considered to be at risk. For example, *S. jejuna*, native to the province of Newfoundland, Canada, is classified as 'endangered' and *S. silicicola*, *S. brachycarpa* var. *psammophila*, and *S. turnorii*, indigenous to the northern part of the province of Saskatchewan, are classified as of 'special concern' (Committee on the Status of Endangered Wildlife in Canada, 2001). Black willow, a common species in the eastern United States, is at the northernmost extent of its range in New Brunswick, Canada, where it forms an

isolated population (Pitcher and McKnight, 1990). In Ohio, *P. balsamifera* and *P. heterophylla* are listed as threatened and potentially threatened, respectively (Pence, 1996).

The objective of this study was to evaluate the effect of two seed moisture contents and four storage temperatures on the viability of *Salix* seed. Seed at two moisture contents from *S. bebbiana*, *S. discolor*, and *S. eriocephala* was stored at 4, -20, -80, and -196°C. The results, after 2 years' storage, may serve as a guide for storage of seed collected from *Salix* spp. at risk.

Material and Methods

Seed from a single clone of *S. discolor* was collected on May 21, 1999 at Hamtown Corner, located just north of Fredericton, New Brunswick (46° 07' N; 66° 44' W). The catkins were immediately brought into the lab and laid on screen trays at 22°C to allow the capsules to dry and open. On May 25, after 4 days of drying, the partially opened catkins were placed in a rotating screen drum and subjected to warm airflow, which dislodged the seed from the cotton. The seed was sieved to remove larger plant material and lightly blown in an air aspirator to remove lighter particles. Percent moisture content (MC) was determined by drying two samples of seed in a forced draft oven for 16 hours at 103°C (ISTA, 1996) and MC calculated on a wet weight basis. The seed lot was halved and one of the sub-samples dried to a lower moisture content by air-drying in a forced draft oven at 30°C. The adjusted MC, or target moisture content (TMC), was calculated using the formula (IPGRI, 1996):

$$TMC = \frac{100 - (100 - IMC)(ISW)}{WDS}$$

Where: TMC = Target Moisture Content
IMC = Initial Moisture Content
ISW = Initial Seed Weight
WDS = Weight Dried Seed

Initial germination testing was carried out on May 26. The high and low moisture content sub-samples were further divided into storage samples weighing 0.35 g each; the seed was placed in 1.8-mL cryogenic vials and stored at 4°C on May 27 for 24 hours before being transferred to a -20°C freezer.

Seed of *S. bebbiana* and *S. eriocephala* was collected on May 28, 1999. The *S. eriocephala* seed was collected at the same location as the *S. discolor* and the *S. bebbiana* seed was collected at Mill Cove, New Brunswick (45° 53' N; 65° 00' W). The *S. bebbiana* seed was a bulk collection consisting of seed from four clones; the *S. eriocephala* catkins were collected from ten clones. The catkins were brought into the lab and laid on screen trays for 3 days at 22°C to allow the capsules to dry and open. The catkins were processed, their moisture content determined, and the lots divided into high and low moisture contents in the same manner described above. On June 1, a germination test was set up for these species and seed was placed in cold storage at 4°C for 24 hours, and then moved to frozen storage at -20°C. Samples of all three species that were to be stored at -80°C and in LN were removed from -20°C on June 11.

In addition to the initial germination test, seed was tested at 3, 6, 12, and 24 months. Two replicates of 100 seed each were used for the initial and 3-month tests, while four replicates of 100 seed each were used for the 6-, 12-, and 24-month tests. Vials of seed stored in LN and at -80°C were removed and placed in -20°C for 24 hours, and then these vials plus those stored at -20°C were placed at 4°C for 24 hours. Following this, all vials were left at room temperature for 4 hours before they were opened. The seed was then placed on moistened Kimpak™ in Petawawa germination boxes (Wang and Ackerman, 1983) and germinated for 10 days in Conviron™ G30 germination cabinets set at 20°C for 16 hours without light and 30°C for 8 hours with light and at a constant relative humidity of 85%. Germination was considered normal when the germinant had chlorophyll and was erect, the seed coat had shed, the cotyledons were open, and the hypocotyl hairs were capable of firmly anchoring the germinant on the substrate as described by Simak (1982).

Results

Moisture content of the seed after processing ranged from 8.5 to 9.8% and from 5.1 to 7.2% after drying. The greatest range in MC occurred with *S. discolor*, with the high and low MCs at 9.8 and 5.1%, respectively. The difference in MC for *S. bebbiana* and *S. eriocephala* ranged from 8.6 to 7.2% and 8.5 to 7.3%, respectively. Germination test results of the freshly collected seed (tested after processing and before drying) were 89.0% for *S. bebbiana*, 60.5% for *S. discolor*, and 71.5% for *S. eriocephala*.

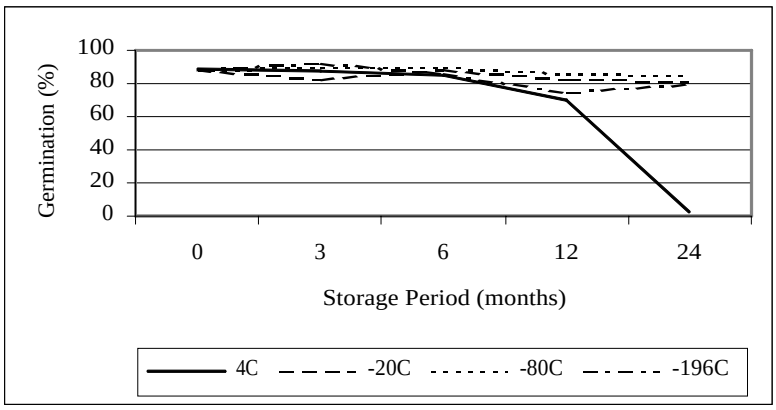

Figure 1. Germination (%) of *S. bebbiana* seed stored at four temperatures over a 24-month period.

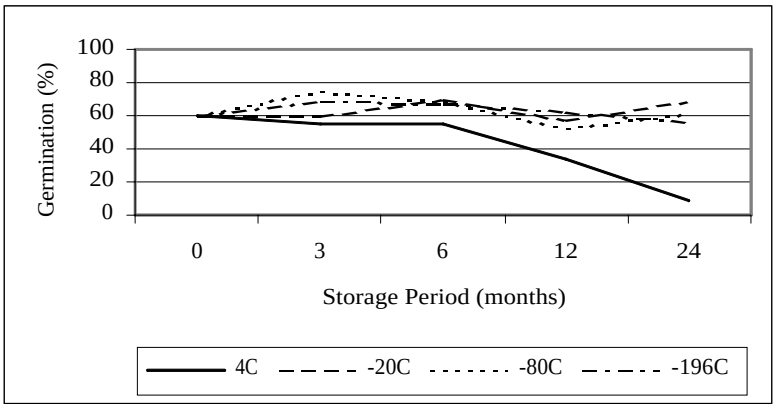

Figure 2. Germination (%) of *S. discolor* seed stored at four temperatures over a 24-month period.

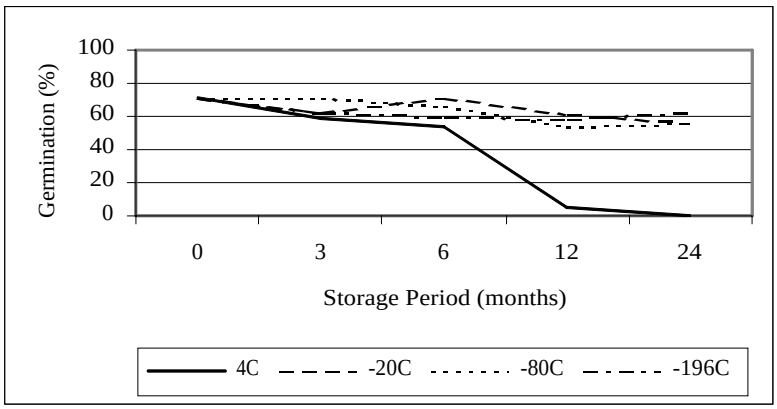

Figure 3. Germination (%) of *S. eriocephala* seed stored at four temperatures over a 24-month period.

Combining the results from each MC, little loss in viability was observed among storage temperatures up to 6 months. In fact, germination of *S. discolor* seed increased. However, after 6 months, seed viability of all three species stored at 4°C showed a marked decline (Figures 1, 2, and 3). The decline in germination over 24 months for seed in sub-zero storage averaged 7% for *S. bebbiana* and 13% for *S. eriocephala*, but results for *S. discolor* showed an increase of 1%.

After 24 months' storage, there was no significant difference in germination between MCs of *S. bebbiana* and *S. discolor* seed ($P < 0.0001$) and *S. eriocephala* seed ($P < 0.05$). Germination of seed for all species in frozen storage with a lower MC was always less than that for seed with a higher MC (Table 1). Germination differed

little among the three sub-zero storage temperatures within a given MC for each species and was only significantly different for *S. discolor* seed at 9.8% MC and *S. eriocephala* seed at 7.3% MC.

Table 1. Mean high-vigor germination of seed from three willow species stored at different moisture contents and four temperatures for 24 months.

Storage Temperature	*S. bebbiana* Moisture Content		*S. discolor* Moisture Content		*S. eriocephala* Moisture Content	
	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	2.0 a[1]	2.0 a	8.3 a	12.5 a	0.0 a	1.5 a
-20°C	81.5 b	79.5 b	68.3 b	55.8 b	56.0 b	43.8 bc
-80°C	84.5 b	72.3 b	61.0 bc	54.0 b	56.0 b	38.3 b
-196°C	80.3 b	79.3 b	56.0 c	53.3 b	62.0 b	52.0 c

[1]significant at $P < 0.0001$

Table 2. Mean abnormal germination of three species of willow seed stored at different moisture contents and four temperatures for 24 months.

Storage Temperature	*S. bebbiana* Moisture Content		*S. discolor* Moisture Content		*S. eriocephala* Moisture Content	
	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	3.8 a	5.0 a	4.8 a[1]	12.0 a	0.0 a[2]	0.8 a[2]
-20°C	5.8 a	6.0 a	12.3 b	15.5 a	16.8 b	18.5 b
-80°C	5.8 a	7.0 a	19.5 bc	15.3 a	19.3 b	28.8 c
-196°C	5.3 a	5.3 a	16.5 c	15.8 a	18.8 b	15.5 b

[1]significant at $P < 0.001$
[2]significant at $P < 0.0001$

Table 3. Proportion of abnormal germinants for seed of three willow species stored at different moisture contents and four temperatures for 24 months.

Storage Temperature	*S. bebbiana* Moisture Content		*S. discolor* Moisture Content		*S. eriocephala* Moisture Content	
	8.6%	7.2%	9.8%	5.1%	8.5%	7.3%
4°C	66.0	71.0	37.0	49.0	-	35.0
-20°C	7.0	7.0	15.0	22.0	22.0	30.0
-80°C	6.0	9.0	24.0	23.0	26.0	45.0
-196°C	6.0	6.0	23.0	23.0	23.0	23.0

There was no significant difference ($P < 0.0001$) in abnormal germination between the two MCs within each species. Abnormal germination was significantly different among the storage temperatures for *S. discolor* seed at 9.8% MC and *S. eriocephala* seed at both MCs (Table 2). Table 3 shows the abnormal germinants as a percentage of the total number of seed that germinated (normal + abnormal). The highest proportion of abnormal germinants occurred in *S. bebbiana* and *S. discolor* seed stored at 4°C. The proportion of abnormal germinants from seed in sub-zero storage was lowest in *S. bebbiana* and highest in *S. eriocephala*.

Discussion

The purpose of the experiment was to determine if *Salix* seed could be successfully stored at extremely low temperatures and what effect moisture content would have on the viability of the seed. Although it is well documented that poplar and willow seed can be successfully stored at sub-freezing temperatures (Zasada and Densmore, 1977; Tauer, 1979; Asakawa, 1982; Simak, 1982; Wang, 1982; Pence, 1996; Simpson and Daigle, 1998; Maroder *et al.*, 2000), the only documented storage temperatures below -20°C were for *P. deltoides* (Pence, 1996).

There exists ample evidence that *Salix* and *Populus* seed are extremely sensitive to storage at any temperature above freezing. In this experiment, storage at 4°C was clearly inferior. The results demonstrate that *Salix* seed

can be stored at temperatures below -20°C, however, no significant differences were found between any of the sub-freezing storage conditions, with the exception of the high MC of *S. discolor* and the low MC of *S. eriocephala* seed. These differences may be due to random variation in quality among the seed sub samples.

The literature suggests MCs between 5 and 15% are acceptable for storing seed of the *Salicaceae* family. Moisture content of seed used in this experiment ranged from 5.1 to 9.8%. Although not significantly different, seed with the lower MC was also less viable and tended to have more abnormal germinants. We do not believe that this is directly attributable to the decrease in MC of the seed. In order to lower the MC, the seed was placed in a forced draft oven at 30°C for 1 hour. This process may have injured the seed resulting in slightly less high-vigor germination and higher abnormal germination than observed at the higher MCs.

Time of collection has a profound effect on seed quality (Pence, 1996). Seed collected too early is of lower initial quality and does not store as well. Seed with low viability (e.g., stored seed) germinates more slowly and is often abnormal (von Wettstein, 1936). This was borne out by our findings. The seed stored at 4°C rapidly lost viability and had a higher proportion of abnormal germinants. The proportion of abnormal germinants was also high in *S. discolor* and *S. eriocephala* seed from frozen storage. In contrast, *S. bebbiana* seed had a low proportion of abnormal germinants. This may reflect the initial quality of the seed as *S. bebbiana* had the highest initial germination at 89%.

Based on these results, the following recommendations can be made:

1. *Salix* seed should be collected when the seed is ready to shed naturally. This ensures high quality seed with high germination and vigor that is able to withstand storage for a long period of time.
2. Storage at -20°C is sufficient. Although seed can survive storage at lower temperatures, no advantage is gained and the costs are higher.
3. Moisture content below 10% (fresh weight basis) should be used when storing seed at sub-zero temperatures. *Salix* seed loses moisture very quickly and lowering the MC to below 10% should not pose a problem. Moisture content should be lowered by air-drying or by placing seed in a desiccator. Avoid force-drying seed in an oven.
4. Seed should be processed and put in storage as soon as possible following collection. Willow seed deteriorates very quickly in nature and at room temperature. Also, when temporarily removing seed from storage for testing or distribution, the remaining seed should be returned to storage as soon as possible.
5. Seed should be stored in several small containers to mitigate the negative effects of taking the seed in and out of storage (i.e., for testing or distribution).

Acknowledgement

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The Importance of Intra- and Inter-seed Variation in Water Content for Assessing the Desiccation Tolerance of Recalcitrant Tree Seeds

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Abstract

Recalcitrant seeds loose viability during desiccation. However, there is uncertainty regarding whether there is a single critical moisture content at which seeds within a seedlot loose viability or whether there is a range of water contents, within a seedlot, at which viability loss occurs. The analysis of germination survival curves and hence the determination of critical water contents may be confounded by an uneven distribution of water, both within and between individual seeds. Here we illustrate this problem for recalcitrant seeded African tree species, including *Vitellaria paradoxa* and *Syzygium cumini*. Gravimetric and biophysical approaches revealed significant differences in water content between different components of individual seeds (seed coat, embryonic axis and cotyledons) both before and during desiccation. Consequently, whole seed water contents may not be reflective of the embryonic axis water content and hence not represent the point at which viability is lost. In addition, within a seedlot, whole seed water contents before and after drying were approximately normally distributed. Consequently, following desiccation to low average whole seed water contents (approximately 12 %) some seeds of *V. paradoxa* were still at high water contents (22-34 %), and hence viable. Despite inter-seed variation in water content it appears that, at least for *V. paradoxa*, drying to less than a narrow range of moisture contents triggers viability loss.

Effects of Pre-treatments on the Germination of Seeds of Four Panamanian Pioneer Tree Species

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Abstract

Previous studies on the germination of tropical pioneer tree seeds have been biased towards comparatively small-seeded species, which require an irradiance cue for germination. Consequently, little is known about the germination requirements of larger seeded species. This study investigated the germination requirements of four comparatively large-seeded pioneer species from Central Panama, to determine optimal germination pre-treatments. Results indicate that germination of *Ochroma pyrimidale* and *Luehea seemannii* was stimulated by hot water treatments (70°C for 2 or 10 minutes) and acid scarification (concentrated sulphuric acid for 2 or 10 minutes). *Guazuma ulmifolia*, only germinated in the acid scarification treatments, while *Apeiba membranaceae* germinated equally well in all germination treatments. These results provide simple methods for effectively germinating these species.

Keywords: germination, pioneers, scarification

Introduction

Pioneer tree species typically grow rapidly, only occur in forest gaps and open areas, have seedlings able to tolerate the harsh dry conditions associated with large clearings and produce large quantities of comparatively small seeds (Swaine & Whitmore 1988). These characteristics make them suitable for use in reforestation programmes and for restoring degraded areas. However, because they have little economic value for timber, seed germination of pioneer tree species has generally received little attention in scientific studies.

Since seedlings of pioneer species can only survive in forest gaps, pioneers often have seeds that require a specific environmental cue for germination to occur. Previous germination studies have generally found that pioneer species often require a high ratio of red:far red light (which indicates the presence of a gap in the vegetation) for germination to occur (Holthuijzen & Boerboom 1982; Vázquez-Yanes & Smith 1982; Orozco-Segovia & Vázquez-Yanes 1989; Vázquez-Yanes & Orozco-Segovia 1990), but have largely neglected alternative germination cues such as soil temperature fluctuations. In addition, previous work has not been representative of pioneers in general, because work has been biased towards small-seeded species which are likely to require a light cue for germination because they can only emerge from close to the soil surface (Vázquez-Yanes & Orozco-Segovia 1993; Milberg *et al*. 2000). Several studies have suggested a role for temperature as a germination cue for some pioneer species. For example, Vázquez-Yanes & Orozco-Segovia (1982) showed maximum germination in the comparatively large seeded (seed mass 1 mg; Ibarraman-Riquez & Oyama 1992) pioneer tree *Heliocarpus donnell-smithii* when seeds were exposed to a 15 °C daily temperature fluctuation. However, the generality of this finding to other large-seeded pioneers is unknown.

In this study, germination of four comparatively large-seeded pioneer species was investigated to determine optimal germination pre-treatments. Furthermore, the results of the germination tests are discussed in relation to the regeneration ecology of the study species.

Materials and Methods

Seed lot details

Table 1. Some characteristics of the study species.

Species	Family	Seed mass (mg)
Apeiba membranaceae Spruce	Tiliaceae	14.2
Guazuma ulmifolia Lam.	Sterculiaceae	4.6
Luehea seemannii Tr. & Planch.	Tiliaceae	1.9
Ochroma pyrimidale (Cav.) Blume.	Bombacaceae	6.6

Fruits of the four study species (Table 1) were collected from Barro Colorado Island, Panama (9°10´N-79°50´W) between April and July 1999. Fruits were collected from a minimum of 3 individual trees. Seeds were removed from associated fruit tissue prior to being air-dried in the dark. In July 1999, seeds were air-freighted to Aberdeen, UK, and stored at room temperature in the dark prior to the commencement of experiments in September 1999.

Response to alternating temperatures

One batch of 25 seeds (*Ochroma*, *Apeiba* and *Luehea*) or 30 seeds (*Guazuma*) was placed in each of 49 germination cells on the surface of a two-way thermo-gradient plate (Model GRD1, Grant Instruments, Cambridge, UK). The thermo-gradient plate created independent gradients of day (five hours at 25-45 °C) and night (19 hours at 17-36 °C) temperatures. During the five four day period seeds were exposed to an irradiance of 200 μmol m^{-2} s^{-1} with a ratio of red/far red light of 1.8.

Germination pre-treatments

Three replicates of 25 seeds each (*Apeiba*, *Luehea* and *Ochroma*) or two replicates of 25 seeds each (*Guazuma*) were subjected to the following treatments:

1. Untreated control (L).
2. Untreated control kept in complete darkness (D).
3. Mechanical scarification (MS; not done for *Luehea*, because the testa was too thin).
4. Cold water (20 °C) for 2 minutes (CW2).
5. Cold water (20 °C) for 10 minutes (CW10).
6. Hot water (70 °C) for 2 minutes (HW2).
7. Hot water (70 °C) for 10 minutes (HW10).
8. Concentrated sulphuric acid for 2 minutes (ACID2).
9. Concentrated sulphuric acid for 10 minutes (ACID10).

Seeds were subsequently sown on the surface of 1 % agar in water in petri-dishes and placed in a growth cabinet (Fi-totron 600H, Fisons Environmental Equipment, Loughborough, UK) at 26 °C and 12 hour day length. Irradiance during the 12 hour day period was 22.3 μmol m^{-2} s^{-1} with a ratio of red/far red light of 1.2.

Data analysis

The effect of fluctuating temperatures on germination percentage was tested by plotting percentage germination against the amplitude of temperature fluctuation in each germination cell, followed by least-squares regression. To determine the effects of the pre-treatments on germination percentage, germination data was arc-sine transformed prior to one-way ANOVA followed by *post hoc* Fisher's LSD test.

Results

Alternating temperatures

Diurnally fluctuating temperatures had no effect on the germination of *Apeiba* ($R^2 = 0.206$, $P > 0.1$), *Guazuma* ($R^2 = 0.188$, $P > 0.1$) or *Luehea* ($R^2 = 0.036$; $P > 0.1$); germination was low in all treatments. Conversely, there was a significant positive relationship between amplitude of temperature fluctuation (x) and percentage germination (y) of *Ochroma* ($y = 1.01x + 22.6$, $R^2 = 0.277$, $P < 0.01$).

Germination pre-treatments

The results indicate that none of the four species requires light for germination (Fig. 1). The two hot water treatments resulted in significantly higher germination percentages for *Ochroma* and *Luehea* ($P < 0.05$), but not for *Guazuma* and *Apeiba* (Fig. 1). However, the acid treatment improved germination of *Guazuma*, as well as *Ochroma* and *Luehea*. The mechanical scarification treatment significantly ($P < 0.05$) improved germination of *Guazuma* although the effect was comparatively small (Fig. 1).

Discussion

None of the four species used in this study requires light for germination. This is in contrast to many earlier

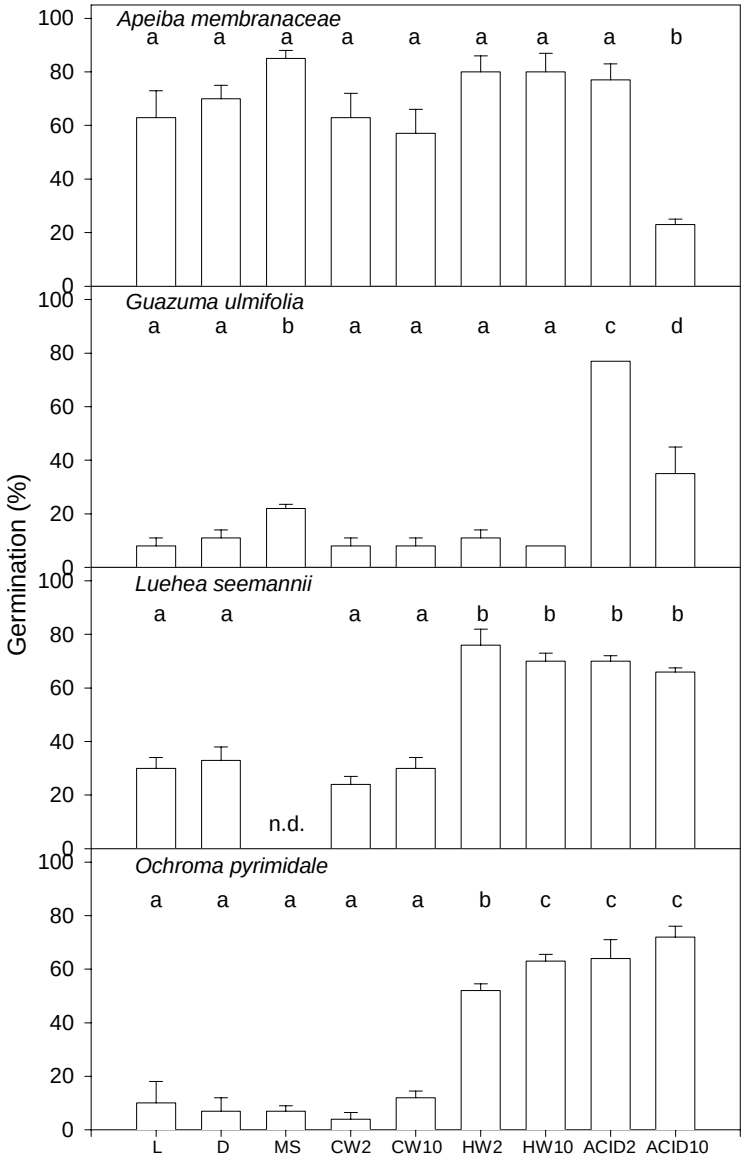

Figure 1. The effect of various pre-treatments on seed germination of the four species. Treatments follow the abbreviations given in the methods section. Different letters within a species, indicate significant differences in percentage germination between treatments. Bars are ± 1 standard error.

studies on the germination of pioneer species that found that light stimulated the germination of many pioneer species (Holthuijzen & Boerboom 1982; Vázquez-Yanes & Smith 1982; Orozco-Segovia & Vázquez-Yanes 1989; Vázquez-Yanes & Orozco-Segovia 1990). This difference may arise because much of the earlier work has focused on comparatively small-seeded species, which can only emerge from close to the soil surface. For these species, a light cue for germination is an effective means of ensuring germination at depths from which the seedling is able to emerge. However, larger seeded species have seedlings with the resources to emerge from greater depth (Bond *et al.* 1999). Emerging from greater depth is advantageous, since the soil remains wetter during dry periods and hence the seedlings have a greater chance of survival. Therefore, since irradiance only penetrates a few mm into soil (Tester & Morris 1987) it would be an inappropriate germination cue for larger seeded species.

Both *Ochroma* and *Luehea* responded positively to hot water treatments, although only *Ochroma* responded to fluctuating temperatures. This suggests that temperatures on the thermo-gradient plate may have been too low to stimulate germination of *Luehea*. Both *Ochroma* and *Luehea* occur in large clearings, along roadsides and in abandoned pastures (Croat 1978; Dalling *et al.* 1999). Therefore, the response to hot water treatments may simulate the effects of either large diurnal soil temperature fluctuations or fire, both of which indicate the absence of established vegetation. Temperature as a germination cue for larger seeded species may be

advantageous since elevated soil temperatures will occur at depths greater than light can penetrate to (Hanks 1992).

The high germination percentages of *Apeiba* in all the treatments, except the 10 min acid treatment, suggests that this species is more of a generalist with seeds that will germinate in any environment, as long as sufficient moisture is present. Seedling establishment is thus presumably dependent on either seed dispersal to open sites or the chance creation of clearings above seedlings.

Guazuma germinated to a low percentage in all treatments, except the 2 min acid treatment. Fruits of this species are readily eaten by vertebrate herbivores (Janzen 1982) and passage through the gut may soften the seed coat. Again, like *Apeiba* this species may rely on dispersal to open sites for successful establishment.

These results, and studies by previous workers (see above) indicate that, as a group, neotropical pioneer trees have a diverse range of germination requirements and consequently few generalisations can be made about optimum treatments for overcoming seed dormancy.

Conclusions

Seeds of the four study pioneer species exhibited different responses to the range of experimental treatments.

1. *Apeiba* germinated across the range of treatments suggesting that it will germinate in a wide range of environments.
2. *Ochroma* and *Luehea* were stimulated to germinate by hot water and acid treatments. The hot water treatment may simulate the effect of fire or the large diurnal temperature fluctuations in large clearings.
3. *Guazuma* was only stimulated to germinate by the acid treatment; this may simulate the effects of ingestion and gut passage by herbivores.

Acknowledgements

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Incidence of New Seed-Borne Disease of Kamagong [*Diospyros philippensis* Des. (Gurke)] Seeds

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Abstract

Preliminary study on the cause of a new seed borne disease in three (3) seedlots of *Diopyros philippensis* collected in Mt Makiling Los Baños Experimental Station of ERDB and UPLB Campus was conducted.
The yellowish mycelial mat of the fungus was found underneath the seed coat and this ramified covering enveloped the entire endosperm. Two types of fruiting bodies emerged from the embryonic axis and from the dorsal portion of the seeds: the club shaped and corraloid basidiocarps. The club shaped basidiocarp has the following characteristics: 2-5 per seed, arises from a single point, simple, erect, has tooth like outgrowth which varies in size and later branches into another set of basidiocarps. The young basidiocarp is yellowish green and turns into olive green when aged with measurements ranging from 2.0 to 3.3 cm x 0.2 to 0.5 cm. On the other hand, the corraloid type is characterized by: 5-30 per seed, does not arise from a single point, erect, some with 2-3 branches, without tooth like outgrowth, light yellow when young and becomes olive green when aged and measures 2 to 13 cm x 0.1 to 0.3 cm.
Based on the morphological structures of the causal fungus, the two types of basidiocarps belong to the *Claveria* sp, however, anatomical structures such as metulae and phialides formation show that the pathogen belong to Fungi Imperfecti similar to *Penicillium* spp. Pure culture isolates of the pathogen was found similar with the fruiting structures observed from affected seeds.

Effects of Various Factors on Germination of *Cedrus atlantica* M. Seeds

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Abstract

Germination of *Cedrus atlantica* seeds was studied by testing the effects of various factors (i.e. seed weight, period of cone harvesting, duration of cold stratification, seed versus cone exposure to low temperatures (4°C), concentration of H_2O_2, AIA and GA_3).

Results revealed that germination rates are enhanced in the following cases: heavy seeds, delayed seed harvesting (i.e. in winter instead of autumn), a cold stratification of 30 days; seed versus cone exposure to low temperatures; H_2O_2 at a concentration of 30%).

Keywords: Algeria, *Cedrus atlantica* Manetti, germination, seeds, pretreatment

Introduction

Cedrus atlantica is an autochthonous tree of the North African mountains (i.e. Morroco and Algeria). Boudy (1950 and 1952) noted on this species a patchy natural recruitement, which can be also capricious, some years. Le Poutre (1961 and 1963), Le Poutre and Pujos (1963), Pujos (1964) studied the factors and mechanisms of natural regeneration of *Cedrus atlantica*. Toth (1978) made field and laboratory germination tests taking into account various factors (i.e. temperature, light…).

Our interest in *Cedrus atlantica* seeds includes various aspects (variation in dimensions among Algerian provenances, the effect of period of conservation on seed germination, and field establishment of the seedlings) in addition to the present contribution which consists in testing the effects of various factors on germination of *Cedrus atlantica* seeds according to authors concerned directly by the genus (Odabasi 1967; Chaudy 1972; Martin 1934; Zaki 1970; Toth 1978, Abourouh 1983, Muller et al.1984) and to other plant physiologists (Evenari 1957, Binet and Brunel 1968; Come 1970 and Mazliak 1982).

Materials and Methods

The germination tests were conducted in petri dishes on cotton covered with filter paper and moistened with distilled water. The petri dishes were put in a germination room at 19°C, 60% relative humidity and a photoperiod of 8h/16 hours. The basic number of seeds per test was 100 (5 petri dishes with 20 seeds/ petri dish). The duration of germination tests was of 21 days. Every 3 days the petri dishes were moistened and the germinated seeds counted. Seeds were recorded as germinated after the radicle emerged through the micropyle. The tests were undertaken separately and independently from each other. Details on materiel and methods applied for each test are given below.

The measured parameters are the germination rate and the germination mean time. The germination rate is the ratio of the final number of germinated seeds on the initial number of seeds tested. For any test the counts over the petri dishes were cumulated. The germination mean time is expressed as follows:

$$GMT = \frac{N_1T_1 + N_2T_2 + N_3T_3 + \ldots\ldots + NnTn}{N_1 + N_2 + N_3 + \ldots\ldots + Nn}$$

Where N_1T_1 is the product of the number of germinated seeds (N_1) with the time T_1. T_1 is the number of days elapsed since the test started. Since seeds were counted every 3 days, T_1, T_2, T_3, T_4, T_5, T_6, T_7, refer to 3, 6, 9, 12, 15, 18, and 21 days.

Effect of seed weight

For seed of the same origin, three seed lots were constituted according to their weight: 0.04 to 0.07; 0.08 to 0.09; and 0.10 to 0.11 g.

Effect of period of cone collecting

Cones were harvested in a single provenance over two successive years at the following dates: May 30th, July 14th, Sept 4th, November 12th, and December 15th, for the first year; February 2nd and March 23th for the second year. Seed lots were constituted with 200 seeds for each harvesting date. Scales of the cones harvested during February were opened, while the ones of the cones sampled during July were closed. Closed cones were opened by placing in water for 48 hours.

Effect of cold and dry conditions

5 seed lots were constituted, each one containing 200 seeds. One seed lot was used as a control (seeds not submitted to any treatment), the remaining ones were put in perforated plastic bags and then placed in a refrigerator (where the temperature ranged between –2 to 5°C) for different periods (i.e. 9, 15, 24, and 30 days). At each date, a seed lot was extracted and tested for germination.

Effect of the mode of seed storage

Cones were harvested at the same period from the same origin. The test was conducted both on seeds extracted from the cones and non extracted ones. The entire cones (i.e. seeds in cones) and seeds extracted from the cones were exposed during 75 days to the same conditions which consisted of a cold and controlled temperature for one part and an ambient (i.e. non controlled) temperature for another part.

Effect of the duration of cold and wet stratification

5 seed lots were constituted, one was used as a control while the others were put in a refrigerator and exposed to wet and cold conditions (i.e. cold stratification) for different periods: 4, 9, 15, 24, and 30 days.

Effect of seed coats, endosperm and resin vesicles removal

Cones were harvested within the same provenance during the fall of November. Seed lots (each one containing 100 seeds) were constituted as follow:
a- the control (i.e. the whole seed)
b- seeds without coats (i.e. embryos and endosperm only)
c- seeds without resin vesicles
d- seeds without endosperm (embryos only)
e- embryos covered with resin.
The seed lots were not submitted to any additional treatment before germination test.

Effect of hormones

Solutions of the AIA and GA_3 hormones were prepared separately at the following concentrations: 100, 50, 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} mg/l. For each hormone, seed lots were exposed to a solution of a given concentration at low temperature (i.e. 4°C) during 24 hours. After this treatment they were moved to petri dishes for germination tests. The seed lots were moistened with the hormonal solutions during the germination test. For the GA_3 the petri dishes were exposed to darkness during 03 weeks before the germination test started.

Effect of oxygenated water (H_2O_2)

Solutions of H_2O_2 were prepared at two concentrations, 15 and 30 %, each of them was applied 1 hour to a seed lot of 100 seeds.

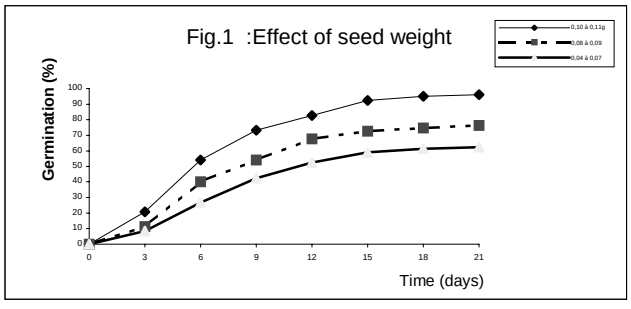


Fig.1 :Effect of seed weight

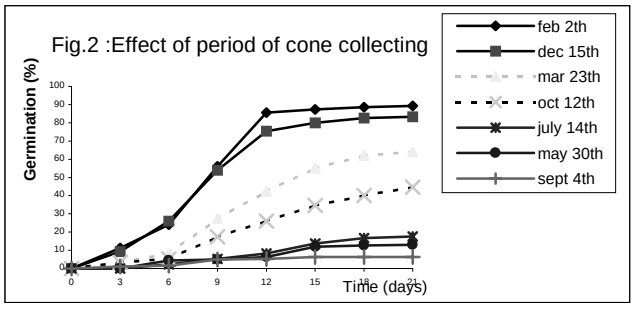


Fig.2 :Effect of period of cone collecting

Results

Effect of seed weight

Germination rates are higher in case of heavier seeds, while the germination time showed a slight variation (cf. fig 1).

Table 1. Germination of seeds of different weight.

seed weight (g)	germination rate (%)	germination mean time (days)
0.04 - 0.07	62	9.06
0.08 - 0.09	83	8.37
0.10 - 0.11g	96	8.15

Effect of date of harvesting

The results showed that a later harvesting (i.e. during winter) is better than harvesting during summer and the beginning of autumn (cf. fig. 2).

Table 2. Seed germination for different dates of harvesting.

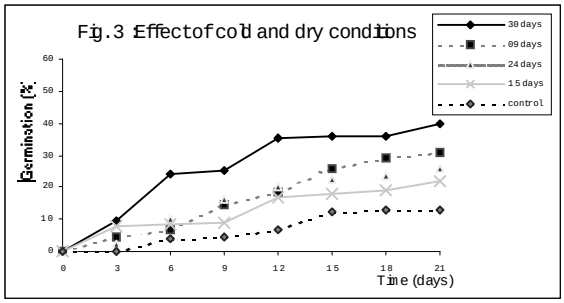
dates	germination rate	germination mean time (days)
May 30th	12.50	11.64
July 14th	17.00	13.14
September 4th	06.00	09.50
November 12th	51.00	12.81
December 15th	82.00	09.07
February 2nd	89.00	09.05
March 23th	06.00	11.00

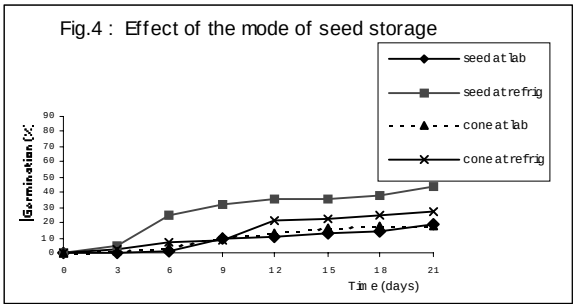
Effect of the duration of cold and dry conditions

The results revealed a higher germination amount for seeds submitted to cold and dry conditions during 30 days (cf. fig. 3) and also a gain of two days in germination time.

Table 3. Seed germination for different periods of cold and dry conditions.

Period of treatment (in days)	germination rate	germination mean time
control	12.50	11.64
9	30.50	11.36
15	22.50	10.46
24	26.00	09.88
30	40.00	08.62





Effect of mode of seed storage

Germination rates are better in case of seeds and cones exposed to cold temperatures, and the highest amount (i.e.44.50%) is obtained for seeds extracted from the cones (cf. fig. 4).

Table 4. Germination of seeds stored in different ways.

mode of storage	germination rate	germination mean time
cones at a cold temperature	26.50	12.90
seeds at a cold temperature	44.50	09.53
cones at ambient temperature	18.00	10.90
seeds at ambient temperature	19.00	13.34

Effect of period of cold stratification

The results showed a higher germination rate and also a better germination speed for seed stratified during 30 days (cf. fig. 5).

Table 5. Germination of seeds exposed to different periods of cold stratification.

period of stratification (days)	germination rate	germination mean time
0 (control)	12.50	11.64
4	34.00	10.50
9	52.00	08.36
15	61.00	06.00
24	61.50	06.58
30	85.50	04.42

Effect of seed coats, endosperm and resin vesicles removal

The whole germination rates are relatively low although the χ^2 test revealed significant difference in germination rates between the control and seeds without coats.

Table 6. Germination of seeds with or without removal of some parts of the seed.

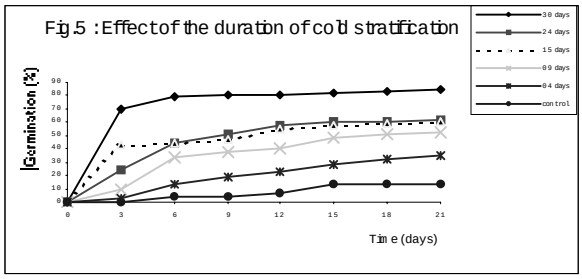
seed lots	germination rate	germination mean time
the control (i.e. the whole seed)	17.00	13.14
seed without coats	23.00	09.25
seeds without resin vesicles	00.00	-
embryos	05.00	21
embryos covered with resin	05.00	21

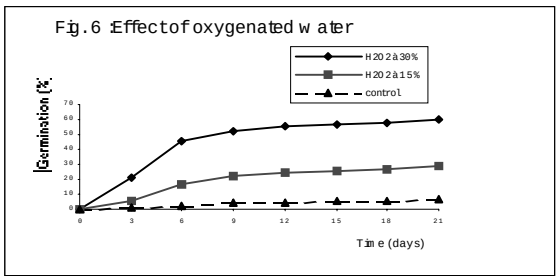
Effect of hormones

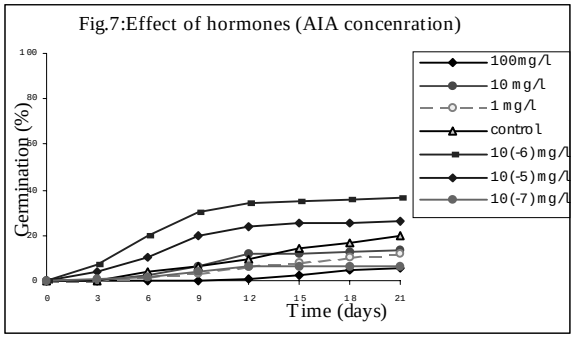
Although the concentration of 10^{-6} mg/l revealed higher germination rates in case of the two hormones when compared to the control, the values obtained for this test remain relatively low (cf. fig. 7 and 8).

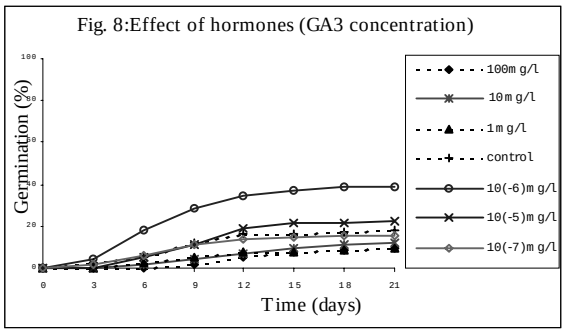
Table 7. Germination of seeds exposed to AIA and GA3 hormones.

Hormone concentration (mg/l)	AIA		GA3	
	germination rate	germination mean time	germination rate	germination mean time
0 (control)	16.00	12.93	17.00	08.82
100	05.00	13.20	09.00	13.66
50	09.00	12.00	07.00	11.57
10	13.00	11.30	12.00	12.75
1	11.00	11.72	08.00	11.25
10^{-1}	17.00	10.05	14.00	11.78
10^{-2}	20.00	09.00	18.00	11.33
10^{-3}	23.00	07.95	16.00	09.75
10^{-4}	23.00	08.73	17.00	10.76
10^{-5}	25.00	08.52	22.00	10.50
10^{-6}	35.00	07.62	38.00	08.36
10^{-7}	06.00	08.50	16.00	09.00









Effect of H_2O_2

The germination rate obtained on seeds treated with H_2O_2 at 30 % were higher (i.e. 60%) than the control (6%). A gain of 2 days in germination time was also obtained for this concentration.

Table 8. Germination of seeds exposed to H_2O_2.

H_2O_2 concentration (%)	germination rate	germination mean time
0 (i.e. the control)	06.00	09.50
15	28.00	08.03
30	60.00	06.25

Discussion

Our results revealed a positive effect of seed weight on germinative capacities of *Cedrus atlantica* seeds in accordance with Toth (1978) for the same species. A delayed seed collecting revealed also a favourable effect in accordance with Toth (1978) and Muller et *al.* (1984). To explain the results found for different collecting dates one should suppose that seeds collected during summer are not sufficiently mature, the ones collected during autumn are mature but not exposed to sufficient cold and rainfall, while the ones harvested during winter have experienced cold temperatures and rainfall, which allowed their dormancy breakage.

Cedrus atlantica seeds are disseminated at the fall of autumn and during winter they are naturally exposed to cold temperatures (and often covered by a layer of snow) and rainfall before germination take place during spring. Laboratory pretreatments consisting in exposing seeds to low temperature combined to moisture are in fact similar to the ones occurring at field during winter. Seed dormancy is in fact a phenomenon, which affects 60% of the species in the temperate climatic zones (Bonnet-Massimbert et Muller 1984).

In accordance with authors (Odabasi 1967 for *Cedrus libani*, Zaki 1970 and Toth 1978 for *Cedrus atlantica*) we consider stratification as the best pretreatment to break seed dormancy. But the duration of this pretreatment must not be prolonged over 4 weeks (Odabasi, 1967) or 6 weeks (Abourouh 1983, Muller et al. 1983) since a prolonged stratification does not result in any additional germination rate.

After 4 weeks of stratification we obtained a germination rate of 85% with a germination test of 21 days). Muller and al. (1984) found a germination rate of 75% after a stratification of 06 weeks with a germination test of 35 days. Toth (1978) obtained values ranging from 87 to 94 % respectively for 4 and 6 weeks stratification with a germination test of 21 days.

Removal of any part of the seed does not seem beneficial to its germination. Gaussen (1955) noted that resin vesicles are indispensable to the germination of *Cedrus* seeds. An inhibiting effect of resin on germination was found by Odabasi (1967) who covered *Pinus nigra* seeds with resin of *Cedrus libani* seeds and according to this author the inhibiting effect is increasing with an increasing quantity of resin.

In our results we observed that endosperm removal resulted in abnormal germination (i.e. the whole part of the embryo grew in synchrony) in accordance with Toth (1978) on seeds of the same species and with Derridj and Satour (2001) on *Abies numidica* seeds.

Our results suggest a non efficient effetc of A.I.A. and GA_3 hormones on germination, although Bulard (1950) indicated for the gymnosperms the efficiency of A.I.A. concentrations ranging between 10^{-3} and 100 mg/l and obtained the best germination rates on *Picea excelsa* for concentrations ranging between 1 and 1000mg/l at darkness. Pittel and Wang (1985) improved germination of *Pinus monticola* by combining GA_3 and kinetin hormones. According to Come (1970) treating the intact seed with GA_3 does not enhance the germination due to the impermeability of coats to this hormone.

In accordance with Abourouh (1983) we noted a positive effect of pretreating *Cedrus atlantica* seeds with H_2O_2 (particularly the concentration of 30%) but we observed a slow emergence of the whole seedling from the remaining integument (the first emerged seedlings were observed only at the 25th day after sowing).

Conclusion

In regards to all tests applied to seeds of *Cedrus atlantica* the stratification revealed to be the best pretreatment although other factors revealed a positive effect (i.e. a cold and dry treatment, exposure to H_2O_2 at 30% during one hour, storing seeds in cold temperature of the refrigerator instead of ambient conditions). Hormone supplementation seems inefficient on germination. Seed coats, endosperm and resin vesicles removal is to avoid.

The effect of stratification seems to depend upon the date of seed collecting (i.e. seed ripeness) and also on seed provenance (data not shown). Further data showed that on freshly collected seeds, high germination rates might be obtained with a shorter period of stratification of 24 to 48 hours (data not shown). This method of breaking seed dormancy represents a combination between the advantage of being cheap and fast and may be very useful to nurserymen interested in obtaining seedlings for reforestation.

Although high germination rates can be reached in controlled conditions attesting of a good intrinsic quality of the seeds, field seedling establishment depends upon external factors such as summer drought and soil structure (non published data).

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Seed-to-Wing Attachments in Important Members of the Pinaceae, with Additional Observations on Members of the Cupressaceae and Taxaceae

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Abstract

Wings of seeds of spruce and many pine species can be removed easily *in toto* because they are not fused to the seed coat. However, confusion has arisen regarding what constitutes "pure seeds" (when testing for trading purposes) in several other genera. A photographic survey of wing-to-seed attachments demonstrated that certain genera, and certain species within a given genus, should be separated when "pure seeds" are defined. Proposals for revising international pure seed definitions are discussed.

Keywords: *Abies, Calocedrus, Cedrus, Keteleéria, Larix, Pinus, Pseudotsuga, Sequoiadendron, Taxus, Tsuga,* integument, pure seed, seed wing

Introduction

Mature seeds of most coniferous trees bear one or more membranous wings; notable exceptions include certain pines, such as *Pinus albicaulis, P. cembra, P. flexilis, P. koraiensis*, as well as *Taxodium distichum*, in which wings are rudimentary or absent. When shed from the parent tree, wings allow air currents to carry them some distance away. For artificial reforestation programs, the same wings are an impediment to sowing, and winged-seeds on the surface of a growing medium are easily disturbed by air movement. Typically, wings and seeds are separated before storage since wing removal greatly reduces the seed bulk. Modern sowing machinery relies on clean, wingless seeds for greatest efficiency. Seeds of the Cupressaceae typically bear more than one wing that are difficult to remove without damaging the seeds, so these seeds are left intact.

When traded, tree seed containers typically indicate the pure seed percentage, an indication of how "clean" is the lot with regard to the species being marketed, seeds of any other species, and any inert matter. Inert matter includes seeds from which the seedcoats have been entirely removed, broken or damaged seeds, cone scales, wings, bark, etc., and the analyst must be able to recognize and separate these components. As an aid in this, a series of "pure seed definitions" have been provided by the International Seed Testing Association (ISTA 1999); the companion Handbook of Pure Seed Definitions with illustrations (Felfoldi 1987) excludes forest tree seeds. Similar descriptions, including tree seeds, have been provided by the Association of Official Seed Analysts (of North America) (AOSA 1998). All are aimed at standardizing what are to be classified as pure seeds, and periodically they revised. The focus here will be on the ISTA (1999) rules.

Of the 63 Pure Seed Definitions (PSDs) included in the International Seed Testing Rules (ISTA 1999: 133-144), 5, *viz*., PSD 10, 47, 49, 50 and 51, cover conifer species; the complete list of PSD numbers by genus and family can be found on pages 123-132. The vast majority of coniferous genera are covered currently in 3 PSDs as follows:

PSD 47 includes seeds of all spruces (*Picea*) and most pines (referred to collectively as *Pinus* II), but excluding longleaf pine (*P. palustris*) and pitch pine (*P. rigida*) (which are referred to as *Pinus* I – see PSD 51);

PSD 49 includes seeds of false cypress (*Chamecyparis*), cryptomeria (*Cryptomeria*), cypress (*Cupressus*), redwood (*Sequoia* - but not *Sequoiadendron*, which is currently assigned to PSD 50), plus arborvitae (*Thuja*).

PSD 51 includes seeds of firs (*Abies*), incense cedar (*Calocedrus*, formerly *Libocedrus*), cedars (*Cedrus*), larch (*Larix*), longleaf pine (*P. palustris*) and pitch pine (*P. rigida*, together referred to as *Pinus* I – see PSD 47), Douglas-fir (*Pseudotsuga*) and hemlocks (*Tsuga*).

Other conifer genera (*Juniperus*, *Taxodium*, *Taxus*) are assigned to PSD 10.

Due to space requirements of these Proceedings, the main focus of this report will be limited to PSD 51, the text of which states that a pure seed is a:

> "seed, without wing, with (but occasionally without) integument, provided a portion of the testa is attached. Piece of seed larger than one-half the original size, without wing or integument, provided a portion of the testa is attached."

PSD 51 then goes on to describe that "integument":

> "refers to the tissue attaching the wing to the seed. In Pinaceae with this definition, the integument is fused to the seed, is rarely removed in processing, and is impossible to consistently remove, without causing damage. Hence, seed with integument attached is considered to be 'pure seed'. Winged seed – i.e. seed with an integument plus wing still attached must be weighed and reported as a separate percentage from 'pure seed' according to paragraphs 3.5.2.A.9 and 3.7. After weighing, the winged seed and pure seed fractions should be recombined and used in representative portions for counting out the germination replicates."

Note that this use of the term "integument" differs from the strict, botanical definition which is variously given as "the tissues covering or surrounding the ovule, usually consisting of an inner and outer layer which comprises the seedcoat of the mature ovule" (Copeland 1976), or "the one or two layers of tissue, often fused, that enclose the nucellus of an ovule and that develop after fertilization into seed coats" (Bonner 1984). In conifers, the seed coat forms even when the ovule is not fertilized.

The objective of the study reported here was to review the current PSDs mentioned above, with the focus on PSD 51, to determine if species' assignments were appropriate. Seed features to which PSDs referred, particularly attachments of wings to the seeds, were recorded on macrophotographs.

Materials and Methods

Seeds of 39 species in 13 genera (Table 1) from the reference collection at the Pacific Forestry Centre (Canadian Forest Service), Victoria, British Columbia, were examined, and photographed using 35-mm colour-transparency film (ASA 200). For larger seeds, a camera fitted with a 28-85 mm AF lens (macro setting) combined, as required, with a 12 mm extension ring and ring flash was used; to highlight special features, the ring flash was supplemented occasionally with a fibre optic light source. For intermediate and very small seeds, or for very close detail, dissecting microscopes equipped with 35-mm cameras, a ring lamp, or one or more fibre optic lamps, were employed. Computer images were obtained using a slide scanner at maximum (2700 dpi) resolution.

Table 1. Genera and species examined in relation to ISTA (1999) Pure Seed Definitions.

Genus	Species
Abies	*alba*, amabilis*, balsamea*, concolor*, fabri*, grandis*, lasiocarpa*, magnifica*, nordmanniana*, numidica*, pinsapo*, procera*, sibirica**
Calocedrus	*decurrens**
Cedrus	*libani**
Chamaecyparis	*nootkatensis*
Cupressus	*funebria*
Keteleéria	*davidiana**
Larix	*decidua*, leptolepis*, lyalli*, occidentalis*, sibirica**
Picea	*alba, glauca, pungens, purpurea*
Pinus	*contorta, coulteri, palustris, ponderosa, rigida*
Pseudotsuga	*macrocarpus*, menziesii**
Sequoiadendron	*gigantea*
Thuja	*plicata, occidentalis*
Tsuga	*heterophylla*, mertensiana**

* Species covered by this report (but not all illustrated).

Results and Discussion

Because photographic depth of field becomes very shallow the closer the camera lens approaches the subject, all critical details, especially of the larger seeds, could not be obtained within a single frame for most specimens.

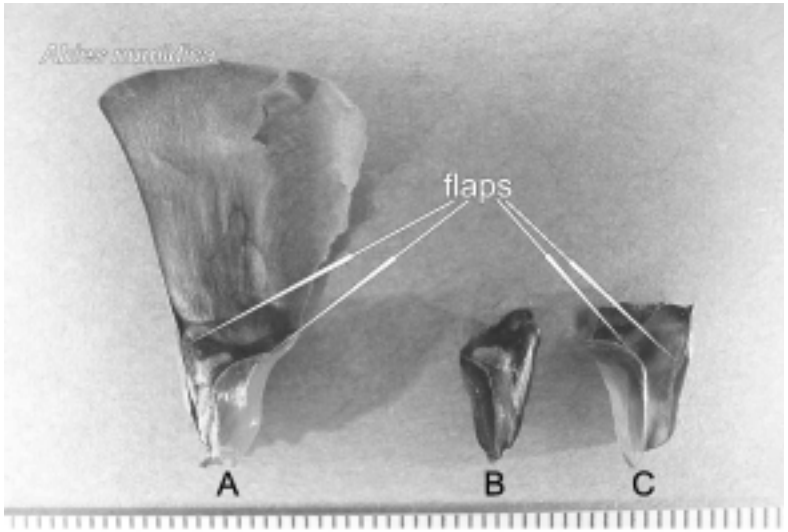

Figure 1. Abaxial view of *Abies numidica* seeds. A – seed with entire wing and integument showing flaps. B – naked seed. C – undamaged integument (wing broken off) from which seed B was removed.

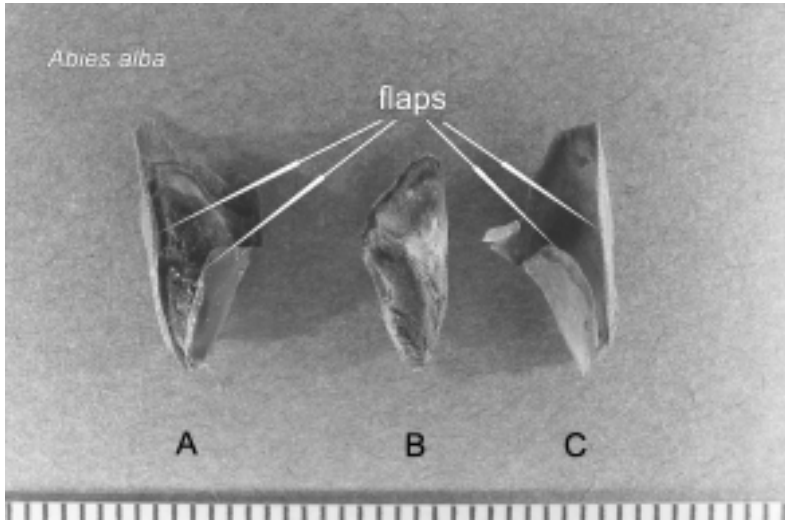

Figure 2. Abaxial view of *Abies alba* seeds. A – seed with integument (wing broken off), a "pure seed" according to PSD 51. B – naked seed. C – undamaged integument from which seed B was removed.

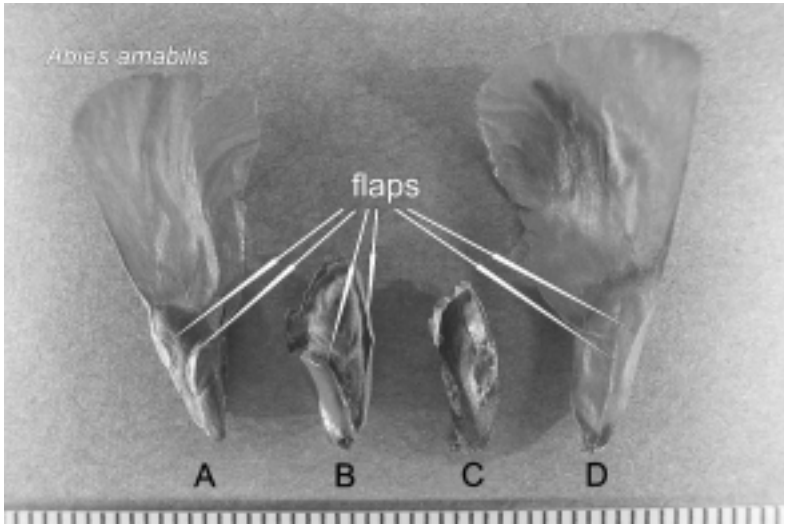

Figure 3. Abaxial view of *Abies amabilis* seeds. A – seed with entire wing and integument showing flaps. B – seed with integument (wing broken off), a "pure seed" according to PSD 51. C – naked seed. D – intact wing and integument from which seed C was removed.

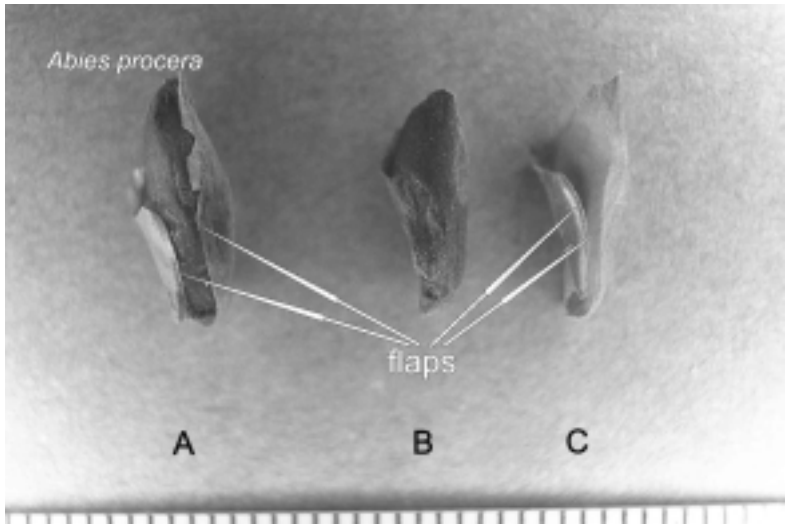

Figure 4. Abaxial view of *Abies procera* seeds. A – seed with integument (wing broken off), a "pure seed" according to PSD 51. B – naked seed. C – undamaged integument from which seed B was removed.

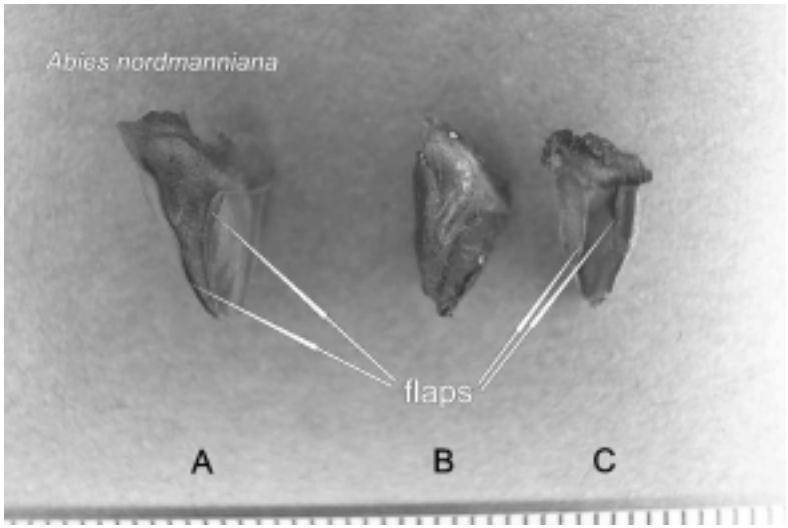

Figure 5. Abaxial view of *Abies nordmanniana* seeds. A – seed with integument (wing broken off), a "pure seed" according to PSD 51. B – naked seed. C – undamaged integument from which seed B was removed.

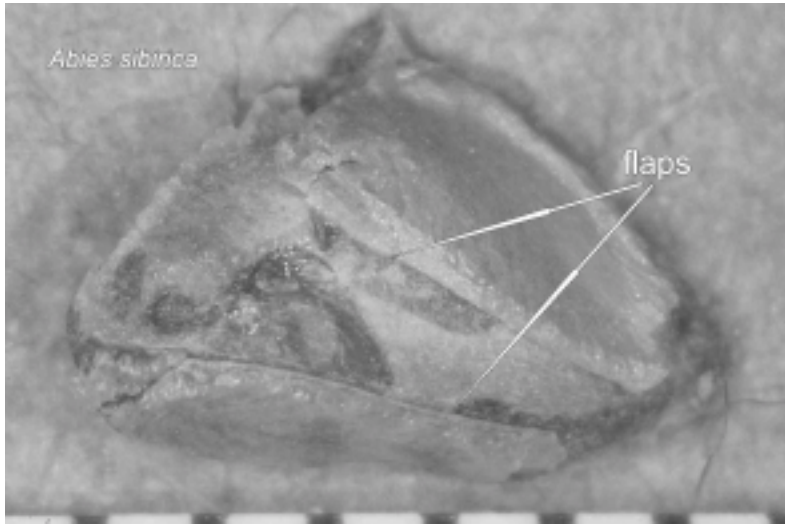

Figure 6. Abaxial view of an *Abies sibirica* seed with integument (wing broken off). This is a "pure seed" according to PSD 51.

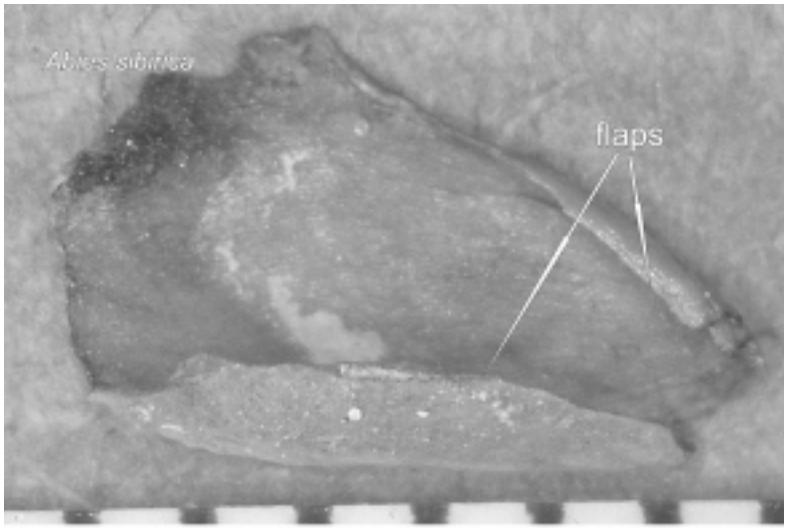

Figure 7. Abaxial view of the "empty" undamaged integument from which the *Abies sibirica* seed in Figure 6 was removed.

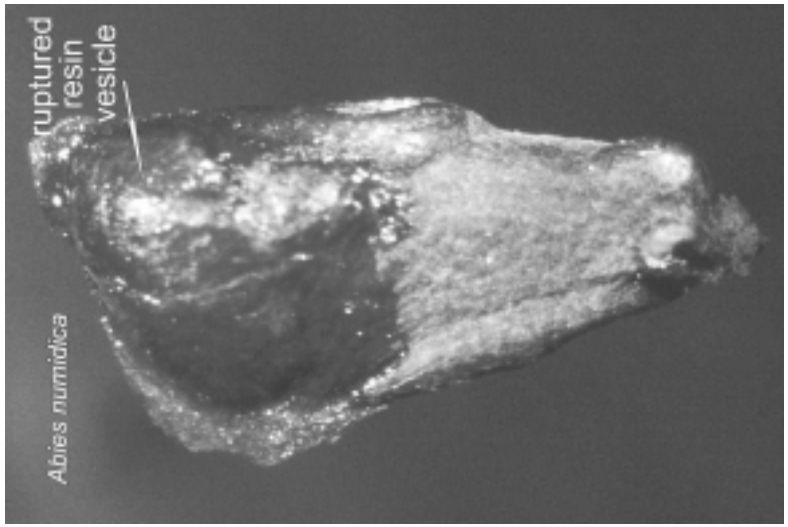

Figure 8. A naked *Abies numidica* seed. The glossy area in the upper half of this seed image was caused by resin from a vesicle that was ruptured in preparing the seed for photography. Seed length ~ 9 mm.

Thus, several macrophotographs were made with slight focus adjustments between frames. Of the more than 250 exposures made 40-45% were scanned into the computer. Representative illustrations for species in PSD 51 only are presented here. In all figures the scale bar (when shown) indicates millimetres; arrows generally point to the edges or margins of the structures indicated, and unless otherwise indicated, all views are of abaxial surfaces. The following observations were made on the 7 genera (in alphabetical order, plus *Keteleéria*) currently assigned to PSD 51.

Abies

Fir seeds have been assigned, ostensibly, to PSD 51 on the basis that the integument "is rarely removed in processing, and is impossible to consistently remove, without causing damage. Hence, seed with integument attached is considered to be 'pure seed'". It was determined for all 13 fir (6 North American and 7 European/Asian/North African) species examined (Table 1) the integuments are not "fused" to the seeds as the first part of PSD 51 states. For the seeds that still retained entire wings (plus integuments), or those that retained integuments only, these structures were removed easily by hand. Moistening the seeds (placing them on moist blotter for two days, as for a germination test) made this separation straightforward. Many germination analysts will know that fir integuments – being highly hygroscopic, which causes them to expand when moist, and loosen their "grip" on the seeds – often separate from the seeds as the latter are moved around to assess germinant status.

Whereas the proximal end of the fir wing, the integument, completely covers the adaxial surface of the seed, only two "flaps" wrap around the long margins to "clasp" the seed on the abaxial surface. This is clearly shown in *A. numidica* (Fig.1), and was described previously (Cermak 1987) for *A. alba* (Fig. 2). These abaxial flaps vary in size, one (on the margin of the seed nearer to the centre of the ovuliferous scale on which it formed) being narrow, and the other wider, sometimes almost making contact with the narrower flap, forming a "pocket" into which the seed rests.

Except for minor variations in flap size, no differences were found between North American firs, as exemplified by *A. amabilis* (Fig. 3) and *A. procera* (Fig. 4), and those from other parts of the world, as exemplified by *A. nordmanniana* (Fig. 5) and *A. sibirica* (Figs. 6-7). Fir seed coats are characterized by a number of resin vesicles that are readily ruptured (Fig. 8). Some seed workers may have found integuments fastened to the seeds because leaking resin has permeated between the seed coat and the integument/wing and, on drying, has become an adhesive. However, the integuments of fir seeds are not "fused" to the seeds in the same manner as those in other genera, notably *Pseudotsuga* (see below). Based on this there appears to be no reason why *Abies* and *Pseudotsuga* should be included in the same PSD.

Calocedrus (formerly *Libocedrus*)

PSD 51 specifically refers to "wing", rather than "wing(s)" (see PSD 49). To a casual observer, incense cedar seeds appear to bear only one wing, but actually they have two (Fig. 9). A large wing extends lengthwise beyond the seed on one side, and a second, much smaller one, barely emerges alongside the first from the opposite side. Both wings are persistent and project beyond the micropylar (rather than the cotyledon) end of the seed (Stein 1974). This genus appears to have been mis-assigned to PSD 51 for two reasons: (a) it is a member of the Cupressaceae, and (b) all Cupressaceae have two persistent wings that are difficult to remove without damaging the seeds. Therefore, *Calocedrus decurrens* should be included with other Cupressaceae seeds in PSD 49.

Cedrus

Cedrus libani seeds showed the same integument characteristics as fir seeds. That is, the integument covers the entire adaxial surface, but two small flaps clasp the seeds on the abaxial surface (Fig.10). Although the seeds examined here could not be separated easily from their integuments (as were firs), it appears that *Cedrus* correctly belongs in PSD 51, or the PSD that includes *Abies* also.

Larix

The integument of larch seeds covers the entire adaxial surface (as in *Abies* and *Cedrus*). Close examination of abaxial surfaces showed what appeared to be integumental margins projecting slightly beyond the seed coat surfaces, as exemplified by *L. sibirica* (Fig. 11) and *L. lyalli* (Fig. 12), but no separable integumental layer was visible, and integuments could not be separated. In this regard, *Larix* seeds resembled those of *Pseudotsuga* (see below). Some analysts have reported difficulty in distinguishing larch wings and integuments, implying that there

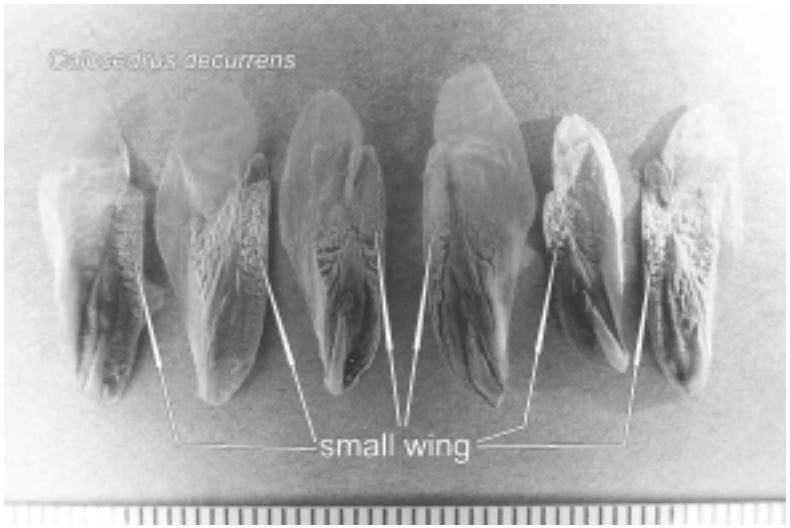

Figure 9. *Calocedrus decurrens* seeds (surfaces not known) each with two wings, one much smaller than the other, extending beyond the micropylar end of the seeds.

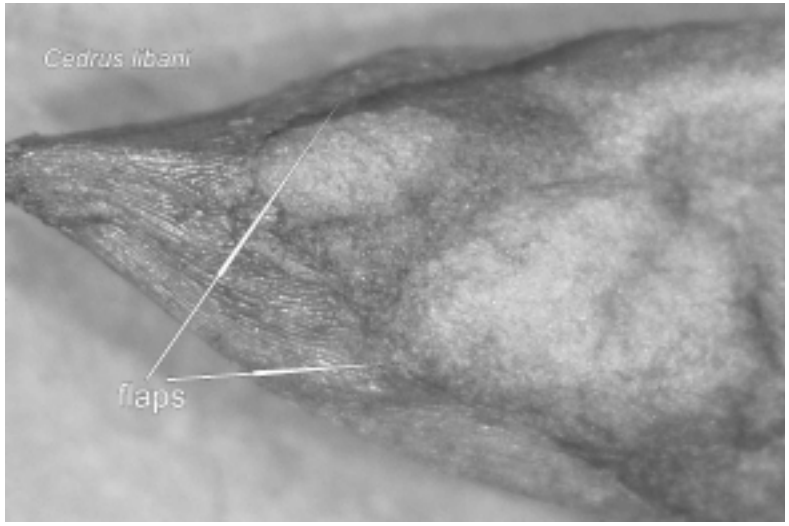

Figure 10. Abaxial view of a *Cedrus libani* seed showing integumental flaps that meet near the micropylar end. Portion of seed in view ~ 6mm.

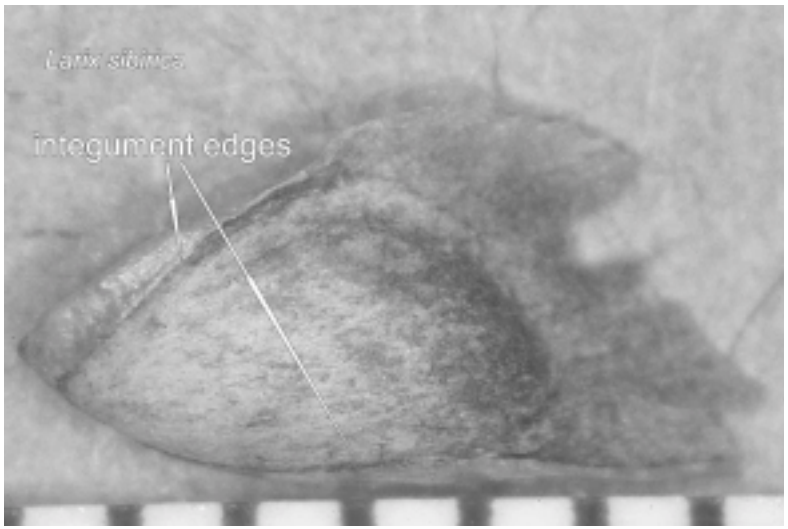

Figure 11. Abaxial view of a *Larix sibirica* seed. Edges of the integument (wing still attached) extend around both seed margins, the upper edge forming a small "pocket" over the micropylar end of the seed.

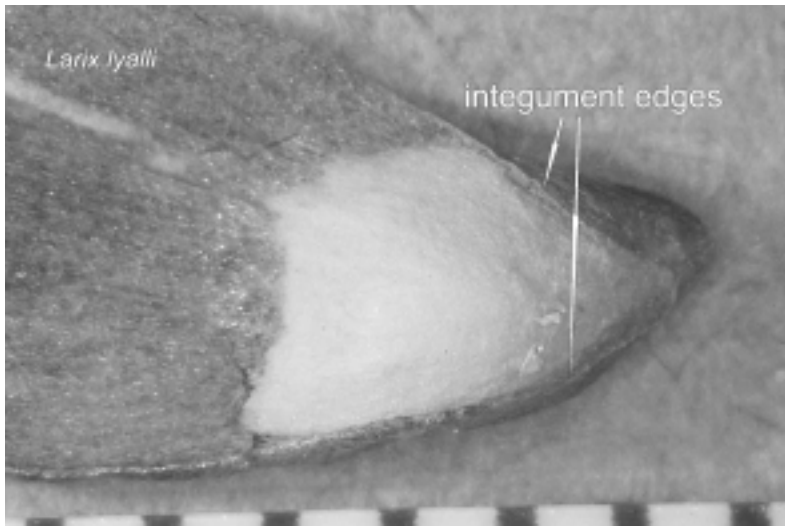

Figure 12. Abaxial view of a *Larix lyalli* seed. Edges of the integument (wing still attached) extend around both seed margins, the upper edge forming a small "pocket" over the micropylar end of the seed.

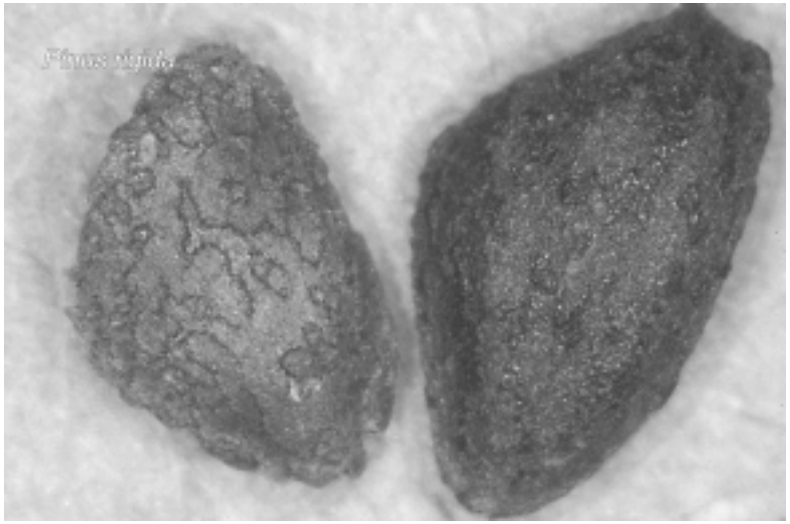

Figure 13. Two *Pinus rigida* seeds (surfaces not known) with no trace of wing or integument. Seed length ~ 4mm.

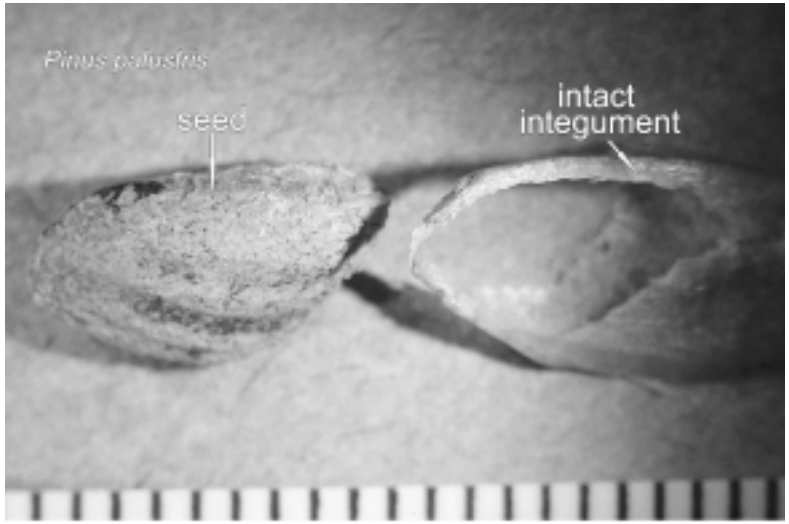

Figure 14. Adaxial view of a *Pinus palustris* seed (left) removed from its intact integument (right). The "claw"-like nature of the integument, typical of many pine seeds, does not completely cover the adaxial or abaxial surfaces.

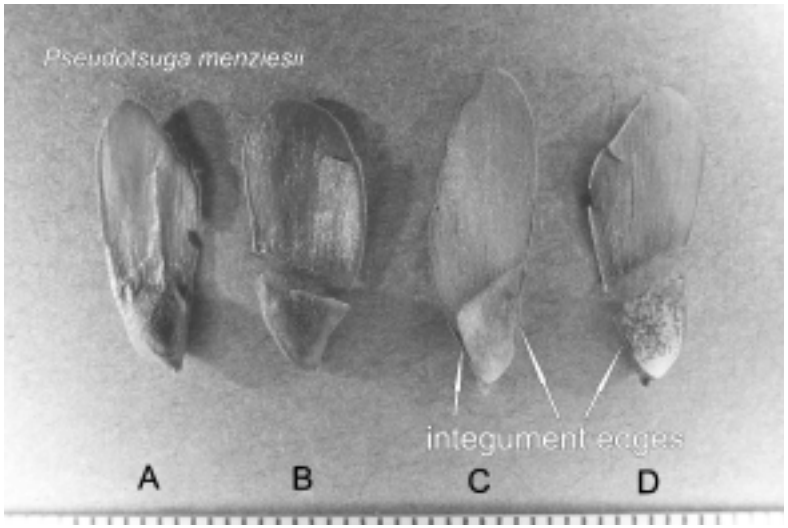

Figure 15. Four *Pseudotsuga menziesii* seeds. A – intact seed, adaxial view. B – seed with wing broken off, adaxial view. C – intact seed, abaxial view, showing integument edges. D – seed with wing broken off, abaxial view. B and D would be "pure seed" according to PSD 51.

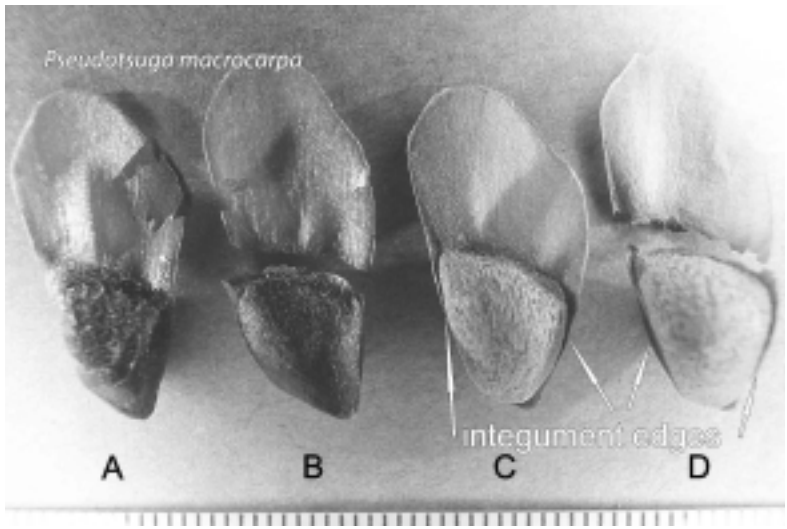

Figure 16. Four *Pseudotsuga macrocarpa* seeds. A – intact seed, adaxial view. B – seed with wing broken off, adaxial view. C – intact seed, abaxial view. D – seed with wing broken off, abaxial view. Integument edges can be seen in C and D. B and D would be "pure seed" according to PSD 51.

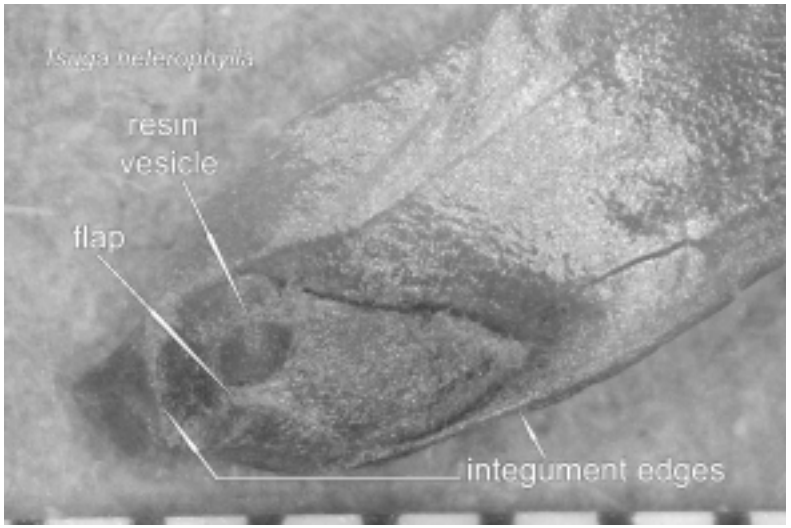

Figure 17. Abaxial view of a *Tsuga heterophylla* seed showing integument edges extending around seed margins, joining at the micropylar end to form a small "pocket". In this view the lower integument edge extends around the seed to form a flap. The oval structure near the seed centre is a large resin vesicle.

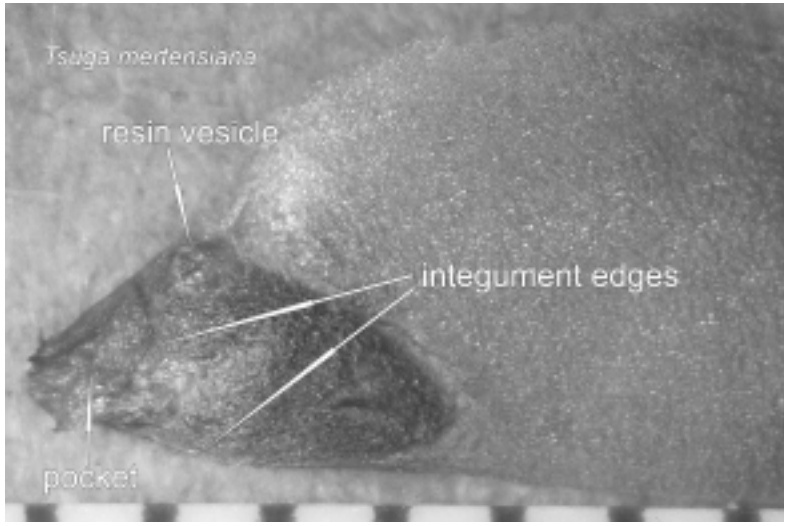

Figure 18. Abaxial view of a *Tsuga mertensiana* seed showing integument edges extending around seed margins, joining at the micropylar end to form a small "pocket". In this view, the circular structure near the upper edge of the seed is a large resin vesicle.

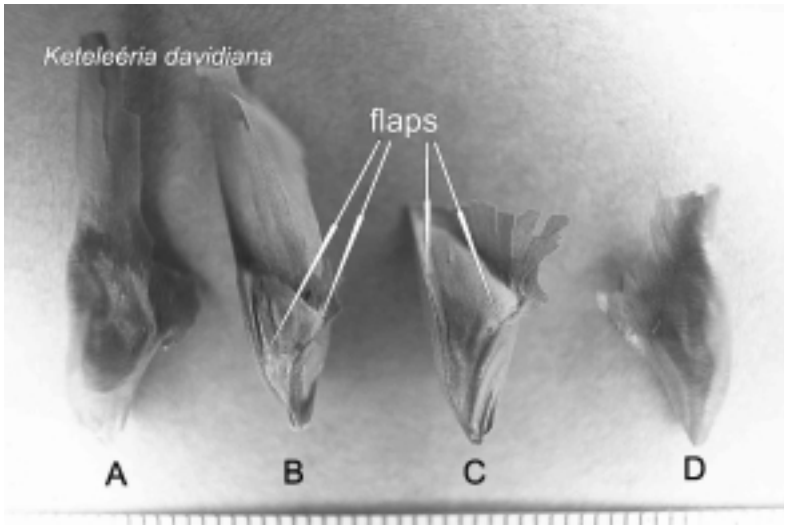

Figure 19. Four *Keteleéria davidiana* seeds. A – adaxial view of integument covering entire surface, with partial wing attached. B – abaxial view of integument flaps extending around seed margins, and partial wing. C- abaxial view of integument with flaps extending around seed margins, wing almost completely broken off. D – adaxial view integument covering entire surface (wing broken off). C and D probably would be "pure seed" according to PSD 51.

may be integumental separation on some occasions (perhaps due to seedlot differences?). Presently it would best to leave *Larix* and *Pseudotsuga* seeds in the same PSD, but to separate them from *Abies* and *Cedrus*.

Pinus I (*P. palustris* and *P. rigida*)

All other pine seeds (referred to as *Pinus II*, see above) are assigned (along with spruce) to PSD 47 in which pure seeds are completely without wing or integument, the latter not being intimately associated with the seed, and thus easily removed. Unprocessed *P. rigida* seeds (that is, with wings and/or integuments) were not available for comparison, but it appeared that whatever wings and integuments originally had been on the seeds examined here, they had been completely removed without apparent seed damage, and there was no trace of wing or integument on either adaxial or abaxial surfaces (Fig. 13). *P. palustris* seeds were easily separated from the wing/integument (Fig. 14), the integument resembling the same "claw" observed in most *Pinus II* species (not illustrated).

Seeds of two other pines, *viz*., *P. aristata* and *P. sylvestris*, together with those of *P. palustris*, have been described as difficult to dewing and susceptible to mechanical damage (Krugman and Jenkinson 1974). No mention was made of *P. rigida* seeds. Also, no mention was made of wings or integuments remaining "fused" to these seeds. Whereas it is possible that the relatively small samples of *P. palustris* and *P. rigida* seeds available may not have been representative of all seeds of these two species, it would appear that justification for their separation from other pine seeds (*Pinus II* species) requires confirmation.

Pseudotsuga

PSD 51 describes the integument as being "fused to the seed", and "is rarely removed in processing, and is impossible to consistently remove, without causing damage." Fused means joined as if melted together (Anon. 1987). Seeds of *Pseudotsuga menziesii* (Fig. 15) and *P. macrocarpa* (Fig. 16), fit this description. Despite large differences in physical dimensions between these two species, identical seed/wing characteristics were observed. Although margins of what appeared to be integuments were discernible projecting beyond the abaxial surfaces of many seeds, to all intents and purposes the single wing appeared to be an "outgrowth" of the adaxial surface of seed coat. That is, the integument was fused to the seed coat, which also fits the botanical description.

When seeds of this genus are processed, the wing is broken off from the seed, usually leaving a very small fringe of wing/integument tissue at the chalazal (cotyledon) end. This tissue remnant may become more abraded, and turn into dust or very small pieces, as the seeds are further handled. Although these loose impurities are easily removed, a small amount may persist after the seeds are packaged for trade. Usually *Pseudotsuga* seeds are among the more impurities-free conifer seeds marketed. However, because of this very trait, they are in conflict with the PSD definition that the "integument … is impossible to consistently remove, without causing damage" for two reasons. First, in *Pseudotsuga*, the integument is impossible to remove at <u>any</u> time, and second, whereas the wings do appear to be "fused" to the seeds, they are easily and consistently removed without causing seed quality damage. It would appear, therefore, that *Pseudotsuga* seeds do not properly belong in PSD 51.

Tsuga

Entire wings can be removed from hemlock seeds without serious damage to seed quality (Ruth 1974). This does not specify integuments, but the word "entire" may imply this. As with fir seeds, hemlock integuments appear to clasp the seeds. The integument covers the entire adaxial surface, but wraps around the seed on the abaxial surface as two small flaps, shown here for *T. heterophylla* (Fig. 17) and *T. mertensiana* (Fig. 18). One flap (on the margin of the seed nearer to the centre of the ovuliferous scale on which it formed) is very narrow, usually not extending beyond the seed margin. The second flap, extending around the other margin of the seed, is wider and while it wraps around further at the micropylar end, it is quite short so that only the tip of the seed is "enclosed".

Hemlock integuments are highly hygroscopic and expand when wet. Attached integumental remnants often come loose during the germination test (as in fir) as seeds are moved around for germinant assessment. Also similar to fir, hemlock seed coats bear resin vesicles, some being large and prominent relative to seed size (Fig. 17). Although not as easily ruptured as those in fir seed coats, if broken the leaked resin may firmly adhere the remaining integument to the seed coat, thus giving the appearance of the two being "fused". Based on these observations, *Tsuga* (like *Abies* and *Cedrus*) seeds should not be in the same PSD as *Pseudotsuga*.

Keteleéria

Keteleeria, together with fir and cedar, are the only members of the Pinaceae with upright cones. Although *Keteleéria* cones do not disintegrate at maturity (which distinguishes them from *Abies* and *Cedrus*), this genus is considered to be most-closely related to *Abies* (Rehder 1958). Keteleeria seeds resemble those of fir, perhaps more so than those of cedar (Fig. 19). The integument covers the entire adaxial surface, terminating on the abaxial surface as two flaps. Like the cedar seeds examined, it was not possible to separate integuments from these Keteleéria seeds, which were old (>30 years) and quite fragile. Based on these observations, should Keteleeria be included in ISTA prescriptions at some future date, they should be placed alongside fir and cedar in the same PSD.

Sequoiadendron

Sequoiadendron seeds currently are assigned to PSD 50 together with *Acacia*, *Cytisus*, and *Mahonia*. While seeds of the latter-named genera were not examined, it appears that *Sequoiadendron* is misplaced because giant redwood seeds do not form arils – neither does *Acacia* (Whitesell 1974) or *Cytisus* (Gill and Pogge 1974); *Mahonia* forms berries (?). *Sequoiadendron* should be included with other Cupressaceae seeds in PSD 49, along with *Sequoia*.

Taxus

Yew is currently assigned to PSD 10, which covers more than 160 genera ranging from *Abelmoschus* (Malvaceae) to *Vinca* (Apocynaceae) (ISTA 1999, pp: 123-132), and includes three coniferous genera, viz., *Juniperus* (Cupressaceae), *Taxodium* (Taxodiaceae), and *Taxus* (Taxaceae). However, PSD 10 makes no mention of "aril", the fleshy, brightly-coloured, cup-like, fruit that bears the single yew seed, and which is a major recognition feature for the genus. Yet PSD 50, which includes *Acacia*, *Cytisus*, *Mahonia* and, currently, *Sequoiadendron* (*quod vide*), speaks of the presence/absence of the "aril". It would appear that the single-seeded arils of *Taxus* fruits and the several-seeded indehiscent strobili commonly called "berries" of *Juniperus* (Johnsen and Alexander 1974) have been mistakenly compared and, hence, have both been assigned to PSD 10. From work experience with *T. brevifolia* and *J. scopulorum*, it is suggested that these two genera do not belong in the same PSD and that, more likely, *Taxus* seeds would be better re-assigned to PSD 50. The assignment to PSD 10 of *Juniperus* seeds needs to be confirmed, as is the inclusion of *Taxodium distichum*, which produces two-seeded cones (Bonner 1974).

Conclusions

Several anomalies were reported and questions raised concerning the assignments of conifer genera to ISTA Pure Seed Definitions (PSDs). In alphabetical order by genus these were:

1. *Abies* (also *Cedrus* and *Tsuga*; plus *Keteleéria*), with their integuments "intimately associated" with, but <u>not</u> "fused" to, the seeds, should not be in the same PSD as *Pseudotsuga* (and possibly *Larix*). PSD 51 needs revision.
2. *Calocedrus decurrens* bears two non-removable wings, as do other members of the Cupressaceae, and should be re-assigned to PSD 49.
3. *Cedrus* – see *Abies* (and *Tsuga*).
4. *Keteleéria* (not presently covered by ISTA prescriptions), should be included with *Abies*, *Cedrus* and *Tsuga*.
5. *Larix* seeds appear to have integuments "fused" to the seeds, similar to *Pseudotsuga*. However, because the wings are easily removed in operational dewinging, without damaging the seeds, PSD 51 does not entirely apply. A new definition is recommended. (See also *Pseudotsuga*.)
6. *Pinus palustris* and *P. rigida* seeds, assigned to PSD 51, appeared to be no different from other *Pinus II* pines assigned to PSD 47. Unless there is contrary evidence, these two pine species should be re-assigned to PSD 47.
7. *Pseudotsuga* seeds appear to have integuments "fused" to the seeds, similar to *Larix*. However, because the wings are easily removed in operational dewinging, without damaging the seeds, PSD 51 does not entirely apply. A new definition is recommended. (See also *Larix*.)
8. *Sequoiadendron gigantea* does not form arils. Seeds of this species bear two non-removable wings, similar to other members of the Cupressaceae, and should be re-assigned to PSD 49.
9. *Taxus* seeds are formed in fleshy arils, which are not to be compared with juniper "berries". *Taxus* should be re-assigned to PSD 50, and the assignment of *Juniperus* and *Taxodium* to PSD 10 needs to be confirmed.

10. *Tsuga* (also *Abies* and *Cedrus*; plus *Keteleeria*), with their integuments "intimately associated" with, but not "fused" to, the seeds, should not be in the same PSD as *Pseudotsuga* (and possibly *Larix*). PSD 51 needs revision.

The observations on which these conclusions have been made are based solely on surface features of seeds of the genera concerned. No study of tissue development was possible. However, the major details discussed, especially the "fused" integuments of *Pseudotsuga* and (possibly) *Larix*, as well as how the integuments of other genera form, require microscopic examination of developing seeds, that is, prior to and through seed maturation. This is the type of work ideally suited to graduate student research, and should be encouraged.

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Genetic Control of Germination and Aging: Lessons for Practice and Conservation

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Abstract

The underlying assumption of tree seed utilization for regeneration or *ex-situ* conservation purposes is that the seeds (and, consequently the seedlings regenerated from them) constitute genetic representation of the source trees. Over 12 years of research examining the extent of genetic control and effects of simulated aging on germination attributes have indicated that this assumption often is not valid. Studies conducted on several commercial and minor tree species native to British Columbia demonstrated the presence of high genetic control over germination attributes. This implies that any uniform pre-germination treatment(s) applied across a number of seed sources likely will result in non-uniform germination, with potential negative consequences on the genetic representation of source trees. That is, different seedlots of the same species collected in the same year have individual, genetically controlled requirements for optimal germination. It has been demonstrated also that the genetic representation of collected seeds may be adversely affected during *ex-situ* storage. When seeds were artificially aged, significant differences in storability among and within species were observed. For example, Sitka spruce (*Picea sitchensis* [Bong.] Carr.) seeds aged more slowly than Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), mountain hemlock (*Tsuga mertensiana* [Bong.] Carr.) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) seeds. Within species, large differences in the rate of seed aging were found among genotypes. The results have implications with respect to genetic gain and diversity when seeds are used for regeneration and conservation. Somatic embryogenesis as a new biotechnology method for bulking up elite germplasm for seedling production is presented and the need for further research on somatic embryos germination and biology is emphasized.

Keywords: germination, seed aging, genetic diversity, heritability, somatic embryogenesis

Introduction

Tree seeds are collected for a number of purposes. Commonly, seeds from seed orchards or natural stands are stored for short time periods only before they are used to restore stands in harvested areas, to establish new plantations, or for afforestation. Seeds are also preserved in longer-term storage for conservation of genetic resources should natural populations fail. The underlying assumption of seed storage (short- or long-term) is that they and the seedlings regenerated from them constitute a good genetic representation of the seed-donor trees. Maintenance of genetic representation is of paramount importance for both, commercial regeneration and conservation of genetic resources.

The importance of genetic diversity of regenerated forests is widely recognized. During their lifetime, trees experience changes in natural conditions that may affect their survival. These changes can be either slow (e.g. climate change) or quite rapid (e.g. introduction of new pests). Maintenance of genetic diversity increases the chances of plantation survival under unpredictable environmental contingencies. While it is important to maintain genetic diversity in all stages of forest regeneration, it is vital that the genetic gains attained through selection and breeding are not lost due to any unintentional selection that may occur during short-term seed storage and/or at various steps of seedling production.

The primary goal of forest tree gene conservation is the maintenance of genetic resources at their contemporary natural levels. This allows for restoration of genetic diversity in species or populations should their genetic resources become diminished. Seed collections are one of many *ex-situ* conservation methods used for that purpose, and are often regarded as complimentary to *in-situ* conservation. However, there are instances where *ex-situ* conservation in the form of seed collections is advocated as the most appropriate/economic approach. Economically-valuable species often are subject to various forms of *ex-situ* conservation including seed banks, clonal archives, provenance and progeny trials, seed orchards, plantations, and tissue and cell cultures in cryo-banks. For non-commercial species, seed banks are often the primary, or the only, *ex-situ* conservation method. Rare, non-commercial species or populations often are at the greatest risk of severe erosion of genetic resources and extinction due to the changes in their native habitat. Therefore, reliance only on seed banks for conservation

of such species should be evaluated. Regardless of seed bank use as a primary or complimentary conservation method, the caveats of long-term seed storage must be well understood for the development of comprehensive strategies that fully meet conservation goals.

Seedlings can be produced on a commercial scale not only from zygotic seeds, but also using somatic embryogenesis. Even though desiccated, mature embryos produced in somatic embryogenesis are inherently different from zygotic seeds, they ultimately provide the same fundamental seed function, *viz.* future plant regeneration in specific environmental conditions. For somatic embryos, highly-controlled conditions in artificial environments allow for successful germination without the nutritive tissues and protective structures that are characteristic of zygotic seeds. Naturally, almost all of seed-related research, including studies discussed in this review, has been conducted on zygotic seed. However, as somatic embryogenesis is rapidly gaining in importance as a method for commercial seedling production, it is beneficial to evaluate traditional seed production in relation to this novel technique.

This report summarizes the results of over 12 years of research examining the extent of genetic control and effects of seed aging on germination attributes. The investigations included a number of major and minor tree species native to British Columbia. All studied species are wind-pollinated and monoecious, and represent a great range of native habitats with respect to elevation, latitude and longitude, different ecological characteristics and reproductive biology. Somatic embryogenesis and its role as an alternative method for delivery of genetic gains, as well as its function for *ex-situ* preservation of genetic diversity, is also discussed.

Materials and Methods

Genetic control of germination attributes was estimated based on bulked-seed collection from wild populations of paper birch (*Betula papyrifera* Marsh.) and Sitka alder (*Alnus sinuata* Rydb.), on seeds collected from wild populations with preserved tree identities for Pacific silver fir (*Abies amabilis* Dougl. ex Forbes) and mountain hemlock, and from seed orchards seeds of Douglas-fir, Sitka spruce, western redcedar (*Thuja plicata* J. Donn ex D. Don), yellow cedar (*Chamaecyparis nootkatensis* [D. Don) Spach.) and western hemlock. Precise estimation of genetic control of germination attributes is often difficult because of confounding environmental and genetic effects and their interactions. Whereas the environmental effects were possibly of some consequence in seeds from wild stands, they were greatly minimized for seeds from common gardens such as seed orchards.

Standard germination tests were performed following the International Seed Testing Association rules (1985). Depending on species, the seeds were or were not stratified. Typically, four replications of at least 100 seeds each from each seed source were used in the tests. The following attributes were determined: germination capacity (GC), germination speed (R_{50} and R'_{50}), peak value (PV) and germination value (GV). Among these attributes, GC is the percentage of total germinated seeds, R_{50} is the number of days it takes to germinate 50% of the total seeds (Ching 1959) and R'_{50} is the number of days it takes to germinate 50% of the germinating seeds (Thomson and El-Kassaby 1993). To estimate PV, the accumulated number of germinants was divided daily by the number of corresponding days, and the maximum value obtained, PV, represents the mean daily germination of the most vigorous seeds (Czabator 1962). Multiplying PV by mean daily germination, MDG (the accumulated total number of germinants divided by the number of days of the test), gives GV, a parameter representing a combined expression of germination speed and germination completeness (Czabator 1962).

To examine differences in storability at the genotype level, seeds were subject to artificial aging (Delouche and Baskin 1973), a method for evaluating differences among seedlots to survive conditions (high relative humidity and high temperature) that cause rapid deterioration. Artificial aging tests were performed on Sitka spruce (Chaisurisri et al. 1993), mountain hemlock (El-Kassaby and Edwards 1998), western hemlock (El-Kassaby et al. 2002) and Douglas-fir (El-Kassaby unpublished). All seeds were subject to accelerated aging using a modification of the standard seed vigour testing procedure for agricultural crops (Association Official Seed Analysts 1983). Seeds were aged under 37.5^{o}C and 100% relative humidity from 0 to 21 days at 3-day intervals (i.e., 0, 3, …, 21 days), except for western hemlock which was aged from 0 to 15 days. Following aging, stratified and unstratified samples were subject to standard germination tests (ISTA 1985).

Results and Discussion

Genetic control of germination parameters

Estimates of broad-sense heritabilities (the extent of genetic control over a specific attribute) were high to very high for all species, except yellow-cedar, indicating the presence of high genetic control over germination

attributes (Table 1). For example, heritability estimates for GV, the parameter that combines both germination completeness and speed of germination, ranged from 0.72 to 0.97, with estimates exceeding 0.90 for Sitka alder, paper birch, western hemlock and Douglas-fir. Heritability values for speed of germination using PV, R_{50} or R'_{50} were also consistently high across species (Table 1). Even though the heritability values for yellow cedar were lower than those estimated for all other species, they still indicate a significant genetic component of the variations in germination attributes (Table 1). These findings have been supported by reports of significant genetic components in germination attributes of western white pine (*Pinus monticola* D. Don) (Hoff, 1987), palebark Heldreich pine (*Pinus leucodermis* Antoine) (Giannini and Bellari 1995), aspen (*Populus tremula* L. and *P. tremuloides* Michx.) (Gallo, 1985) and yellow poplar (*Liriodendron tulipifera* L.) (Barnett and Farmer, 1978).

Table 1. Examples of broad-sense heritability values for germination parameters[1,2].

Species	Source of Variation	D.F.[3]	GC	R_{50}	R'_{50}	PV	GV
Sitka alder	Among Populations	26	0.96		0.94	0.78	0.97
	Within	81					
Paper birch	Among Populations	17	0.90		0.92	0.87	0.91
	Within	54					
Pacific silver fir	Among Trees	41	0.70				0.72
	Within	126					
Mountain hemlock	Among Trees	19	0.30	0.85	0.82	0.84	0.80
	Within	57					
Western hemlock	Among Trees	19	0.88		0.91	0.91	0.92
	Within	60					
Douglas-fir	Among Trees	18	0.92	0.80		0.91	0.93
	Within	57					
Sitka spruce	Among Trees	17	0.74			0.78	0.74
	Within	54					
Western redcedar	Among Trees	21	0.79			0.78	0.80
	Within	66					
Yellow cedar	Among Trees	11	0.36			0.28	0.42
	Within	36					

[1]Sitka alder (Benowicz et al. 2000), paper birch (Benowicz et al. 2001), Pacific silver fir (Davidson et al. 1996), mountain hemlock (El-Kassaby and Edwards 1998), western hemlock (El-Kassaby et al. 2002), Douglas-fir (El-Kassaby et al. 1992), Sitka spruce (Chaisurisri et al. 1992), western redcedar and yellow cedar (El-Kassaby et al. 1993).
[2]If both stratified and unstratified seeds were used in tests, the values presented are for stratified seeds only.
[3]D.F. = degrees of freedom.

Although these observations suggest a broad pattern of high genetic control of germination attributes in tree species, the case of yellow-cedar should serve as an example that the pattern is not universal. The lower heritability values obtained for this species may relate to the presence of phenotypic plasticity observed in its reproductive cycle (El-Kassaby 1995). Its long natural reproductive cycle of (3 years in British Columbia at high elevation and the northern end of its natural range) means that it is dependent on the presence of a favourable environment during the 2-year period from strobilus development to seed maturation. However, in more central regions of its range, and lower elevation and latitude this species has a 2-year reproductive cycle. Because of the occurrence of such an abbreviated reproductive cycle, cones collected from lower or shaded parts of the crown of trees grown in milder climate (seed orchard trees) may be immature at the time of cone sampling. Yellow cedar cone production is often poor (Karlsson and Russell 1989) and it forces cone sampling from many different places on the tree. Success in breaking dormancy may vary widely within individual trees if cones are collected from different crown locations. Differences in seed maturity and dormancy expressed at the individual level would lower the estimated heritability values.

Some heritability values might be overestimated, particularly in case of seeds collected from wild populations. Confounded genetic and environmental effects very likely played a role in heritability estimation for the hardwood species (paper birch and Sitka alder). This seems to be of less consequence for mountain hemlock and Pacific silver fir. Mountain hemlock seeds were collected from two natural populations and the data were analyzed on individual-population basis. For Pacific silver fir, the large individual tree effect argued for a significant genetic component in germination characteristics. It should be noted also that the comparison of heritability values based on seeds collected from wild populations (4 species: paper birch, Sitka alder, mountain

hemlock and Pacific silver fir) with those based on seeds collected in seed orchards (5 species: western hemlock, Douglas-fir, Sitka spruce, western redcedar and yellow cedar) reveals that the range of estimated values was comparable for both types of collections (Table 1).

Genetically based differences among genotypes in germination attributes ensure that not all seeds germinate at the same time in the same environmental conditions. Sudden change in conditions in spring (e.g. late frost) would cause high mortality rates if uniform germination occurred. Moreover, genetically based variations in germination attributes ensure successful germination over a greater range of environmental conditions. Even though the majority of seeds fall in close proximity, there may be large differences in micro-site conditions within the same location that may affect seed germination. These effects help to ensure the survival of the species.

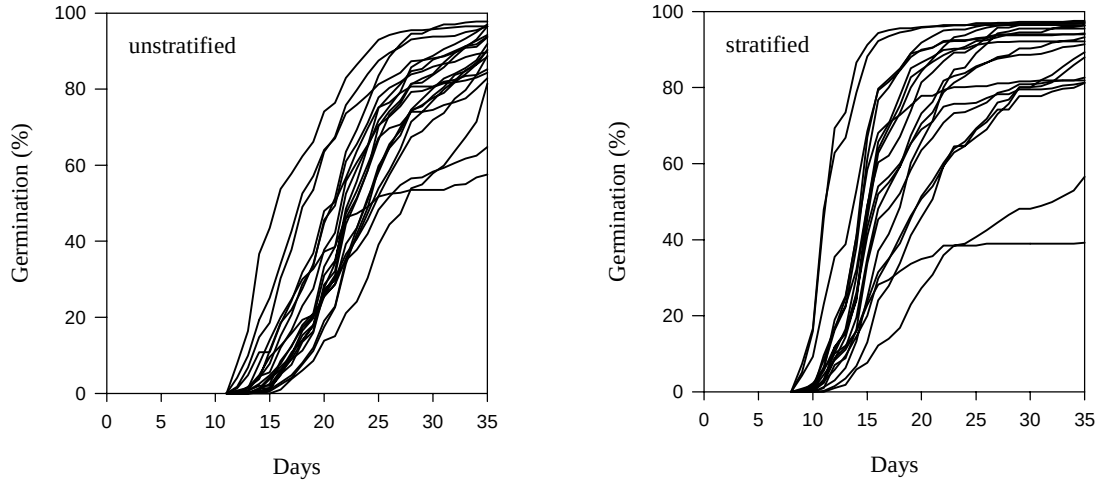

Figure 1. Germination course of 20 western hemlock families for unstratified and stratified seeds (from El-Kassaby et al. 2002).

Genetic differences in germination attributes found among different seed sources imply that uniform pre-germination treatments, as well as uniform germination conditions utilized across the seeds collected from different seed-donors, will likely result in non-uniform germination. The germination course for western hemlock (Figure 1) is presented as an example of differences in speed and germination capacity among genotypes. When seedlings are grown for forest regeneration and multiple seeds are sown (to ensure at least one seedling) in each container, such non-uniform germination may lead to the unintentional reduction in genetic diversity through the selection of earlier/faster germinating seeds (giving taller germinants) during thinning of supernumerary germinants (Figure 2). Depending on the magnitude of differences among seedlots, and the purpose of regeneration, germination conditions may have to be customized for different seed sources if the loss of genetic diversity is to be minimized during seedling production. To accomplish this, that is to provide varying germination conditions to different seed-donors, and thereby to ensure a balanced genetic representation of all the seed sources in the produced seedlings, prior bulking of seeds from different sources should be avoided.

Seed aging

Considerable differences were found among species with respect to the rate of seed aging. Seeds of Douglas-fir (El-Kassaby unpublished), mountain hemlock (El-Kassaby and Edwards 1998) and western hemlock (El-Kassaby et al. 2002) aged at a greater rate than seeds of Sitka spruce (Chaisurisri et al. 1993). Douglas-fir and mountain hemlock seeds completely lost their germinability after approximately 10 days, and western hemlock after 12 days of accelerated aging. In contrast, Sitka spruce seeds lost their germination capacity only after 21 days. At advanced stages of the aging process, unstratified seeds tended to germinate better than stratified seeds of Douglas-fir and both hemlocks.

In addition to among-species differences, within-species variations (among genotypes) in the rate of aging were observed for all four species (see Figure 3 for examples). While all individual seed-donors showed a steady decline in germination ability over aging duration, they differed in the rate of decline. In the case of western hemlock, whereas the initial (before aging) range of germination varied between 90 - 98% (stratified seeds), the range increased to 49 - 93% after 3 days of aging. Similarly, among mountain hemlock seedlots the initial viability ranged from 76 to 96%. Aging treatment caused a broadening in this range after 3 and 6 days, indicating

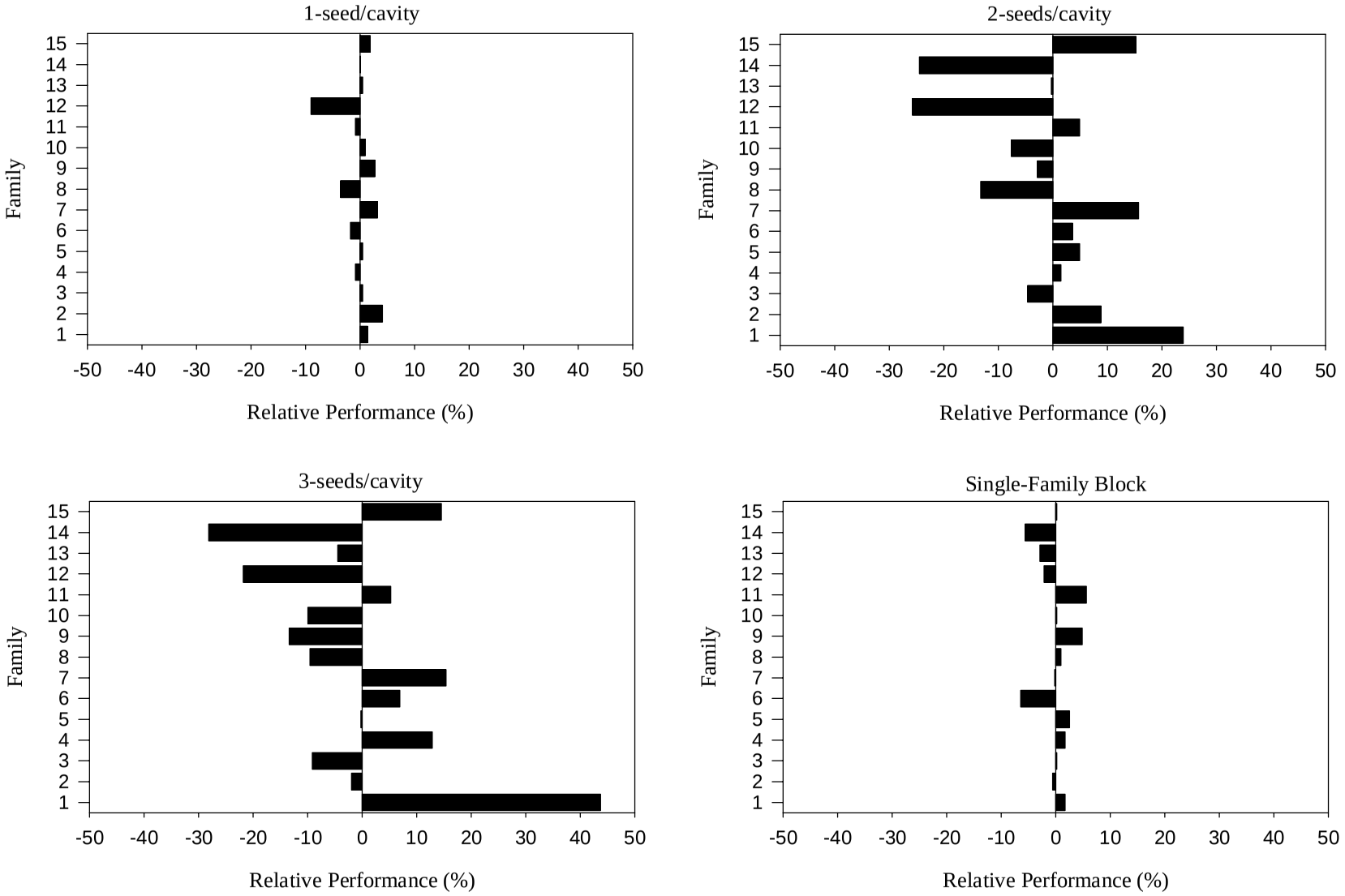

Figure 2. Relative performance of the 15 Douglas-fir families from seeds to seedlings in four treatments: 1-seed/cavity, 2-seeds/cavity, 3-seeds/cavity and single-family block sowing. Relative performance was determined as the % deviation from the expected equal representation among families to the resultant seedling crop (from El-Kassaby 2000).

differences among seedlots in their propensity to lose viability. Similar responses to aging were observed in Douglas-fir. The initial (before aging) GC of stratified Douglas-fir lay between 83 and 99%, but after 4 days of aging, GC ranged from 35 to over 88%. For this species, some seed-donors lost almost all their germination capacity after aging for 7 days, while other donors were still able to germinate as high as 35%. Even though the rate of decline in GC with aging was lower in Sitka spruce than in other species, the differences among the genotypes were just as strongly pronounced. The initial GC of stratified Sitka spruce lay between 85 and 99%, but after 9 days of aging, GC ranged from 12 to almost 80%. Whereas some clones lost all their GC after aging for 18 days, others were still able to germinate, and one genotype germinated 35% after 18 days of aging.

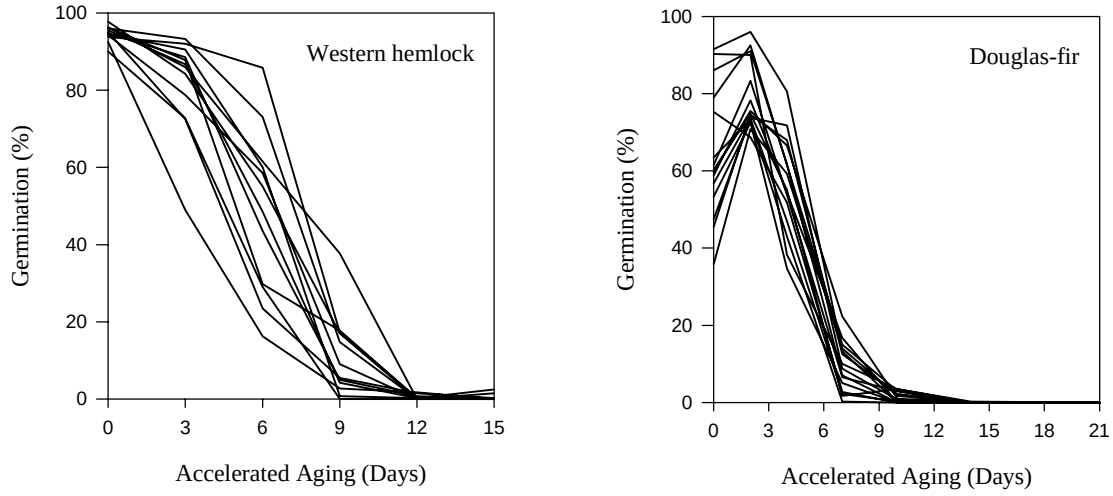

Figure 3. Germination capacity of 12 western hemlock families (stratified seeds) (from El-Kassaby et al. 2002) and 15 Douglas-fir families (unstratified seeds) (El-Kassaby unpublished) after simulated aging.

The fact that seeds from different genotypes deteriorated at different rates during artificial aging suggests that they may also age differently in long-term storage. It is not clear how artificial seed aging and aging under normal storage conditions are related, whether the relationship may be universal, or if the changes are species (or even seedlot) dependent. However, there is evidence from agriculture that accelerated aging can be effective in detecting differences in seed vigour reflected in field performance (e.g. Powell and Matthews 1985; Hall and Wiesner 1990). Furthermore, seedlots of some agricultural species that had high survival after accelerated aging stored well under normal storage condition, while those that had high mortality deteriorated rapidly in storage (Delouche and Baskin 1973). With respect to tree species, Bonner (1998) reported that germination after accelerated aging correlated significantly and positively with germination after five years of storage in seeds of sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.).

Differential rates of seed aging at the genotype level should be taken into consideration when storing seeds for conservation purposes. *Ex-situ* conservation such as seed banking is often thought of as a "static" approach to conservation. This is in contrast to *in-situ* "dynamic" conservation, where the species can respond to the changing environment. However, using the term "static" conservation for seed banks can be misleading. As described above, research in seed aging has shown that the genetic diversity of a seedlot changes over time. The most important consequence of the changes taking place during seed storage is that, in terms of gene richness and diversity, the disparity between the original source population and the collected seeds widens with the length of storage. Thus, for species or populations where *in-situ* conservation is impossible or impractical, a range of *ex-situ* methods should be explored in addition to the use of seed banks.

There are several other implications of the artificial seed aging tests. It should be clear that bulking of seeds collected from several genotypes into a "single seedlot" should be avoided for long-term storage for conservation purposes. This will allow for more balanced regeneration in terms of genetic diversity when the seeds are eventually sown. It should also be noted that germination pretreatments and conditions for aged seeds will likely be different from those for seeds stored for a short period of time, or not stored at all. For example, stratification of severely aged seeds of western and mountain hemlock seems to have no merit, or may even be detrimental. As has also been shown in the data referred to above, because there is a differential decrease in GC across genotypes initial germination attributes may not serve as good indicators of seed behaviour during storage. Finally, for some species short-term aging may have a similar impact as stratification in terms of dormancy breaking (see

example of Douglas-fir in Figure 3) and therefore it may be used as a much faster way to break dormancy compared to the typical several weeks of cold stratification.

Somatic Embryogenesis

The predicaments of traditional seed production and use identified above clearly indicate that there is need for new technologies to be included as complementary forms of *ex-situ* conservation and as precise delivery systems of genetic gains. One such technology is somatic embryogenesis, which offers a unique remedy to the potential problems of unequal genetic representation of source trees. Storage of genotypes as desiccated embryos and as cryopreserved embryogenic tissue allows the production of virtually an unlimited number of seedlings. Thus, the number of seedlings from genotypes with low germination capacity can be increased as needed; in contrast, the number of seedlings produced from zygotic seeds is limited to the number of viable seeds. This approach is of particular importance for species with low and/or unreliable seed production. Moreover, because somatic embryogenesis permits strict control over the genetic composition of the crop, it also allows for the precise delivery of genetic gains and production of the crop at the desired level of genetic diversity.

Embryogenic cultures have been produced for a majority of commercially-important conifers and hardwood species (Merkle and Dean 2000). Commercial production of tree seedlings through somatic embryogenesis is rapidly gaining importance, but more research is needed with respect to the biology of somatic embryos. The somatic embryogenesis process typically includes the following steps: induction, cryopreservation, liquid culture multiplication, somatic embryo maturation, desiccation, germination, and transplanting of embryos to seedling containers for subsequent growth in the nursery (Figure 4) (El-Kassaby 2001).

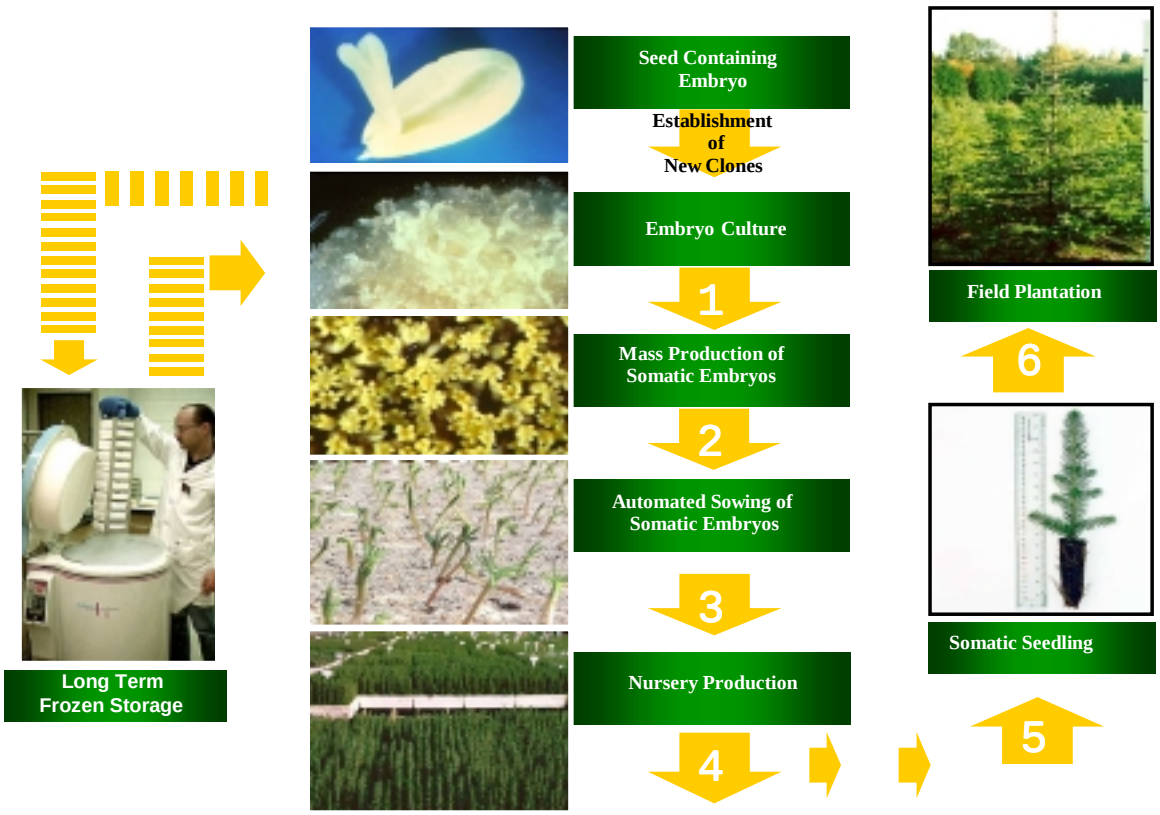

Figure 4. Production process of somatic seedlings (photo is courtesy of CellFor Inc.).

Induction

Zygotic embryos excised from mature or immature seeds are placed on a sterile culture medium with plant hormones stimulating development of somatic (embryogenic) tissues. The tissue developed from each individual zygotic embryo consists of a mass of proembryos (early stage somatic embryos) with one single genetic identity that is capable of continuous proliferation.

Cryopreservation

Embryogenic tissues produced during the induction are frozen in liquid nitrogen and stored. The purpose of this step is to maintain long-term somatic tissue viability in a juvenile condition (i.e. in a state capable of producing embryos). Cryogenic storage, therefore, is essentially a clone bank or gene conservation method of unique genotypes and provides tree breeders and foresters the time required to conduct clonal testing. As a form of *ex-situ* conservation it is particularly appropriate for endangered species or populations with unreliable, or low, seed production, or for species with recalcitrant seeds.

Liquid culture multiplication

The induced tissues are exponentially multiplied (commonly known as "bulking up") in a step that represents true cloning. The multiplied tissues are undifferentiated and are used for the actual production of mature embryos (next step). The ability to produce an unlimited number of genetically-identical somatic embryos is the key advantage of somatic embryogenesis technology.

Somatic embryo maturation

The undifferentiated tissue produced by liquid culture multiplication is exposed to maturation media where it proceeds through more advanced stages of embryogenesis. As a result, mature, well-differentiated cotyledonary embryos are produced that are similar in all respects to embryos in zygotic seeds. However, high numbers - ranging from tens of thousands to millions - of mature embryos can be produced.

Desiccation and storage

Mature embryos are subject to a desiccation process mimicking natural seed development as they would mature on the mother tree. However, somatic embryogenesis allows for the production of embryos at any time of the year and the desiccation process removes the restrictions imposed by the production of embryos with high moisture content that cannot be stored. Desiccated embryos can be stored and subsequently used to produce large number of seedlings at the desired time during the relatively narrow biological window suitable for seedling production.

Sowing and germination

The above steps are similar, in principle, to the sowing and germination of zygotic seeds in a greenhouse environment. However, there are special challenges because the somatic embryo lacks a megagametophyte ("endosperm") and seed coat. Encapsulated or naked embryos are germinated. The embryos are mechanically sown into mini-plugs, acclimatized to *ex-vitro* conditions, and transplanted into either container or bare-root nurseries for seedling production.

Conclusions

A decade or more of studies of several tree species to examine within-tree differences in germination attributes and seed storability have shown that seedlings produced by traditional means are likely to display variable genetic fidelity to the source trees. As a result, genetic diversity of forest regeneration for commercial and/or conservation purposes may be negatively impacted, with consequences for long-term tree survival. This is particularly important for the species or populations most vulnerable to extinction since they often rely on seed banks as a main form of conservation. Additionally, adverse effects on the genetic gain in commercial plantations can be expected in some circumstances. New technologies such as somatic embryogenesis should be considered as complementary forms of *ex-situ* conservation and as precise delivery systems of genetic gains. More research is warranted to evaluate the benefits and risks of such new technologies. There are several other implications of the germination and artificial aging tests worth exploring in the future. These include the use of short-term artificial aging as a substitute to cold stratification (and its impact on plant vigor/performance in the field), characterization of any change in optimal germination conditions for seeds stored for long periods, and the development of reliable indicators of seed storability.

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Germinative Behavior of *Leguminosae* Seeds under Different Temperature Treatments

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Leguminous plants has a hard seed coat whose impermeability, theoretically, cause the low germination of seeds of numerous wild species. Temperature regimes can affect the percentage and rate of germination (Kebreab & Murdoch, 1999). A way of decrease dormant seed in some legumes is to cause alterations to the seed coat by temperature alternation (Baskin et al., 1998).

But a few works study the alternating temperature effects in *Leguminosae* and less even that cover a wide range of constant and alternating temperatures with wild species of this family.

The objective of this research work are to compare seed germination behaviour of various species belong to different genus of *Leguminosae* at a wide range of constant and alternating temperatures. The implication of the effect of temperature as a regulating factor in the germinative process is discussed.

In a preliminary germination studies with two *Leguminosae* species we noticed that the best results was at constant temperatures; alternating temperatures decreased germination percentage, according Gonzalez-Andres & al. (1993).

The germination of different arboreal and shrub species of *Leguminosae* are evaluated under constant and alternate temperature regimes. We study 11 species of different collecting year and different storage conditions. The evaluated species, storage time and conditions, as well as the scarification time are the following:

Anthyllis cytisoides L.	70 months	20ºC	10 min
Astragalus lusitanicus Lam.	70 months	20ºC	15 min
Ceratonia siliqua L.	8 months	20ºC	180 min
Colutea arborescens L.	70 months	20ºC	80 min
Coronilla juncea L.	70 months	20ºC	5 min
Cytisus heterochrous Webb ex Colmeiro	46 months	20ºC	40 min
Dorycnium rectum (L.) Ser.	81 months	–25ºC	10 min
Genista umbellata (Desf.) Poir.	9 months	20ºC	90 min
Medicago arborea L.	22 months	20ºC	30 min
Teline linifolia (L.) Webb	10 months	20ºC	30 min
Ulex parviflorus Pourr.	1 month	20ºC	40 min

Seeds were germinated in petri dishes with blotting paper. In each experiment four replicates were used per treatment. The germination is tested under darkness conditions and the following temperature regimes: 10º, 15º, 20º, 25º, 30º, 40º, 25º/10º, 25º/15º, 25º/20º, 35º/15º, 35º/20º and 40º/20ºC.

Given the physical or external dormancy that present the seeds of this group, we compare all the tests with scarified seeds too. We use concentrated sulphuric acid to erode the external coat. The scarification time was determined by anatomical analysis of the seed coat.

Observed the results of the different tests, there are not a definite behaviour that show a clear distinction between constant and alternate temperature regimes. Each species has concrete preferences of temperature that could show ecological adaptations to their characteristic habitat.

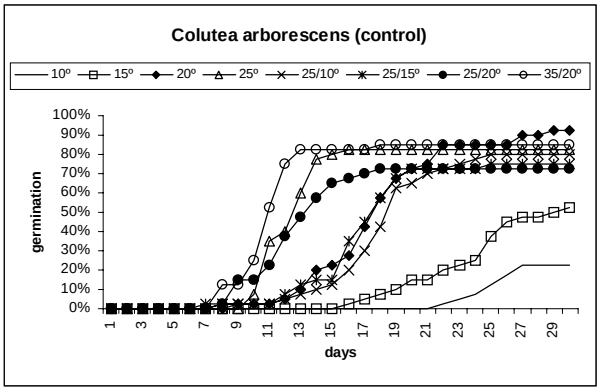
The seeds stored up to 70 months don't show primary dormancy, at least at optimal conditions. Maybe the separation of cell walls of the coat cause the loss of impermeability (Ballard, 1973).

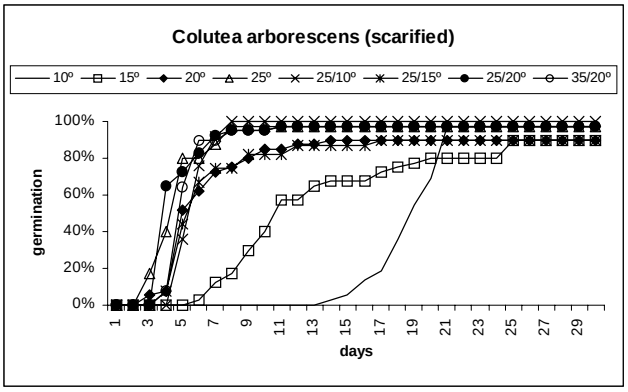
D. rectum has maintained all its viability during storage at –20ºC. While *A. cytisoides*, *C. arborescens* and *C. juncea* stored at room temperatures show lower percentages, maybe there was some loss of viability during its storage.

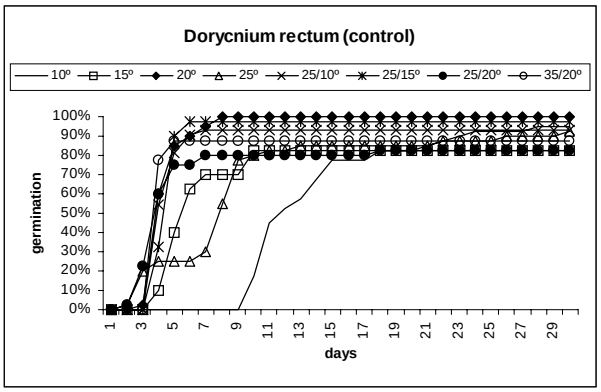
C. arborescens which have a thick seed coat maintain a germination percentage near to 100% after 70 months of storage.

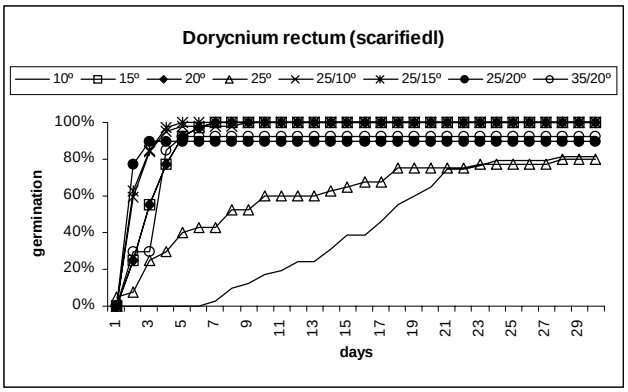
A. lusitanicus, *C. arborescens* and *D. rectum* don't like temperatures lower than 15ºC. On the contrary *C. juncea* show slow germination at high temperatures.

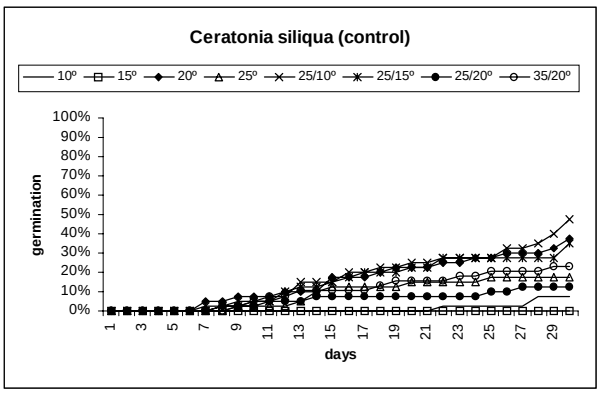
A. cytisoides has doubtful preferences in a moderate range of temperatures. Only decreases the germination rate in extreme conditions.

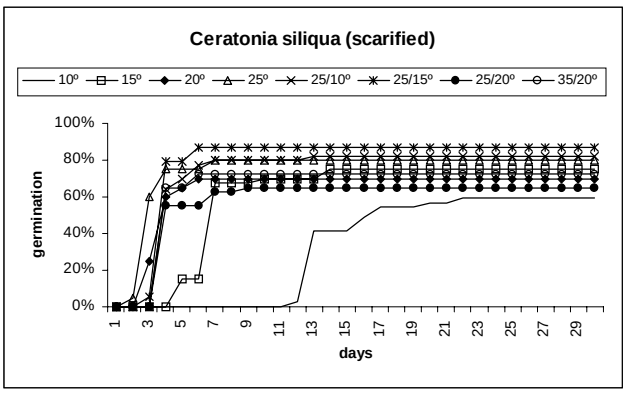


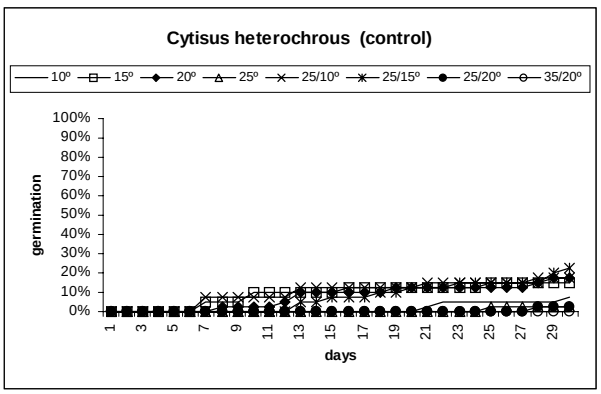


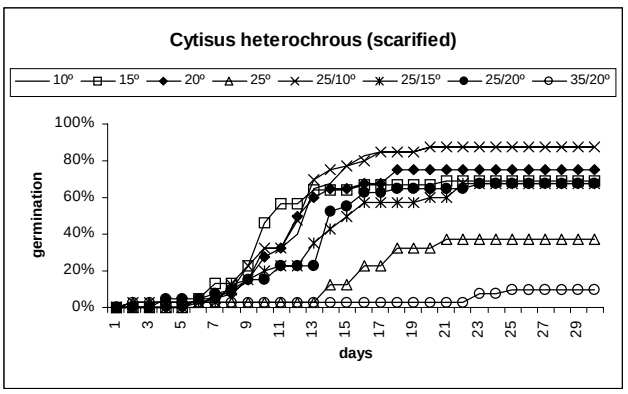












The rest of the species stored less than 46 months show physical dormancy that is broken with acid scarification.

The final percentage of *C. siliqua*, *G. umbellata* and *M. arborea* is always below 50% in non scarified seeds. After scarification the percentage reached is close or higher than 90%. The results at the different temperature regimes are compact and don't show significant preferences. Only *C. siliqua* shows a slow response al 10ºC.

C. heterochrous, *T. linifolia* and *U. parviflorus*, the only ones whose seeds has stophiole, have a wide variability in their germination capacity at the different exposed temperatures after the scarification. These three species have a clear tendency to reach the high germination percentages at average temperatures below 20ºC.

The global analysis of the obtained results shows that, although exceptionally, when we try with few conditions, the constant temperatures can be better than the alternating ones, but when increasing the studied interval of temperatures, we could conclude that there are not significant differences between both types of regimes.

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Desiccation Sensitivity in *Inga vera* subsp. *affinis* Seeds

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Abstract

Inga vera Willd. subsp. *affinis* (DC.) T.D. Penn. is a tropical tree species of the Leguminosae family, widely used in restoration of riparian forests in Southeastern Brazil. Its seeds are recalcitrant (desiccation-sensitive) which makes their storage very troublesome. In order to proceed toward a long-term storage of such kind of seeds, e.g. for seed bank purposes, one of the first steps is to know the level of desiccation sensitivity. Therefore, we carried out an experiment in which seeds of *Inga vera* were desiccated to 5 moisture contents (MC, fresh weight basis): 58% (control; without desiccation); 50%; 44%; 36.5% and 28.5%, in 2 desiccation rates (fast and slow, which took 27 and 106 hours, respectively, to reach the lowest MC). The seeds were set to germinate at 8 temperatures (5, 10, 15, 20, 25, 30, 35 and 40^{o}C), on top of sand, in constant light. Seeds not dried (control), reached 100% of germination at temperatures between 15^{o}C and 40^{o}C; 85% in 10^{o}C; and only 6% in 5^{o}C. The fastest germination occurred at 35^{o}C and 30^{o}C. Seeds with 50% of MC showed similar behaviour to the fresh seeds, while those with an MC of 44% attained, at maximum, 70% of germination. At an MC of 36.5%, seeds germinated only at low rates (not higher than 26%) and, with 28.5% of MC, seeds did not germinate anymore. In general, fast-dried seeds performed better than slow-dried seeds. The results allow to conclude that seeds of *Inga vera* subsp. *affinis* are very desiccation-sensitive.

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Seeds of *Zelkova abelicea*, an Endemic Tree of Crete

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Introduction

The genus *Zelkova* (Ulmaceae) comprises five species, three of which are distributed in Asia and two in Europe. *Zelkova abelicea* is a relatively short tree or shrub, endemic to the island of Crete (the other European species being a recently described one, *Z. sicula*, an endemic to Sicily).
Z. abelicea is a vulnerable species. It is relatively widespread in the mountainous massif of Lefka Ori (White Mountains, Chania prefecture, western Crete) with some stands on the other mountainous regions of Crete (Turland 1993). It is usually found in a shrubby form as a result of sheep and goats overgrazing. Its timber is durable and is used by the locals for the construction of traditional walking sticks. Only a few large and regularly fruiting individuals, approximately 100, can be found in the natural populations. The protection and conservation of this species is a matter of urgency (Phitos et al 1995). *Zelkova abelicea* is protected by national law (Presidential decree 67/81, Greece) and the collection and exportation of any plant material is prohibited unless a special permit is granted by the Ministry of Agriculture. Despite this protection, thousands of seeds and wild growing seedlings have been collected from their natural populations and exported abroad for 'scientific' reasons (e.g. Egli 1997). Recent investigations under nursery conditions showed a low germination capacity (only 10 %) for *Z. abelicea* seeds; however no dormancy release treatments were tested (Egli, 1997).
Information concerning collecting, handling and storing the seeds of this taxon is scarce and the present study aims to contribute in filling up this gap. The parameters that are studied include: description of seed structure, seed weight, storage behaviour, dormancy types and dormancy release treatments, temperature requirements for germination, rate of germination and seed ecophysiology.

Materials and Methods

Seeds of *Zelkova abelicea* were collected from one of the largest natural populations of the species, at Omalos plateau (1200 m asl) in November 2000. The germination experiments were performed after one year of storage in the drying room of the MAICh Seed Bank (15-20 ^{o}C, 15 % R.H.).
For germination experiments, seeds were sown on filter paper and incubated in 3 growth chambers (AGP 600 – TECNOLAB, Spain) with controlled temperature and light conditions. The temperatures tested were: 10 ^{o}C, 15 ^{o}C, 20 ^{o}C (all ± 0.5 ^{o}C) and the photoperiod (light /dark cycles) was set to 12/12h. The criterion of germination was visible radicle protrusion; germinated seeds were discarded after each count. The tests terminated when no additional seeds germinated. Each value of germination percentage was the average of five replicates of 50 seeds ± standard error (SE).
For germination experiments in the dark, seeds were incubated within light-proof metal containers in the same growth chambers and the seeds were counted in a dark room under a dim, green safelight.

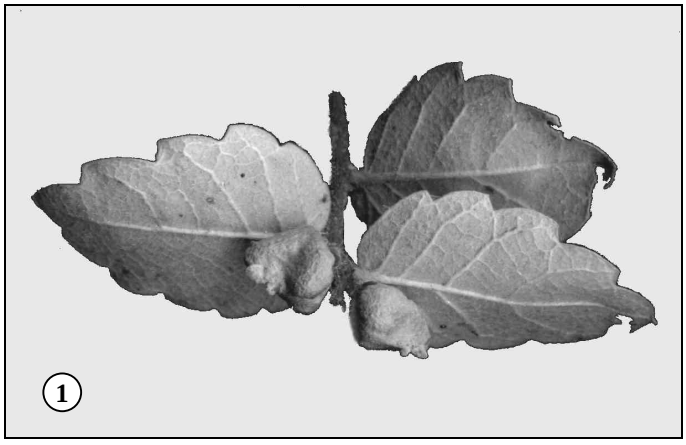
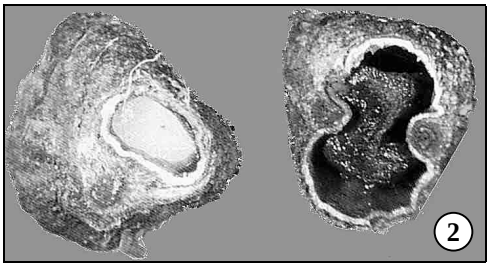


Photo 1 (left). Dispersal units of *Z. abelicea*: the annual shoots with several nuts and dry leaves still attached.
Photo 2 (below). Sound (left) and unsound seeds in transverse sections.

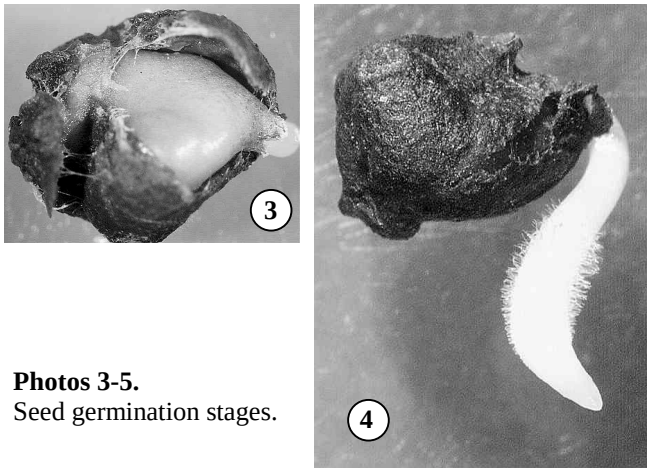


The fruit of *Z. abelicea* is a nut; the seed is non-endospermic with 2 large, flat cotyledons. The seed average

weight is 15.22 ± 0.22 mg (n=120). Empty nuts usually weight less than 10 mg and this characteristic can be used in the assessment of the quality of the seedlot in general and of the soundness of an individual seed in particular. The empty seed portion of the collection used in the present experiments was evaluated to 60 %.

Results and Discussion

Germination of *Z. abelicea* seeds took place very slowly at 10 °C and was completed after 7 months (Fig. 1). On the other hand, germination was fully suppressed at 15 °C and 20 °C. Ungerminated seeds that had imbibed at the 'inhibitory' temperature of 20 °C were observed to germinate upon a subsequent transfer to the 'optimal' temperature of 10 °C. Preliminary results show that germination at 20 °C can be induced by chilling and/or GA_3.



Photos 3-5.
Seed germination stages.

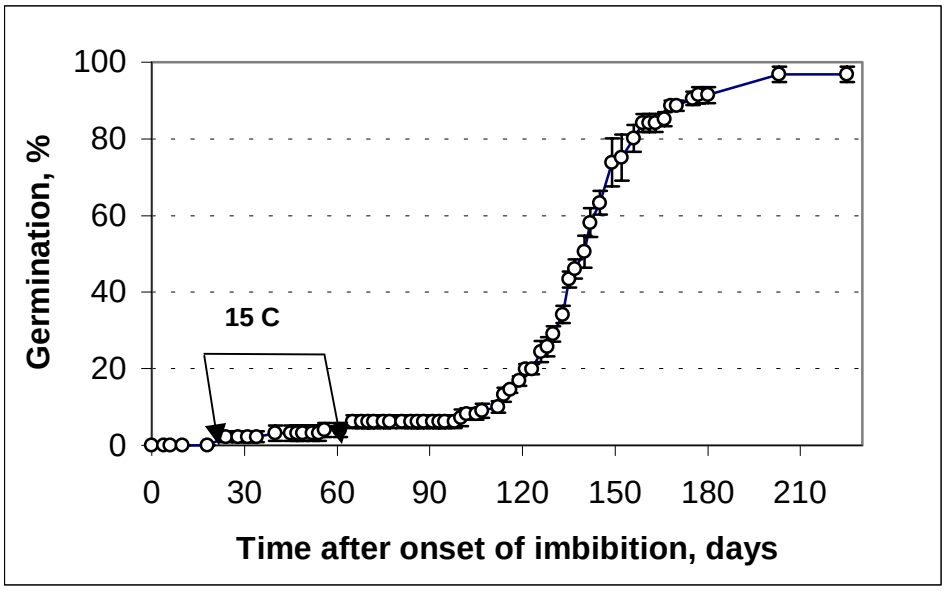


Figure 1. Germination time course of *Z. abelicea* seeds incubated at 10 °C (Light/Dark, 12/12 h) during a 7-month period (seeds were transferred to 15 °C between 20 and 60 days).

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Optimum Moisture Content and Prechill Duration for Dormancy Breakage of Douglas Fir Seeds (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco)

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Abstract

Freshly harvested and dry-stored seeds of Douglas fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) exhibit a mild form of dormancy described as 'conditional', 'relative' or 'shallow'. Untreated seeds only germinate slowly and over a narrow range of conditions, but their germination benefits from prior incubation under moist conditions at 3 – 5°C (a so called 'prechill').

Seeds of Douglas fir were initially germinated at six constant temperatures (10-35°C) following different dormancy breakage pretreatments (0 - 48 weeks prechill). From this preliminary study 15°C was selected as the best, single germination temperature for determining the efficacy of subsequent dormancy breakage treatments.

Seeds from the same seedlot were then adjusted to 10, 15, 20, 25, 30, 35 and 40 % moisture contents (mcfw - fresh weight basis), prechilled for 0, 2, 4, 8, 16, 32, 64 and 128 weeks and transferred to 15°C. A smoothed bivariate spline was used to model results and showed that virtually all combinations of moisture content and prechill duration significantly stimulated germination capacity, but the optimal germination percentage (93%) was only stimulated by various combinations of between 30 - 35 % mcfw and 25 - 48 w prechill. Even at optimal moisture contents (30 and 35%), extending the prechill duration beyond 48w led to a decrease in germination capacity. This was not due to dormancy reintroduction but was caused by seed death.

Regression models using a weighting function to account for differences in standard deviations demonstrated significant increases in the mean moisture content of individual seeds at higher moisture contents (25%) and longer prechill durations (64w) which were concomitant with significant decreases in dry weight. The most likely explanation for this was seed respiration. The combined results infer that dormancy breakage in Douglas fir seeds, requires an hydration level sufficient for respiration to take place, and that after maximal dormancy release, seeds at the highest mc's (35 - 40%) may exhaust their food reserves quickest and begin to deteriorate soonest.

Keywords: Douglas fir, prechill duration, moisture content, dormancy breakage, germination

Efecto de Baja Temperatura en la Conservación de Semillas Pregerminadas de *Pinus halepensis* y *Pinus pinaster*

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Abstract

A fast and a homogenous germination is an important requirement for the successful seed use in nurseries and in direct sowing programs. This feature can be achieved by using pregerminated seeds, which have been subjected to specific temperature and humidity conditions that lead to their germination. The advantage of this treatment is lost if the seeds are not used immediatelly, as they deteriorate rapidly. An important goal would be the development of storage conditions in order to halt the germination process maintaining both the initial seed viability and the advantages of the pregerminated seed. We tested whether this can be achieved with *Pinus pinaster* and *P. halepensis* seeds stored at a temperature of -2 ºC for 21, 28 and 35 days. Seed viability was evaluated by the germination test. It was found that low temperature has an adverse effect on *P. pinaster* seeds by reducing germination rate and increasing the amount of abnormal seedlings. In contrast, *P. halepensis* seeds were not affected by the low temperature tested.

Keywords: *Pinus halepensis, Pinus pinaster*, freezing, preconditioned seed, germination

Introducción

El área de distribución potencial de *Pinus pinaster* y *Pinus halepensis* comprende, en su conjunto, dos terceras partes del territorio forestal de la Península Ibérica. Según datos aportados en el Segundo Inventario Forestal Nacional la superficie ocupada por *P. pinaster* está próxima a 1,200,000 Ha de las que unas 600,000 Ha corresponden a masas naturales. *P. pinaster* es una de las especies más destacadas entre los pinos ibéricos, sobresaliendo su adaptabilidad a una gran variedad de suelos y climas. La tolerancia al frío es un carácter adaptativo muy apreciado de *P. pinaster*, dado el amplio grado de diversidad encontrado en sus masas naturales (Alía Miranda 1996).

La distribución de *P. halepensis* en la área mediterránea es de las más amplias de las especies de pinos. Se cita que las masas naturales de pino carrasco ocupan un total de 805,953 Ha en España. *P. halepensis* es una especie marcadamente colonizadora de terrenos desprovistos de vegetación, siendo su rasgo más sobresaliente su resistencia a la sequía (Gil Sánchez 1996). Vegeta en lugares con 1 a 5 meses de periodo seco. El factor más determinante para su distribución parece ser la temperatura, especialmente las mínimas invernales. En estudios realizados por Falusi et al. (1984), comparando la resistencia al frío de *P. halepensis*, *P. brutia* y *P. eldarica*, se manifiesta la menor resistencia del pino carrasco respecto a las otras especies. La amplia distribución potencial de *P. pinaster* y *P. halepensis* unida a su facilidad de arraigo es lo que determina la utilización tan extendida de estas especies en repoblaciónes forestales.

La reforestación de terrenos en España se realiza generalmente por medio de plantaciones precedidas por una serie de trabajos, como son los desbroces y las preparaciones de suelo, que suponen grandes desembolsos de dinero, hasta el punto de absorber la mayor parte del gasto junto con la partida de jornales. De manera que el importe destinado a la compra de planta es un montante menor. En contraposición los semillados directos presentan un coste general mucho menor (ahorros en preparación de terrenos, jornales), lo que constituye su principal ventaja frente a la plantación. Además, la rapidez de actuación permite semillar gran cantidad de hectáreas por campaña.

Los semillados directos y su modalidad de siembra aérea fueron ampliamente utilizados en Norteamérica en los años 40-50 (Derr et al. 1971). En España se han realizado también algunas experiencias con éxito variable. Entre los muchos factores que intervienen en los resultados, como se ve en distintas experiencias publicadas (Castell y Castelló 1996, Domínguez et al. 2001), se pueden citar la predación, la elección de especie, la época y la preparación de la semilla. Resulta fundamental la protección de la semilla contra los depredadores mediante sustancias repelentes. Otra vía para reducir o evitar la predación puede ser la preparación de la semilla de manera que su emergencia se produzca en los días siguientes a la siembra, reduciendo el umbral, que es el número de

días que transcurren desde el inicio del ensayo hasta la germinación de las primeras semillas. Para ello se suele utilizar la técnica de preacondicionamiento o prehidratación. Este último término se viene utilizando en algunos trabajos, si bien su significado es el mismo que preacondicionamiento, llevando en ocasiones a confusión. Sería deseable una normalización de la terminología.

La hidratación de las semillas es una condición indispensable para su germinación. La imbibición está relacionada con la diferencia de potencial hídrico existente entre la semilla y el sustrato húmedo en el que se encuentra. Es un fenómeno puramente físico. Pero es el primero de los cambios que se suceden previos a la germinación, por lo que la idea de propiciarlo en el laboratorio tiene la finalidad de adelantar la emergencia de las semillas. Sin embargo, la imbibición no debe realizarse de manera muy rápida, porque si existe exceso de agua en el sustrato puede ser perjudicial para la germinación y crecimiento de las plántulas. En una hidratación muy rápida, el contenido celular se hace soluble pero las paredes celulares no están aún lo suficientemente hidratadas para funcionar como membranas semipermeables e impedir la salida de solutos (Besnier 1989). En este sentido, se habían realizado ensayos anteriores para testar diferentes duraciones de preacondicionamientos posteriormente comprobadas mediante el ensayo de germinación. Como conclusión se determinó que la duración de 7 días de preacondicionamiento es la más adecuada (datos en prensa) y, en consecuencia, es la que se toma como control en este ensayo. La ventaja del método de preacondicionamiento es fundamentalmente la reducción en los días de umbral, adelantándose el comienzo de la germinación de 13 a 4 días.

Por otro lado, la imprevisibilidad de las condiciones atmosféricas hace que los semillados directos deban a veces decidirse con pocos días de antelación o retrasarse en el caso de cambios atmosféricos que aconsejen no llevarlo a cabo de manera inmediata.

En el presente artículo se describe un ensayo de conservación mediante congelación de semilla preacondicionada de *P. halepensis* y *P. pinaster* y se trata de valorar la incidencia de los tratamientos sobre la viabilidad de la semilla.

Material y Métodos

Para este ensayo se han utilizado semillas de *Pinus halepensis* de la procedencia ES16 Cazorla de cosecha 1995/96. Las semillas de *Pinus pinaster* eran de la procedencia ES20 Sierra Bermeja de cosecha 1989/90. Los ensayos fueron realizados en el año 2000, hasta entonces las semillas estuvieron almacenadas en el almacén frigorífico del C.N.M.F. “El Serranillo”.

Previamente a los ensayos de germinación se había sometido a las semillas a un preacondicionamiento, consistente en disponer las bandejas con las semillas ya colocadas durante 7 días en la cámara de germinación a 20 ºC, buscando el inicio de los procesos de imbibición, la activación de la maquinaria bioquímica y el desencadenamiento de los procesos metabólicos. Antes del comienzo de la apertura de la testa, se sacan las bandejas de la cámara de germinación y se trasladan a una cámara de congelación donde se ha fijado una temperatura de -2 ºC, excepto el grupo testigo, que se mantenía a 20 ºC para provocar la germinación. Las bandejas permanecen en la cámara de congelación por espacios de tiempo variable, desde 20 a 36 días (periodos de 20, 29 y 36 días). Una vez transcurrido ese tiempo se sitúan de nuevo en la cámara de germinación donde se completa el ensayo, para poder apreciar los efectos del preacondicionamiento y posterior conservación en frío sobre su viabilidad.

Los ensayos de germinación se realizaron siguiendo las normas ISTA (ISTA 1999) en las que se determinan los métodos de análisis. En el caso de los pinos citados, prescriben el ensayo sobre papel y a temperatura constante de 20 ºC. En nuestro caso, elegimos como sustrato arena calibrada del número 5 (partículas entre 2 y 5 micras), humedecida con una solución de fungicida (0.1 % quinosol). Las semillas se enterraban y se cubrían las bandejas con un plástico para evitar pérdidas de humedad. Permanecían así durante al menos 30 días, aunque en realidad se mantuvieron más tiempo para comprobar que no se producían más germinaciones una vez transcurrido ese periodo. Las germinaciones de las especies referidas son lentas (Thanos 2000), de manera que con frecuencia se necesitan 35 días para completar un ensayo, y aunque se da por terminado, se sabe que aún hay un porcentaje de semillas cuya germinación puede ocurrir al filo de los 60 días. Los ensayos se mantuvieron en una cámara de germinación con temperatura constante de 20 ºC y atmósfera saturada. Se proporcionó luz a las bandejas durante 8 horas alternando con 16 horas de oscuridad. Se realizaron conteos de germinación en días alternos y se tomó el acuerdo de considerar una plántula como normalmente germinada una vez que se había desprendido la testa de sus hojas cotiledonares y podían apreciarse sus partes como completas y de dimensiones normales.

Las plántulas se clasificaron en normales y anormales. Para las especies arbóreas de germinación epigea, se consideran normales las que excedan en conjunto (la raiz primaria y el hipocotilo) cuatro veces la longitud de la semilla (normas ISTA). Las plántulas anormales son todas aquéllas que presentan tales defectos que no parece que puedan dar lugar a plantas normales en condiciones favorables de cultivo. En el caso de apreciar algún defecto clasificable como los que figuran en la tabla 5.2.5.*A* de las normas ISTA (ISTA 1999), se anotaba y se consideraba como no germinada, por lo que no forma parte del porcentaje de germinación final.

Las semillas no germinadas se clasificaron, una vez terminado el ensayo, en duras, aquellas que tenían sus estructuras intactas pero no fueron capaces de germinar; muertas con sus tejidos deteriorados; y vanas o sin embrión.

La falta de vigor se muestra también en plantas que se consideran normales. Existen algunos métodos que intentan expresar numéricamente el vigor. Como medida del vigor en este ensayo hemos seguido el más ampliamente utilizado para semillas de árboles, desarrollado por Czabator en 1962 (Bonner 1997), que propuso utilizar el término valor de germinación (GV) que viene a expresar de alguna manera la rapidez de la respuesta de la semilla y la contabiliza. Se determina calculando el valor cumbre (Peak Value, PV) y la media de germinación diaria (MDG). El valor cumbre PV, se refiere al día en el que se han producido el mayor número de germinaciones, teniendo también en cuenta la inmediatez de la respuesta.

Resultados y Discusión

Deben analizarse separadamente varios aspectos de los resultados: umbral, facultad germinativa y otros efectos atribuíbles a la conservación en frío.

Dado que el semillado directo basa gran parte de su éxito en una rápida nascencia que disminuya los efectos negativos de la depredación, tiene gran valor el uso de técnicas conducentes a disminuir el umbral. Cuanto menor tiempo pasen las semillas en el campo expuestas sin germinar, menores posibilidades tienen de ser ingeridas o trasladadas a comederos. Los umbrales obtenidos se representan en la Tabla 1. No se observan cambios importantes al someter las semillas a congelación hasta 21 días, es decir, en general se mantiene el umbral sin pérdidas relevantes para ninguna de las dos especies. Sin embargo, si congelamos las semillas durante 35 días se observa un retraso en el umbral con respecto al testigo para las dos especies.

Tabla 1. Resultados obtenidos tras el ensayo para *Pinus pinaster* y *P. halepensis*. La letra minúscula detrás del valor numérico indica igualdad de significación en el Test de Tukey para intervalos de confianza de 0.95.

Especies	Tratamiento	Umbral (días)	Germinación (%)	GV	Anormales (%)	Muertas (%)
P. pinaster	Testigo	7	83a	11.4	2a	5a
	21 días a -2 °C	8	73b	9.79	5a	16b
	28 días a -2 °C	11	66b	3.03	6a	23b
	35 días a -2 °C	14	71b	4.43	9b	13ab
P. halepensis	Testigo	9	70a	5.61	1a	28a
	21 días a -2 °C	9	73a	4.20	1a	25a
	28 días a -2 °C	10	73a	5.12	1a	25a
	35 días a -2 °C	14	71a	5.50	5b	24a

Como puede apreciarse en la Figura 1, los resultados de facultad germinativa obtenidos para los distintos tratamientos de *P. halepensis* son muy similares, no encontrando diferencias significativas entre los totales obtenidos (p=0.7509, F=6.4069), lo que puede interpretarse como un éxito de los tratamientos ensayados.

En cuanto a *P. pinaster*, las germinaciones totales van decreciendo a medida que los tratamientos suponen conservaciones más largas, encontrándose diferencias significativas entre la duración del período de congelación y la facultad germinativa (p=0.0001, F=18.664). Así, al aumentar el período de frío, disminuye la germinación.

Existe tambien un paralelismo entre los resultados de facultad germinativa y los valores de GV que se muestran en la Tabla 1, de manera que la información dada por GV viene a matizar el dato de porcentaje de germinación en el sentido de incluir en su cálculo de alguna manera, la cadencia en la que se han sucedido las germinaciones.

Tambien se ha observado que al aumentar el tiempo que permanece la semilla en frío (congelación), aumenta la cantidad de plántulas que presentan algún tipo de anormalidad. Las anormalidades deben su origen a diferentes causas: deficiencias nutritivas en las plantas madres, falta de maduración, infección por microorganismos y

plagas, daños mecánicos ocasionados en su manejo, daños provocados por tratamientos inadecuados, o por productos fitosanitarios.

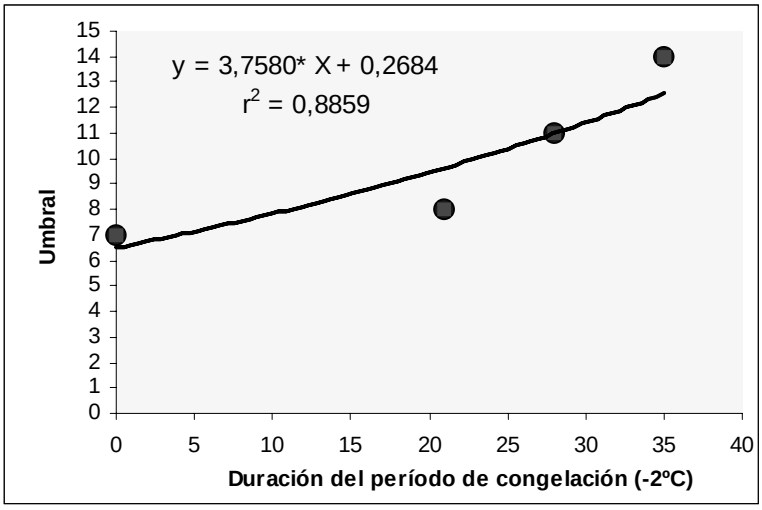

Figura 1. Gráfica de regresión para *P. halepensis.* Se indica la ecuación de regresión entre la duración del período de congelación y el umbral obtenido en cada tratamiento (p= 0.058).

Se encuentran diferencias significativas entre la duración del período de congelación y el porcentaje final de plántulas anormales, para las dos especies (*P. halepensis*: p=0.0020, F=9.1176; *P. pinaster*: p= 0.0058, F=18.664). En el caso de *Pinus pinaster* este efecto de aumento de plántulas anormales está correlacionado positivamente con la duración de la congelación (Fig.2). Los datos indican que la conservación no tiene efectos muy negativos si la duración no es mayor de 28 días.

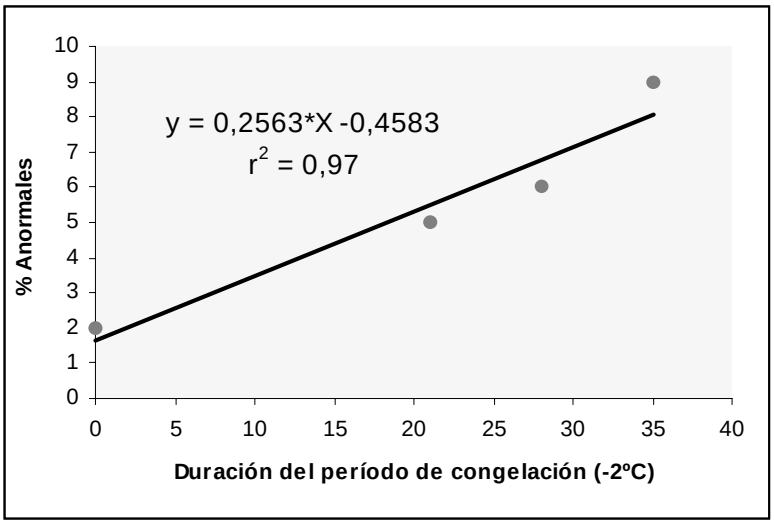

Figura 2. Gráfica de regresión para *P. pinaster*. Se indica la relación entre la duración del período de congelación y el porcentaje final de plántulas anormales obtenido (p=0.0157).

En el caso de *P. pinaster* la muerte de las semillas parece otro efecto secundario debido a la intolerancia a los tratamientos ensayados. A medida que la duración de la congelación se alargaba, se encontraban porcentajes mayores de semillas muertas en el ensayo de germinación, siendo las diferencias estadísticamente significativas (p=0.0004, F=13.322).

Sin embargo, lo expuesto más arriba no puede afirmarse en el caso de *P. halepensis*; los ensayos de germinación con esta especie han puesto de manifiesto que la gran mayoría de las semillas que no han germinado se

encuadran en la categoría de muertas, cuyos porcentajes eran bastante altos incluso en el grupo control y se mantuvieron en los mismos niveles para todos los tratamientos.

Conclusiones

La conservación de la semilla preacondicionada por congelación es una técnica útil, pues tiene la gran ventaja de prepararla y disponerla para una germinación rápida, lo que es especialmente valioso en los semillados directos. Es necesario sin embargo, valorarla según las especies a utilizar, pues como se ha visto su respuesta no es fácilmente previsible. Contrariamente a lo que la autoecología de las especies podría hacer esperar, la semilla preacondicionada de *P. pinaster* se ve mas afectada por la conservación en frío, mientras que la de *P. halepensis* no sufre aparentemente, conservando los parámetros de su germinación en los mismos niveles para todos los tratamientos ensayados.

La sencillez del método propicia la posibilidad de utilizarlo en grandes cantidades, pues al no abrirse la semilla durante el preacondicionamiento, su manipulación no es muy delicada, lo que permite el uso de cribas u otros utensilios para la separación del medio y la semilla.

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Germination of Pretreated Scots Pine (*Pinus sylvestris* L.) Seeds after Long-Term Storage

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Introduction

Pretreated seeds are usually sown directly, but due to the augmented quantities immediate sowing is not always possible and pretreated seed lots have to be stored. Germination capacity of non-treated pine seeds is known to preserve well in cold store, but the research on storage of pretreated seeds is scant. The aim of our work was to study the changes in germination indices of pretreated Scots pine seeds.

Material and Methods

Pretreated and non-treated seeds of open-pollinated stands and seed orchard were used. Pretreatments of seeds were carried out with IDS-method. The pretreated seeds were stored for 9 or 10 years at the temperatures of 2 °C and -18 °C. The changes in germination indices were investigated using germination tests and radiography.

Results and Discussion

The germination indices of both pretreated and non-treated seeds impaired during long-term storage. In general the changes were greater in cool than in freeze store. Declination of the germination indices was more obvious in pretreated seed orchard than in forest stand seeds. The germination indices of forest stand seeds whether pretreated or not, preserved equally well in cold storage, while those of seed orchard seeds prevailed better in non-treated seed batches. Forest stand seeds incubated at 10 °C maintained their germination indices better than seeds incubated at 5 °C, while in orchard seeds the effect was not as evident after long-term storage. The results proved that especially orchard seeds collected early in the autumn should be stored non-treated.

Keywords: conifers seeds, IDS-treatment, seed orchard, incubation and storage temperature

Changes in Percentage and Speed of Root and Shoot Germination in *Quercus robur* Seeds after a Controlled Moisture Content Chilling Treatment

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Keywords: *Quercus robur*, controlled moisture content, chilling, speed of germination, critical moisture content of radicle protrusion

Introduction

Field emergence of spring-sown oak seeds (syn. fruits), as compared to autumn sown seeds, are slower and less uniform and result in less vigorous seedling growth. Consequently, several authors have suggested that oak seeds have an epicotyl dormancy when shed in autumn. A chilling period at 3-5°C would be required to break such epicotyl dormancy and reduce the time to shoot emergence. Optimal storage of oak seeds at –1 to –3°C will, however, not allow a release of dormancy which implies that epicotyls may be 'fully dormant' when sown in spring. The aim of this study was to investigate if it was possible to develop a 'controlled moisture content chilling' treatment for *Q. robur* seeds to obtain an increased speed and uniformity of seed germination of both roots and shoots, at the same time avoiding unacceptable radicle elongation during chilling.

Materials and Methods

Seeds of *Q. robur* were dried to 42, 44, 48 and 52 % moisture content (MC, fresh weight) and chilled at 4°C for 0, 4, 8, 12 and 16 weeks in a drum-like container, allowing aeration through a breathing hole covered with an air-permeable cloth that restricted water loss. No water was supplied during the 16 weeks chilling. At each sampling time moisture content was analysed on 50 individual seeds by oven drying. Seeds for testing germination were recorded for premature germination during the chilling treatment and then re-moistened for 48 hours in cold running water at all mc*duration treatments, except for a separate treatment at 0 weeks of chilling where seeds were sown without re-moistening at the chilling moisture content.

Eight replicates of 20 seeds each were sown in transparent acrylic germination cassettes (30 x 40 x 6 cm) allowing visual registration of root germination and shoot emergence in medium. Cassettes were filled with a lightly compressed fine-graded standard peat moss with uniform moisture content. Seeds were placed on top of the peat moss with their 'radicle end' close to the transparent 'window' and covered with 3 cm of fine moist sand. After adding 0.25 l water by spray to the sand, the top of the cassette were sealed by thin plastic cover to avoid evaporation but allow aeration. The cassettes were placed in a slanting position (60° angle) with seeds at the lower window, allowing germinating roots to grow geotropically along the window. Seeds were germinated for 8 weeks at constant 18°C and 16 hours daylength and germination recorded twice each week. Root germination (RG) was recorded visually as a radicle reaching the transparent lower 'window', and shoot germination (SG) as emergence of shoots above the sand. Time for root (TRG) and shoot (TSG) to germinate and time for the root to grow to the bottom of the cassette (= 14 cm) (TR14) were recorded. The time between root and shoot germination (TRSdif) was calculated. All recordings were kept together for a single seed. Data were analysed by the GLM procedure (SAS Institute) after appropriate data-transformation and means separated by the REGW Q-test.

Results

The drum system maintained seed MC stable over the 16 weeks of chilling. After 16 weeks of chilling seeds had germinated 12, 18, 57 and 59 % at 42, 44, 48 and 52 % MC respectively. Thus, the lower critical MC for radicle protrusion at 4°C is below 42 % MC in this seed lot. At 44 and 42 % MC almost all germinated seeds had radicles shorter than 0.5 cm, whereas 24 and 37 % seeds had radicles longer than 0.5 cm at 48 and 52 % MC respectively. A full control of radicle protrusion at 4°C is therefore not within reach in this trial. The percentage root and shoot germination of remoistened seeds at 0 weeks chilling were slightly higher (n.s.) than without remoistening (except for MC=52%), whereas time to root and shoot germination was 1-3 days slower after remoistening (except for MC=42%). TRSdif and TR14 were not significantly different.

There was a strong significant effect of MC, duration of chilling and the interaction between these. Root and

shoot germination at all chilling duration's were lowest in seeds chilled at 42 % MC and increased with higher MC to a maximum in seeds chilled at 48 % MC. Seeds chilled at 52 % MC germinated less than at 48 % MC. Parallel to this speed of root and shoot germination at all duration's was lowest in seeds chilled at 42 % MC and increased with increasing MC. Whereas root germination in seeds chilled at 52 % MC was slower than at 48 %, shoot germination after 4 or more weeks chilling was faster in seeds chilled at 52 % than at 48% MC. Root and shoot germination of seeds chilled at 42 or 44 % MC decreased with increasing chilling duration's, whereas seeds chilled at 48 or 52 % MC either germinated the same or slightly better with increasing duration of chilling. Speed of root germination increased significantly at all MC's with increased chilling duration. Chilling at 48 and 52 % MC for 12 or 16 weeks gave the lowest TRG values. TRG at 48 % MC decreased from 18 days at 0 weeks to 7 days after 16 weeks chilling. Speed of shoot germination only decreased slightly at 42 and 44 % MC, whereas a significant decrease in TSG with increasing duration of chilling was found at 48 and 52 % MC. TSG in seeds chilled at 52 % MC changed from 31 days at 0 weeks to 19 days after 16 weeks of chilling. As the decrease in time to root germination was slightly larger than the decrease in time to shoot germination, TRSdiff remained the same or increased slightly with longer chilling durations.

16 weeks chilling at 48 % MC gave the highest percentage and speed of germination of both roots (90%, 7 days) and shoots (78 %, 20 days) in this seed lot. However, 48 % MC did not restrict radicle protrusion during chilling (total 57 %, 24 % > 0.5 cm after 16 weeks) and therefore cannot be exploited directly by growers with traditional techniques. Chilling at 44 % MC allows better control of radicle protrusion but resulted in significantly lower percentage and speed of germination in this study.

Discussion

The results indicate that remoistening of seeds already at high moisture content (52 %) may reduce both percentage and speed of germination. Also chilling seeds at 48 % MC almost always gave better results than chilling at 52 %, which suggests that metabolism during chilling is slowed down at too high MC. High water content in the pericarp may reduce oxygen transport to the embryo and thus support the hypothesis that low oxygen availability may delay germination in *Q. robur*. The critical MC for radicle protrusion is shown to be below 42 % and thus leaves only a narrow MC interval down to the critical MC for inducing desiccation damage in the most sensitive seeds of a population, known to be close to 38-40 %. The rate of chilling, i.e. the effect on speed of germination, decreases at lower MC but is still present at 42 % MC. Germination percentage, however, decreases significantly at this MC and is therefore not of interest commercially. This reduction in germination may, however, depend on the quality of the seeds, i.e. high vigour seeds may not lose viability as fast as low vigour seeds. Time to root germination is reduced about 50 % after 16 weeks of chilling at 48 and 52 % MC. This is strongly correlated to the high percentage of prematurely germinated seeds at these MC's. The time to shoot germination is only reduced about 30 % after 16 weeks of chilling compared to unchilled seeds and the rate of reduction is much faster at 52 % MC than at 48 %, and much slower at lower MC's. This may suggest that the chilling effect on root and shoot germination is controlled slightly different by MC, meaning that critical MC for improving speed of shoot germination is possibly higher than for improving speed of root germination. As a reduction in MC of seeds cannot restrict premature germination during chilling, the possible effect of reducing the chilling temperature below 4 °C should be studied in order to determine if similar improvements in speed of germination could be obtained without getting premature germination during the chilling treatment.

Conclusion

Speed of root and shoot germination can be increased significantly by chilling seeds at 48 or 52 % MC for up to 16 weeks without affecting laboratory germination percentage negatively. The chilling effect may be interpreted either as a release of dormancy or simply as a germination advancement. As critical MC for radicle protrusion at 4°C is shown to be below 42 % in this seed lot, a significant proportion of seeds will develop radicles during chilling. The higher the MC and longer chilling duration, the higher percentage of germinated seeds and the longer radicles. Thus, following the best treatment (16 weeks of chilling at 48 % MC followed by 48 hours remoistening before sowing) more than 50 % seeds showed radicle protrusion. Radicles longer than a few mm will potentially be damaged during handling and sowing, and this hampers the potential for direct commercial exploitation of the present results.

Resin Removal Effect on Bald Cypress [*Taxodium distichum* (L.) Rich. var. *distichum*] Germination

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Abstract

The resin in Bald Cypress (*Taxodium distichum* (L.) Rich. var. *distichum*) cones prevent effective cleaning of the seed lots. Typically, the cones are crushed, resulting in the seed and cone parts being mixed and often stuck together by the resin. To obtain cleaner seed, a new technique removing the resin was developed by a tree seed company. In this study, typical seed and clean seed from the same lot were tested at various stratification lengths to determine the effect of the resin removal on stratification length and germination. Removing the resin from seed reduced the stratification length needed for germination. The effect of the seed cleaning on seed storability at two temperatures, 2^{o} and -8^{o}C, is also being tested, and this information will be available later.

Keywords: *Taxodium distichum*, Bald Cypress, resin

Introduction

Bald Cypress, *Taxodium distichum* (L.) Rich. var. *distichum*, is an important forest tree in the southeastern United States. The native range of this deciduous conifer in the Redwood family (Taxodiaceae) is the Atlantic and Gulf Coastal Plains and the lower Mississippi and Ohio Rivers to southern Indiana. Bald Cypress often grows in saturated soils in bottomlands with prolonged flooding up to 3 meters deep. Trees often have buttressed bases and sometimes the roots form knees above the water level. Some trees live over 1000 years and trees 400 to 600 years old are common in virgin stands. The larger trees in virgin forests are 215 to 365 cm in diameter at breast height (dbh) and 43 to 46 m in height (Wilhite et al). The dbh of the champion tree is 520.7 cm.

Bald Cypress is important for timber, wildlife, environment, and as a landscape tree. The heartwood timber from older trees is resistance to decay, and the timber has a number of usages. The tree is also important for wildlife habitat and is the dominant tree in many wetlands. It plays an important role in protecting the environment, especially since it grows in areas frequently flooded and withstands high winds. Bald Cypress's natural beauty and wide tolerance to various soil types and drainage conditions have contributed to its wide planting as an ornamental.

Each year large amounts of Bald Cypress seed is collected and planted in forest nurseries. The seed do not disperse from the cones because cone resin holds the seed to the cone scales. To release the seed, the cones are crushed. The seed and cone parts are difficult to separate due to the resin. Insect galls also are commonly present in the cones and are held to the cone parts and seed by the resin. Typically, the seed and the cone parts and insects galls from the crushed cones are planted together. The seed component is often 45 to 50% of the lot. Removing the cone scales would reduce, usually by about half, the bulk of the lot for drying and storage and result in better uniformity in planting.

The key to separating the cone parts, insect galls, and seed is removal of the resin. A new procedure to remove the resin has been developed by a southeastern United States forest seed company. Clean Bald Cypress seed without resin, cone parts, or insect galls is now commercially available.

Materials and methods

To test the effect resin removal on germination and storage of seed, the same lot of fresh seed was tested before and after resin removal. A sample was set aside before the lot was processed and another sample was taken from the lot after processing. Each sample was mixed and divided into 32 equal parts. Eleven parts from each lot were set aside for germination after one to five years storage at 2^{o} and -8^{o}C. This information will be available latter. One part was tested unstratified and the other 20 parts were stratified four different ways and five time lengths. (Table 1). Twelve hundred seed were germinated in each treatment, twelve germination dishes in random distribution in a germinator at 20-30^{o}C with eight hours light. Purity, seeds per kilogram, moisture content, x-ray analysis, and tetrazolium tests were also run on both lots. All tests followed the procedures adopted by the International Seed Testing Association (International Seed Testing Association 1999).

Table 1. Stratification media and lengths.

Stratification Media	Length of Stratification				
Crepe Cellulose Paper (kimpak)	7 days	14 days	28 days	63 days	91 days
Naked 4 ml bag	7 days	14 days	28 days	63 days	91 days
Damp perlite	7 days	14 days	28 days	63 days	91 days
Underwater	7 days	14 days	28 days	63 days	91 days

Results

The results of the moisture, purity, seeds per kilogram, filled seed, and viability tests are given in Table 2. X-ray analysis determined the filled seed. Viability was determined by staining with 1% tetrazolium chloride solution.

Table 2. Comparisons of lots before and after resin removal.

	Moisture	Purity	Seeds/kg	Filled seed	Viable
Before resin removal	8.3%	71.60%	13,874	48%	38%
After resin removal	9.7%	98.39%	14,749	93%	81%

Table 3 lists weekly filled seed germination of cleaned and uncleaned seed. Germination of the filled seed part of the sample only is reported to enable comparisons of lots with unequal empty seed components. Three comparisons are shown: unstratified cleaned seed to uncleaned seed, 28 days stratified cleaned seed to uncleaned seed, and unstratified seed to 28 stratified seed for both cleaned and uncleaned seed. The seeds were soaked overnight at room temperature (20^{o}C), and the stratified seeds were placed on damp crepe cellulose paper (kimpak) spaced apart (not touching) at 2^{o}C in closed boxes.

Table 3. Germination of filled seed on kimpak unstratified and stratified 28 days.

	Unstratified Cleaned seed	Unstratified Uncleaned seed	Stratified 28 days Cleaned seed	Stratified 28 days Uncleaned seed
7 days	0	0	0	0
14 days	50	31	57	50
21 days	69	58	77	65
28 days	75	64	80	67
35 days	79	68	82	68

Tables 4 and 5 show the germination results of clean and unclean seed stratified 28 days on four media. All stratifications began with an overnight water soak at room temperature (20^{o}C), and then the seed were drained and placed on or in the stratification media at 2^{o}C. On the crepe cellulose paper (kimpak), the seeds were spread on the surface of the paper without touching each other and placed in plastic boxes with lids. For the naked bag stratification, the drained seeds were placed in a 2 ml bag and sealed. For stratification in perlite, the seeds were mixed with three parts of damp perlite to one part of seed and placed in plastic boxes with lids. The seeds were stratified underwater by placing the seed in cotton mesh bags submerged in tubs of water kept in a cooler at 2^{o}C.

Table 4. Germination of cleaned seed stratified 28 days on four media.

	% Germination			
Test days/Media	Kimpak	Naked bag	Perlite	Underwater
7 days	0	0	0	0
14 days	57	38	55	43
21 days	77	71	75	71
28 days	80	78	78	78
35 days	82	80	80	80

Table 5. Germination of uncleaned seed germination stratified 28 days on four media.

Test days/Media	% Germination			
	Kimpak	Naked bag	Perlite	Underwater
7 days	0	0	0	0
14 days	50	52	43	62
21 days	65	65	57	70
28 days	67	67	59	70
35 days	68	67	60	71

Discussion

The purity and filled seed of the clean (after resin removal) seed are much improved over the unclean (before resin removal) seed (Table 2). Removing the resin singularized the seed and made the seed free flowing, allowing processing to remove most of the cone parts and empty seed. Viability testing shows that 87% of the filled cleaned seed are viable, while 79% of the filled unclean seed are viable. Cleaning the lot removed some of the dead seed as well as the empty seed and cone parts.

Germination results demonstrate that both stratified and unstratified seed germinated well (Table 3). Stratifying the seed 28 days increased the rate of germination for both the clean and unclean seeds. Stratification had a greater effect on uncleaned seed than on cleaned seed.

The stratification method resulted in little difference with cleaned seed, while the uncleaned seed stratified underwater germinated more rapidly. Uncleaned seed stratified in perlite germinated lower than seed stratified by other methods.

Conclusion

Long stratification, up to 90 days, is the current recommendation for breaking dormancy, and the general practice is to submerge the seed for the entire stratification period (Bonner 1974). No benefit was shown for stratifying the cleaned seed underwater. A short stratification period is adequate for cleaned seed.

Removal of the resin allows for cleaning of the lot to improve the purity and filled seed of the lot. Handling singularized, free flowing seed in the nursery is much easier than seed with resin, and less area is needed to store the bulk lot after the cone parts and empty seed are removed. Much higher germinations were achieved with the cleaned seed due to removal of the empty seed.

The resin removal does not adversely affect germination in fresh seed. Tests after one to five years storage will determine the effect, if any, of resin removal on the storability of the seed.

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The Effect of Seed Size and Germination Temperature on the Germination of Three Different Acacias

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Abstract

The objectives of the study were to evaluate the germination of seeds of three species of *Acacia* using three seed size-classifications and three temperature regimes. *Acacia auriculiformis*, *Acacia crassicarpa* and *Acacia mangium* seeds were collected from Seedling Seed Orchards in Wonogiri (Java), Riam Kiwa and Pleihari (South Kalimantan). Seeds of the three *Acacia*s were grouped into small, medium and large classes based on seed length. The seeds were germinated in three different germination cabinets with temperatures of 25, 30 and 35 ^{o}C. Germination stages were observed, and data were analyzed using a factorial design for the parameters Germination Percentage (GP), Germination Speed (GS), and Germination Value (GV). Results indicated that in general *A. auriculiformis* and *A. mangium* had relatively better germination than *A. crassicarpa,* and the differences were statistically significant. Among the three different temperature regimes, all species had better germination at temperatures of 25 and 30 ^{o}C than at 35 ^{o}C. Seed size classes had no significant effect on the three parameters.

Keywords: *Acacia*, germination, temperature

Introduction

The optimum conditions for different stages of germination and seedling growth vary between and within species. In general, maximum germination can be obtained when environmental conditions for germination are optimal, and the seeds are in the best physiological state. Seed germination is also controlled by maternal effects, and seedling growth is controlled by both genetic and environmental conditions. However, temperature and moisture are essential in triggering the metabolic activity within the seed prior to germination. Conditions are considered optimal when a large proportion of a seed lot germinates almost uniformly within a short period of time. The optimum temperature for most seeds ranges from 30 to 40 ^{0}C (Copeland and McDonald 1985). In the germination process, seeds with normal and relatively larger size can have better germination than smaller and abnormal size seeds (Susko and Doust 2000). Variation in seed size for *A. auriculiformis* has been reported in an earlier study (Komar 2001). In this species, variation exists although it was not significant between provenances. However, the overall variation in seed size for provenances and families was significant, as indicated by seed weight. The variation in seed size based on weight showed significant differences in the cumulative germination and early seedling growth (Komar and Indrati 2001). This study was devised to evaluate the response of the different seed sizes and temperature regimes for germination of three *Acacia* species.

Materials and Methods

Seed Source

Species used in this study were *A. auriculiformis, A. crassicarpa and A. mangium.* Seeds of *A. auriculiformis* were collected in 1999 in a seedling seed orchard located in Wonogiri, Central Java as per Komar (2001). Seeds of *A. mangium* were collected in 2000 also from Wonogiri Seedling Seed Orchard. Seeds of *A. crassicarpa* were collected in October 2000 from the Seedling Seed Orchard in Riam Kiwa, South Kalimantan.

Seed Size Classification

Seeds of the three species were divided into three different seed size classes: small, medium and large as described in Table 1. Seed size classification was obtained by picking the smallest and largest seed from the seed lot. The size of smallest seeds was expanded several millimeters toward the largest size, and the largest seeds were narrowed toward the smaller size until a relatively equal proportion of seeds for those three seed size class were obtained. A lot of 450 seeds per seed size class was randomly selected. Then, 150 seeds (3 replication @50 seeds) were germinated at each of three different temperature regimes.

Table 1. Seed size classes for the 3 different *Acacia* species.

Species	Small	Medium	Large
A. auriculiformis	< 3 mm	5-3 mm	> 5 mm
A. crassicarpa	< 4 mm	4-5 mm	> 5 mm
A. mangium	< 2 mm	4-2 mm	> 4 mm

Pre-treatment

Seeds from each size class were placed inside porous bags in order to keep them separate. They were then pretreated to break the dormancy by soaking in hot water (approximately 90-100 ^{o}C) overnight (24 hours). After pretreatment, 150 seeds for each size class were divided into three sets of 50 seeds. This procedure was used for all species. The pretreated seeds were then germinated on paper media inside petri dishes. Three layers of paper were prepared before seed sowing. They were sprayed with water and kept moist during the entire observation period. The petri dishes were placed in three different temperature-controlled germinators, set for 25, 30 and 35 ^{o}C (constant).

Response variables and parameters

a. Germination Percentage (GP) was evaluated at the end of the first, second and third week. GP was obtained by dividing by the number of germinated seed by the total seeds sown and then multipling by 100%. Only normal germinated seeds were included in the calculation.

b. Germination Speed (GS) (Peak Value). Germination speed used in the analyses was the peak value (PV) of mean daily germination speed (MDG) (Djavanshir and Pourbeik 1976), where

   MDG = Cumulative daily germination divided by the number of days since sowing

c. Germination Value (GV) was calculated by using the following formula (Djavanshir and Pourbeik, 1976) and Czabator (1962):

   $GV = (\Sigma\ DGS / N) \times GP / 10$ where:

   DGS = Daily germination speed
   N = The number of daily count, starting from the date of first germination
   GP = Germination percentage at final day of the test

Statistical analyses

Data were analyzed using a split plot design. Species was the main factor, and temperature and seed size class were the second and the third factors, respectively. The mean seed size was different among the three species. To evaluate the difference between two means, Duncan's Multiple Range Test was used.

Results

Germination percentage

The average of germination for all *Acacia* species tested ranged from 30 to 83.33%. The germination of *A. auriculiformis* and *A. mangium* seeds was better than that of *A. crassicarpa* seeds. The different temperature regimes for seed germination of the three species had similar results. Germination temperatures of 25 and 30 ^{o}C were better than that of 35 ^{o}C for germination of all three species. At the two lower temperatures, seeds tended to germinate faster than at the higher temperature, especially *A. auriculiformis* and *A. mangium* seeds. Based on seed size class, small and medium seeds of *A. auriculiformis* had better germination at 25 and 30 ^{o}C. *A. crassicarpa* and *A. mangium* seeds had higher germination percentages at 25 ^{o}C, regardless of the seed size class.

Analyses of variance for germination percentage showed a significant effect of species and germination temperature (Table 2). Germination percentage of *A. auriculiformis* and *A. mangium* is significantly higher than that of *A. crassicarpa.*

Table 2. F-value for Germination Percentage (GP), Germination Speed (GS), Germination Value of Czabator (GV-CZA)) and Germination Value of Djavanshir and Pourbeik (GV-DJP).

No	Source of Variance	GP	GS	GV-CZA	GV-DJP)	F-table
1	Species (S)	19.29**	54.76**	37.49**	40.59**	4.98
2	Temperature (T)	31.43**	35.83**	34.26**	41.28**	4.98
3	Seed size class (W)	1.92	0.45	0.76	0.66	4.98
4	Replication	0.17	0.95	0.45	0.72	4.98
5	Species x Temperature (SxT)	2.64	3.38	5.16**	5.22**	3.65
6	Species x Seed size class (SxW)	1.53	2.18	1.65	1.82	3.65
7	Temperature x Seed size class (TxW)	1.28	2.53	1.99	1.98	3.65
8	Species x Temp x Seed size class (SxTxW)	2.47	2.36	2.26	1.79	2.82

Note: ** indicates highly significant

Germination speed

The effect of species and temperature on germination speed is significant (Table 2), whereas seed size class has no significant effect on germination speed. *A. auriculiformis* and *A. mangium* have more rapid germination speed than does *A. crassicarpa* at all germination temperatures (Table 3).

Table 3. Duncan's Multiple Range Tests of Germination Percentage (GP), Germination Value of Czabator (GV-CZA) and Germination Value of Djavanshir and Pourbeik (GV-DJP) of three different species in combination with temperature.

Species	Germination temperature			Average
	25 ^{o}C	30 ^{o}C	35 ^{o}C	
A. Auriculiformis				
– Germination percentage	74.89a	79.78a	54.67b	69.78a
– Germination speed	5.60a	6.05a	3.51b	5.03b
– Germination Value (Cza)	20.47a	23.26a	9.62b	17.78b
– Germination Value (Dja.P)	32.10a	35.71a	14.69b	27.5b
A. Crassicarpa				
– Germination percentage	56.67a	52.00a	39.56b	49.41b
– Germination speed	3.05a	2.83a	2.03b	2.64b
– Germination Value (Cza)	8.35a	7.37a	4.17b	6.63b
– Germination Value (Dja.P)	13.65a	11.22a	5.51b	10.13b
A. Mangium				
– Germination percentage	78.89a	63.56a	42.00b	61.48a
– Germination speed	6.22a	5.51a	3.29b	5.00a
– Germination Value (Cza)	23.75a	17.46a	6.96b	16.06a
– Germination Value (Dja. P)	36.65a	26.28a	9.85b	24.26a

Note: The values followed by the same letter indicate a non-significant difference between the two means.

Germination value

Germination value is a cumulative value of germination percentage and germination speed. *A. auriculiformis* and *A. mangium* had better germination speed than did *A. crassicarpa*. The effect of temperature was similar; the best temperatures for germination of all three species were 25 and 30 ^{o}C.

Discussion

The three different species had different germination percentages regardless of temperature regimes and the seed size classes. This was probably a result of initial physiological and genetic quality. Based on earlier records, seed germination of *A. crassicarpa* is mostly lower than other two *Acacias* even when using similar dormancy breaking procedures (Komar 2001). This difficulty with *A. crassicarpa* was also indicated by the germination speed. The germination speed of *A. auriculiformis* and *A. mangium*, regardless of the seed size class, was significantly higher than that of *A. crassicarpa*. Earlier results (Komar 2001) also indicated that the germination percentage of *A. crassicarpa* was lower than that of the other two species. This is probably due to the fact that *A. mangium* and *A. auriculiformis* are genetically closely related, while *A. crassicarpa* is more distantly related.

The optimum temperature for germination of the three species, regardless of the seed size class, was 25 and 30 ^{o}C. There was little variation among seed size classes and species. At 25 and 30 ^{o}C, seeds tended to germinate faster. The interaction between seed size and temperature could be ignored. However, the effect of seed size on germination performance has been observed in many species. Seed weight (seed size) did not cause significant variation in the germination of *A. nilotica* and *A. abbida* (Oboha and Ali 1985). However, it did have a significant effect on the germination, seedling growth, number of leaflets, biomass production and the growth rate in other species. In *Gliricidia sepium*, the effect of size (thickness) on germination was significant (Bumatay and Supino 2001). The larger seeds had higher germination capacity.

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Effect of Treatments and Seed Collection Time on Seed Germination of *Albizia julibrissin* Durazz. Seeds

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Abstract

The seeds of *Albizia julibrissin* Durazz. are dormant because of their hard coat and they need a pretreatment in order to germinate. In this paper the following issues were examined: (a) various treatments for the successful breakage of seed dormancy and their effect on the germination value and (b) the effect of seed collection time on the percentage of germination. The treatments that were applied were: seed soaking in concentrated H_2SO_4, soaking in warm or cold tap water and dry heating of seeds. The treatment that was the most successful in breaking the dormancy was seed soaking in concentrated H_2SO_4 for 2 hours (germination percentage 100.0%). Soaking in warm water also resulted in high germination percentages. When the seeds were soaked in 40^{o}C water for 4 hours the germination percentage was 94.0% and when they were soaked in 50^{o}C water for 3 hours the percentage was 93.0%. Finally, it has been estimated that the later the seeds are collected (between October and March) the deeper the dormancy they acquire.

Keywords: *Albizia julibrissin*, germination, germination value, seed dormancy

Introduction

Fifty *Albizia* species have been identified up to the present (Young and Young 1992). The best-known one is probably *Albizia julibrissin* Durazz., a small tree, that is very popular for its ornamental value. *Albizia* is propagated with seeds that, like most legume seeds, have a hard coat, which contributes to the longevity of the seed (Rice 1989, Fenner, 1993, Pandita et al. 1999 Arianoutsou and Thanos 1996). Seeds that have a hard coat require pre-treatment to germinate to a satisfactory percentage. The practices most commonly used for breaking dormancy by softening the hard seed coat are: (a) mechanical or chemical scarification; (b) dry-heat; and (c) soaking in hot water (Baskin and Baskin 1989, Fenner 1993, Rehman et al. 1999, Thanos and Georghiou 1988, Thanos et al. 1992, Takos et al. 2001, Tsakaldimi and Ganatsas 2001).

To break the dormancy of *A. julibrissin* seeds, Prinsen (1986) recommends soaking them in boiling water (which is allowed to cool gradually) for 24 hours. Yap and Wong (1983) recommend the same method for breaking the *A. facataria* seed dormancy, doubling the time the seeds remain in the water to 48 hours. On the other hand, Bowen and Eusebio (1983) state that the seeds must be soaked in cooler water because seed mortality occurred in water at 100^{o}C temperature. According to Dirr and Heuser (1987), when *A. julibrissin* seeds are soaked in water for 24 hours and then subjected to mechanical scarification, their dormancy is broken and they demonstrate high germination percentages. However, they consider that the best method for breaking the dormancy is the chemical scarification of the seeds for 15-45 min. Likewise, Hartmann et al. (1997) consider that 30 min of seed chemical scarification are enough to break the dormancy. Young and Young (1992) also recommend chemical scarification for 45 minutes for *A. falcata*, *A. chinensis* and A. *ricchardiana*. Finally, Das and Saha (1999) recommend the use of either mechanical scarification with sand paper or chemical scarification through soaking in concentrated H_2SO_4 for 30 minutes to break the dormancy of *A. procera* seeds.

The purpose of the present research was to: (a) break the dormancy of *A. julibrisssin* seeds using techniques for the softening of the seed coat that may develop a different degree of hardness under the conditions prevailing in Greece than in other countries with a different climate; (b) investigate the impact that the exact time of seed collection has on coat hardness and seed germination; and (c) investigate the impact that short-term storage in conjunction with seed collection time has on coat hardness and seed viability.

Materials and methods

A. julibrissin seeds were collected successively at the beginning of each month from October 2000 to March 2001 inclusive. The trees from which seeds were collected were part of a row of trees in the city of Drama in North Greece. The area has an elevation of approximately 90 m above sea level. The first collection of seeds

took place in early October. Immediately after collection, the seeds were subjected to four different types of treatment for the purpose of softening their hard coat to break their dormancy and then their germinability was tested with a germination test, and compared with seeds that had not been treated (controls). The treatments used to break the dormancy were:

(a) Seed soaking in hot tap water, which was allowed to cool gradually in room temperature. The seeds were soaked in water for 1, 2, 3, 4, 5 and 6 hours in water at a constant temperature of 30°C, 40°C, 50°C and 60°C.
(b) Seed soaking in cool tap water. The seeds were soaked in cool tap water for 1, 2, 3, 4, 5 and 6 days. Every two days the water was renewed for the seeds that remained for further durations in the water.
(c) Chemical scarification with concentrated sulfuric acid (H_2SO_4). The seeds were soaked for 15, 30, 60, 90 and 120 min in concentrated (98%) H_2SO_4, and then were thoroughly rinsed with tap water for approximately ten minutes.
(d) Heating in an oven. The seeds were heated in an oven at a constant temperature of 40° C, 50° C, 60° C, 70° C, 80° C, 90° C and 100° C for 10, 20, 30, 40, 50 and 60 min.

Regarding seeds collected from November till March, their germinability was tested in the germinator immediately after collection without any pre-treatment (controls). At the same time, the germinability of seeds subjected to chemical scarification for 120 minutes was also tested. Chemical scarification for 120 minutes was selected because it was the treatment that broke the dormancy of seeds collected in October with complete success and 100% of the seeds germinated. Seeds collected at all collection dates, including those collected in October, were stored under normal, alternating room conditions (25°C day/15°C night) until early March. In the spring, germination tests were performed again on the stored seeds both after pre-treatment (120 min in H_2SO_4) and without pre-treatment (controls).

For every treatment and collection time, four replicates of 100 seeds were placed on moist paper in 10 cm-diameter glass Petri dishes. The dishes were placed in a germinator, together with four replicates of untreated seeds (controls). The temperature in the germinator was set at 25°C for 8 hours with 1000-lux light coming from cold light bulbs and at 20° C for 16 hours in the dark. The seeds were kept moist during the germination test. The germinating seeds were counted once a week for 6 weeks until all viable seeds had germinated (ISTA 1999). The appearance of a 2-mm-long radicle was the criterion for germination (Bonner at al. 1994). The total germination percentage was counted as the average of the four replicates. The germination value was calculated using the equation GV=PV x MDG (Czabator 1962), where GV is the germination value, PV is the peak value, which is calculated as the quotient of the highest value of the cumulative germination percentage divided by the number of days from the beginning of the test, and MDG is the average daily germination.

An ANOVA was carried out to determine the effect of the treatments on the germination percentage as well as the best time of collection. A Duncan's test (p=0.05) was further used for the comparison of the means in the SPSS statistical program (Norusis 1997).

Results

Germination percentages produced by the various treatments on seeds collected in October

The various treatments affected the seeds differently. Seeds that were heated in the oven revealed very low percentages, with germination percentages ranging from 0.0 to 38.0%, lower than the control seeds .On the contrary, the seeds that were exposed to the effect of water (hot or cool) and were chemically scarified displayed high germination percentages that were significant different from control seeds. The final germination percentages of seeds are shown in Table 1. The table does not show the germination percentages displayed by the seeds that were heated in the oven.

The highest germination percentage (100.0%) was achieved after soaking seeds for two hours in concentrated H_2SO_4. Moreover, the germination percentage was very high (92.0%) after the seeds were soaked for 90 minutes in the acid and fairly high (70.0%) after the seeds were soaked for 60 minutes in the acid. Soaking the seeds in the acid for 30 and 15 minutes did not break the dormancy and the germination percentages, 21.0% and 12.5% respectively, were even lower than the germination percentage of the control seeds (32.5%).

Soaking the seeds in hot water (30°C) for 1 hour increased the germination percentage (70.5%) in comparison to the control seeds, but increasing the soaking time (up to 6 hours) did not improve germination percentages, and hardly any of the differences were significant (Table 1). These percentages were lower and differed significantly from the corresponding percentages resulting after soaking seed in 40°C hot water and 50°C hot water.

Table 1. Germination percentages in the various treatments.

Treatment	Duration	Germination (%)
Hot Water 30°C	1h	70.5 ghi*
	2h	69.5 ghi
	3h	70.0 ghi
	4h	67.5 fghi
	5h	62.5 ef
	6h	67.8 fghi
Hot Water 40°C	1h	89.3 lmno
	2h	88.5 lmno
	3h	86.5 lmn
	4h	94.0 op
	5h	89.0 lmno
	6h	89.0 lmno
Hot Water 50°C	1h	85.5 klmn
	2h	83.8 kl
	3h	93.0 no
	4h	89.8 lmno
	5h	82.8 kl
	6h	84.3 kl
Hot Water 60°C	1h	49.5 d
	2h	63.5 efg
	3h	60.3 e
	4h	62.0 ef
	5h	79.5 jk
	6h	74.3 ij
Tap Water	1day	66.8 efghi
	2days	73.0 hi
	3days	22.0 b
	4days	23.0 b
	5days	26.0 b
	6days	27.0 bc
Acid Scarification	15min	12.5 a
	30min	21.0 b
	60min	70.0 ghi
	90min	92.0 mno
	120min	100.0 p
Control		32.5 c

*Percentages followed by the same letter do not differ significantly.

Germination percentages after soaking the seeds in 40°C hot water for 1 to 6 hours were very high and did not differ significantly from each other and from the germination percentage produced after soaking in acid for 90 minutes. The germination percentage achieved for a soaking time of 4 hours (94.0%) was an exception and was the only one that did not differ significantly from the percentage produced by the completely successful treatment (120 min in H_2SO_4). Soaking the seeds in 50° C hot water also produced very high germination percentages. At this temperature, hardly any germination percentages showed any significant difference from each other or from the corresponding germination percentage achieved at 40° C. The highest germination percentages at 50° C were attained after three and four hours of soaking in water (93.0% and 89.8%, respectively) and did not differ significantly from the percentage attained through soaking the seeds in acid for 90 minutes. Lower percentages were achieved through soaking in 60° C water and ranged from 49.5% to 79.5%. The only worthwhile percentage was the one attained when the seeds remained in the water for five hours (79.5%); this percentage did not differ significantly from the ones attained through soaking in 50°C hot water for 1, 2, 5 and 6 hours.

The germination percentages obtained after soaking the seeds in cool tap water for 1 to 6 days were for the most part lower than the percentages of the other treatments. The highest percentage (73.0%) was found in seeds that remained in the water for two days and there was no significant difference from the germination percentages achieved through soaking in 30° C hot water and acid (60 min). The germination percentages of the seeds that remained in water for 3, 4, 5 and 6 days were lower (22.0% to 27.0%) and differed even from the percentage of the control seeds (32.5%) significantly.

Germination Value

Table 2. Germination Value.

Treatment	Germination value*
Soaking in hot water of 40 °C temperature for 1h	51.25 ef
Soaking in hot water of 40 °C temperature for 2h	48.25 e
Soaking in hot water of 40 °C temperature for 4h	11.00 b
Soaking in hot water of 40 °C temperature for 5h	18.69 c
Soaking in hot water of 40 °C temperature for 6h	16.95 c
Soaking in hot water of 50 °C temperature for 3h	38.55 d
Soaking in hot water of 50 °C temperature for 4h	14.92 bc
Acid scarification for 90min	53.75 f
Acid scarification for 120min	101.53 g

*Percentages followed by the same letter do not differ significantly

The Germination Value was calculated only for treatments that gave high germination percentages (>88.0%) while manifesting insignificant differences from each other, as shown in detail in Table 1. As shown in table 2, the highest germination value occurred after chemical scarification for 120 minutes, while the lowest **one** occurred after soaking in 40°C hot water for 4 hours. The germination value obtained after chemical scarification for 120 minutes is much higher than all the other values as 99.5% of the seeds germinated in the first week (Table 3). Seeds scarified in acid for 90 minutes germinated in the first week to a percentage of 85.0%, seeds soaked in 40°C water for 4, 5 and 6 hours germinated in the first week to a percentage of 25.0%, 61.8% and 56.0% respectively and, finally, seeds soaked in 50°C water for 3 and 4 hours germinated in the first week to a percentage of 81.3% and 40.8% respectively. Seeds treated as shown in Table 2 completed the overall germination percentage in a longer period of time than the ones that underwent chemical scarification for 120 minutes, as shown in table 3.

Table 3. Germination rate for the various treatments.

Treatment	Week					
	1	2	3	4	5	6
	Germination percentage					
Acid scarification for 90min	85.0	5.0	2.0	0.0	0.0	0.0
Acid scarification for 120min	99.5	0.5	0.0	0.0	0.0	0.0
Soaking in hot water of 40°C for 1 h	85.0	3.0	1.3	0.0	0.0	0.0
Soaking in hot water of 40°C for 2 h	80.0	5.0	3.5	0.0	0.0	0.0
Soaking in hot water of 40°C for 4 h	43.5	25.0	20.0	3.8	1.0	1.0
Soaking in hot water of 40°C for 5 h	61.8	15.0	4.5	5.5	1.3	1.0
Soaking in hot water of 40°C for 6 h	56.0	15.0	8.0	6.0	2.0	2.0
Soaking in hot water of 50°C for 3 h	81.3	6.5	3.3	2.0	0.0	0.0
Soaking in hot water of 50°C for 4 h	40.8	25.0	15.0	6.0	3.0	0.0
Control	1.5	24.5	2.0	2.0	1.5	1.0

Other collections

Table 4 shows the germination percentages resulting when seeds were germinated immediately after their collection, and includes both the seeds that did not undergo any treatment (control seeds) and the ones that underwent chemical scarification in relation to the various collection dates (from October till March inclusive).

Table 4. Germination percentages occurring immediately after seed collection.

Month of seed collection	Untreated seeds (Controls)	Chemically scarified seeds (120 min in H_2SO_4)
	Germination (%)*	
Oct	32.5a	100
Nov	5.0bc	100
Dec	6.0bc	100
Jan	5.5 bc	100
Feb	5.0bc	100
March	2.0c	100

*Percentages followed by the same letter do not differ significantly.

Germination percentages of seeds not subjected to treatment (controls) ranged from 32.5% (October collection) to 2.0% (March collection) (Table 4). The germination percentage of the October control seeds was much higher than the germination percentages of the control seeds of other months with a statistically significant difference even from the November percentage. On the contrary, the seeds scarified in H_2SO_4 for 120 minutes achieved the absolute germination percentage (100.0%), which demonstrates that this treatment breaks dormancy with complete success even when the seeds remain on the tree longer so their coat hardens and therefore dormancy becomes deeper.

The germination percentages estimated in the spring for stored seeds of all collections are shown in Table 5. Germination tests were performed on seeds without treatment (controls) and on seeds scarified in H_2SO_4 for 120 minutes.

Table 5 shows that there is a gradual reduction in the germination percentage of seeds not subjected to treatment,

Table 5. Germination percentage of stored seeds in the various collection times.

Month of seed collection	Untreated seeds (Controls)	Chemically scarified seeds (120 min in H_2SO_4)
	Germination (%)*	
Oct	10.3a	100
Nov	7.5b	100
Dec	6.0b	100
Jan	7.0b	100
Feb	3.0c	100
March	4.0c	100

*Percentages followed by the same letter do not differ significantly.

depending on the seed collection time. The earlier the collection takes place, the softer the coat is, which results in more seeds germinating. Therefore, the germination percentage of seeds collected in October and stored until early March was significantly higher (10.3%) than the corresponding percentages displayed by stored seeds of the remaining collections (7.3% to 3.0%). On the contrary, the germination percentage of the chemically scarified stored seed was 100% for all collections. Therefore, whatever the extent of seed coat hardness and the extent of dormancy it can be broken with H_2SO_4.

Discussion

It is a well-known fact that the existence of a hard coat acts as an obstacle against water imbibition and therefore against seed germination (Baskin and Baskin 1989, Fenner 1993, Rehman et al. 1999). *Albizia julibrissin* seeds belong to this group of seed because, as this research has demonstrated, chemical scarification and soaking in warm water softens the seed coat and enables the seeds to germinate successfully to a high percentage unlike the seeds that do not undergo a treatment to soften their coat. The method of seed dry-heating was unsuccessful although it is a well-known method for the softening of hard seeds and it is considered suitable for breaking the dormancy that is due to seed coat hardness. The most successful treatment was chemical scarification for 120 minutes because it broke dormancy completely (seed germination percentage equal to 100.0%). However, this outcome contradicts other researchers' work. Dirr and Heuser (1987) and Hartman et al. (1997) recommend a shorter time of treatment (30 to 45 minutes) to break the dormancy. On the contrary, in this research, chemical scarification for 30 minutes resulted in a germination percentage that was lower even than the percentage of the controls. Nevertheless, chemical scarification resulted in faster germination in all cases, as stated also by Babeley et al. (1986).

Soaking seed in hot water also proved an exceptionally successful method for the breaking of dormancy. Seed germination after water soaking was significantly higher than that of the controls. In particular, the water temperatures of 40^{o}C and 50^{o}C had an impact similar to the one of chemical scarification for 90 and 120 minutes. However, in this research, the germination percentage was maximized by soaking seed in water temperatures lower than the boiling water recommended by Prinsen (1986) and Yap and Wong (1983).

Germinability after soaking in cool water for 1 and 2 days was considerably greater than the germination of the controls, but was lower than the germination of the chemical scarification and hot water soaked seed. However, after soaking seed in water for more than 2 days, germinability decreased gradually as seed soaking time increased. Dry heating gave disappointingly low germination percentages. Given that all possible temperatures up to 100^{o}C were used for the time periods ranging from 10 to 60 minutes, new research must be carried out in which temperatures will be increased with decreasing exposure times or, exposure times will be decreased with temperatures remaining the same as the ones used in this research. Nevertheless, germination percentages equal to zero were observed after exposure to a temperature of 100^{o}C. Therefore, it is very likely that seed mortality occurs at this temperature, as already stated by Bowen and Eusebio (1983). The control seeds collected in November, December, January, February and March had very low germinability percentages. Both the seeds that were stored and induced to germinate in March, and the seeds induced to germinate immediately after their collection displayed significantly lower germination percentages than the corresponding ones of the October collection. This means that the seed hardens gradually and this lowers germinability. Storage under normal room conditions had no impact on the germination percentage as the seeds stored had a germination percentage almost equal to that of seeds induced to germination after collection. An exception to this was the lot of seeds collected in October, which showed a considerably lower germination percentage after storage. However, chemical scarification was a highly successful treatment for the breakage of dormancy in all cases mentioned above and gave germination percentages equal to 100%.

Conclusions

The dormancy of *Albizia julibrissin* seeds is successfully broken after chemical scarification for 120 minutes. Moreover, this treatment results in an exceptionally fast germination. However, dormancy is broken with equal success by soaking the seeds in 40°C water for 4 hours or 50°C water for 3 hours. The percentage germination obtained after soaking in tap water for 2 days are also satisfactory. It has to be pointed out that a treatment such as soaking in hot water is a practical, safe and easy method, especially for the treatment of large quantities of seeds containing an indeterminate percentage of dormant and non-dormant seeds. In such a case, even soaking in tap water for two days may be considered a satisfactory treatment, despite the fact that it gives a lower germination percentage.

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Seeds as Indicators of Genetic and Reproductive Status in Red Spruce and Eastern White Pine

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Abstract

Tree seeds provide a useful source of information for assessing the genetic processes and evolutionary forces that determine the reproductive and vegetative fitness of individuals and populations. Our aim was to use seed analysis to characterize the relationships of stand traits such as age, height, and stem diameter, with genetic and reproductive traits reflecting the status of natural populations of red spruce (*Picea rubens*) and eastern white pine (*Pinus strobus*) across their respective geographic ranges in eastern Canada. In eastern white pine, multi-locus outcrossing rates were significantly correlated (i) negatively with the average distance to its five nearest neighbours (a surrogate measure for within-stand density of mature stems, and (ii) positively with the proportion of filled seeds per cone. Filial seed progeny fixation index was positively correlated with both (i) average nearest neighbour distance, and (ii) proportion of empty seeds per cone. Therefore, strong relationships were detected between stand density and both outcrossing rates and filled seed production. In red spruce, we wanted to test the idea that old-growth forests serve as important reservoirs of genetic diversity. The negative relationship between average tree height and percent monomorphic loci; and the strong positive correlations between tree height and (i) percentage of polymorphic loci, (ii) measures of allelic richness, and (iii) observed heterozygosity, indicates strong relationships between growth performance (a fitness trait) and measures of genetic diversity in red spruce. The negative relationship between height growth and the proportion of rare alleles suggests that these rare alleles may be deleterious to growth performance; whereas latent genetic potential showed a significant and positive relationship with height. Age was not correlated to average stand (population) height but age was correlated to seedling progeny height growth. In late-successional species such as red spruce, age and size (e.g., height and stem diameter) relationships may be strongly influenced by local stand disturbance dynamics that determine light availability, growing space, etc. In larger, older stands, age appeared to provide a good surrogate measure or indicator for genetic diversity and progeny growth performance. However, in smaller, more isolated populations, these age and fitness relationships may be strongly influenced by the effects of inbreeding depression and genetic drift. Therefore, older populations or old-growth forests can represent superior seed sources, but only if they are also of sufficient size and density to avoid the deleterious effects of inbreeding and genetic drift. Thus, larger and older forests appear to have an important evolutionary role as reservoirs of both genetic diversity and reproductive fitness. Under the rapid environmental changes anticipated as a result of climate change, increasing population isolation through fragmentation, or following the introduction of exotic pests and diseases, these older populations of trees may function in maintaining the adaptive potential of tree species.

Keywords: conservation, genetic diversity, inbreeding, old-growth forests, reproductive fitness

ASP52: A Large LEA-like Protein in the Cell Walls of the Seeds of the Camel Thorn Tree, *Acacia erioloba*, Protects Proteins against Stress-induced Conformational Changes

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Abstract

The objective of this study was to investigate whether a desert tree seed contained (a) novel LEA-like protein(s) and, if so, to determine the location and the biological role.
A 52 kDa hydrophilic protein, Asp 52, with LEA-like properties was isolated from the mature seeds of *A. erioloba*. Immunocytochemical analysis showed that this protein was present in the cell walls and in decreasing quantities in the cell walls of germinated seeds. This decline in Asp 52 content was confirmed by SDS-PAGE and could be correlated with loss of desiccation tolerance of the seedlings. Asp 52 was shown to exhibit some interesting biochemical properties in that it prevented the denaturation of and aggregation of thermally denatured proteins and prevented the loss of enzymatic activity of alcohol dehydrogenase. Asp 52 was also shown to stabilise a model membrane system, liposomes, against desiccation-induced damage in a manner analogous to trehalose and the yeast stress response protein Hsp 12.
We therefore conclude that a unique LEA-like protein has a significant biological role in these seeds. The protein sequence is still to be determined.

Physiological Characteristics of Dormant and Germinating Horse Chestnut Seeds

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Abstract

Aesculus hippocastanum L. (horse chestnut) seeds are recalcitrant seeds exhibiting a deep dormancy, which is released during wet, cold stratification. Water content in the axes of dormant seeds is about 62-64% (fr wt), whereas germination starts at a moisture content of 73-74%. Seed dormancy is under hormonal control. It is maintained by ABA and broken only by cytokinins, both natural and synthetic. Endogenous ABA peaks in the embryo axes in the middle of the stratification period when embryo dormancy declines, thus decelerating precocious dormancy release. Endogenous cytokinins decrease at embryo dormancy. Germination of horse chestnut seeds is controlled by the ability of the axis to absorb water and increase the water content up to threshold levels triggering (1) accumulation of osmotica, mainly sugars and potassium ions, and (2) acidification of cell walls due to activation of H^+-ATPase in plasmalemma. Both result in initiation of cell elongation. The hypogeal germination of horse chestnut seeds occurs only by cell elongation, first in the hypocotyl and later in the root, up to the axis length of 3 cm.

Keywords: *Aesculus hippocastanum*, dormancy, germination, hormonal regulation, seeds

Introduction

Aesculus hippocastanum L. (horse chestnut) trees, usually growing in temperate and subtropical zones, were successfully introduced and acclimated for landscape and town gardening in the middle of Russia. The seeds may be classified as recalcitrant, surviving after seed fall only under wet conditions. In general, recalcitrant seeds are characterized by high water content; drying or water loss during winter is fatal for them (Walters 1999). In mid-Russia, with its rather cold winter temperatures, the seed population of *A. hippocastanum* requires cold wet stratification during dormancy for survival and subsequent germination.

We have studied the embryo axes of horse chestnut seeds during dormancy and germination by measuring water content, sensitivity to applied phytohormones, levels of endogenous phytohormones, content of major endogenous osmotica, acidification of cell walls, rate of radicle protrusion, and initiation of growth activity.

Materials and Methods

Freshly-fallen seeds were collected at the very beginning of October during 1996 – 2001 and then kept in moistened sand at 4^0C. Water content in the embryo axes was regularly measured by weighing the samples. At weekly intervals, the seeds were tested for germinability by supplying them with water and incubating them in enameled trays in the dark at 27^0C. Germinability was estimated daily by counting seeds with protruded radicles. At the same time, the seeds were tested for sensitivity to the phytohormones. These experiments were performed with both intact and partially-decoated (above the embryo axes) seeds.

Samples of the axes were regularly frozen in liquid nitrogen for the analyses of hormones. Cytokinins and ABA levels were measured by ELISA in the methanolic extracts purified by using a column with polyvinylpolypyrrolidone (Sigma, USA) in series with an Amprep C18 column (Amersham, UK) (Kotov and Kotova 2000). Starch was measured after acid hydrolysis by the anthrone method. In the cell sap from unfrozen axes, sucrose and fructose were analyzed according to Roe *et al.*; glucose was determined with a GLU 160E kit (Czech Republic) and the K^+ content was estimated by flame photometry. Acidification of the 0.1 M KCl medium around the axes was measured with a pH-meter. The cellular analysis of growth was performed by counting the number of cells and measuring the cell lengths along longitudinal sections of hypocotyl and radicle.

Results

Horse chestnut seeds exhibit deep dormancy, followed by dormancy release, and acquire germinability. This results in early germination and growth of seedling organs. Freshly-fallen mature seeds from various populations collected under mild (Magdeburg, Germany) or more severe (Mogilev, Belarus and Moscow, Russia) climatic

conditions germinated under favourable conditions for 1-2 months, thus indicating the state of deep dormancy. The results below were obtained with seeds collected in Mogilev and Moscow dendroparks in the years 1996-2001.

The time-course of the events in dormant seeds subjected to cold, wet stratification is shown in Fig. 1. Under favourable water supply and temperature conditions, the rate of germination and germination percentage increased as the stratification time lengthened. In partially-decoated seeds with facilitated water inflow, germination occurred more rapidly (Fig. 1B) than in the intact seeds (Fig. 1A), but the general trend was similar. Three time periods can be distinguished, namely (a) deep dormancy or dormancy maintenance (0-5 weeks) when the seeds germinate slowly and germination percentage gradually rises almost to 100 %; (b) dormancy release (6-16 weeks) characterized by gradual acceleration of germination in all seeds, and (c) rapid (1-2 days) germination of all seeds (16-19 weeks) under favourable conditions and resulting in total germination of seeds kept at 4^0C (from week 18 on).

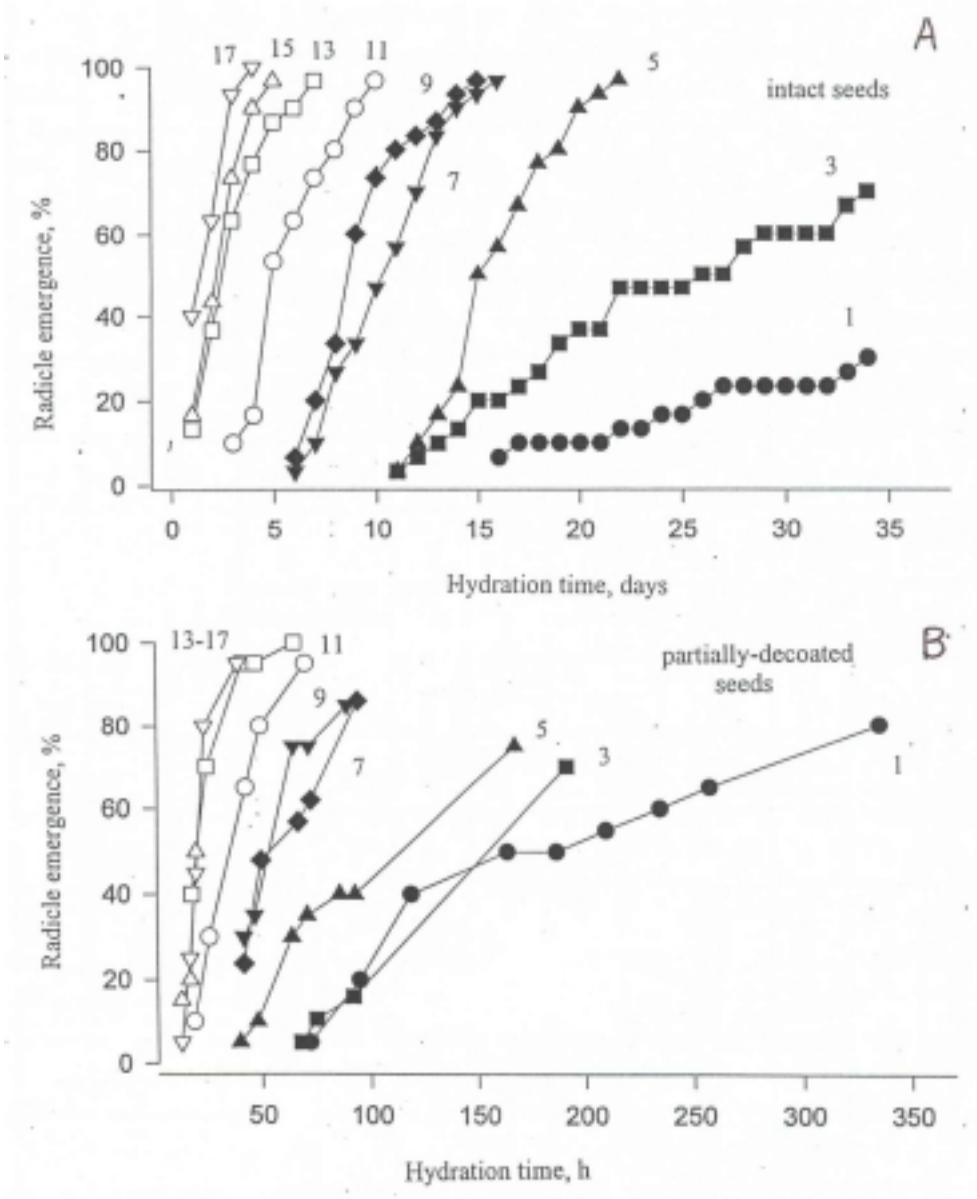

Figure 1. Germinability of dormant horse chestnut seeds. Numbers of the curves indicate the weeks of stratification.

These results also indicate that dormancy maintenance and release reflect various proportions of embryo and coat-imposed dormancy. We estimated the behaviour of intact and partially-decoated seeds in terms of average time by which radicle emergence occurred in 50% of seeds under favourable germination conditions. The time-

course of these values during stratification (Fig. 2) has shown that the inclination angle of curve 1 increased from week 6, thus indicating the deceleration of dormancy loss and transition from maintenance to release. Dormancy release was completed by week 16. The difference between curves 1 and 2 represents the extension of coat-imposed dormancy declining until week 16. Below curve 2, the extension of embryo dormancy is outlined. By week 11, embryo dormancy terminated. The non-dormant germinating seeds need only 19-21 h for the germination of 50% of them (short length below the scheme). Therefore, coat-imposed dormancy mostly determined the dormant state in horse chestnut seeds. Embryo dormancy is a weaker contributor, operating only over 10 weeks.

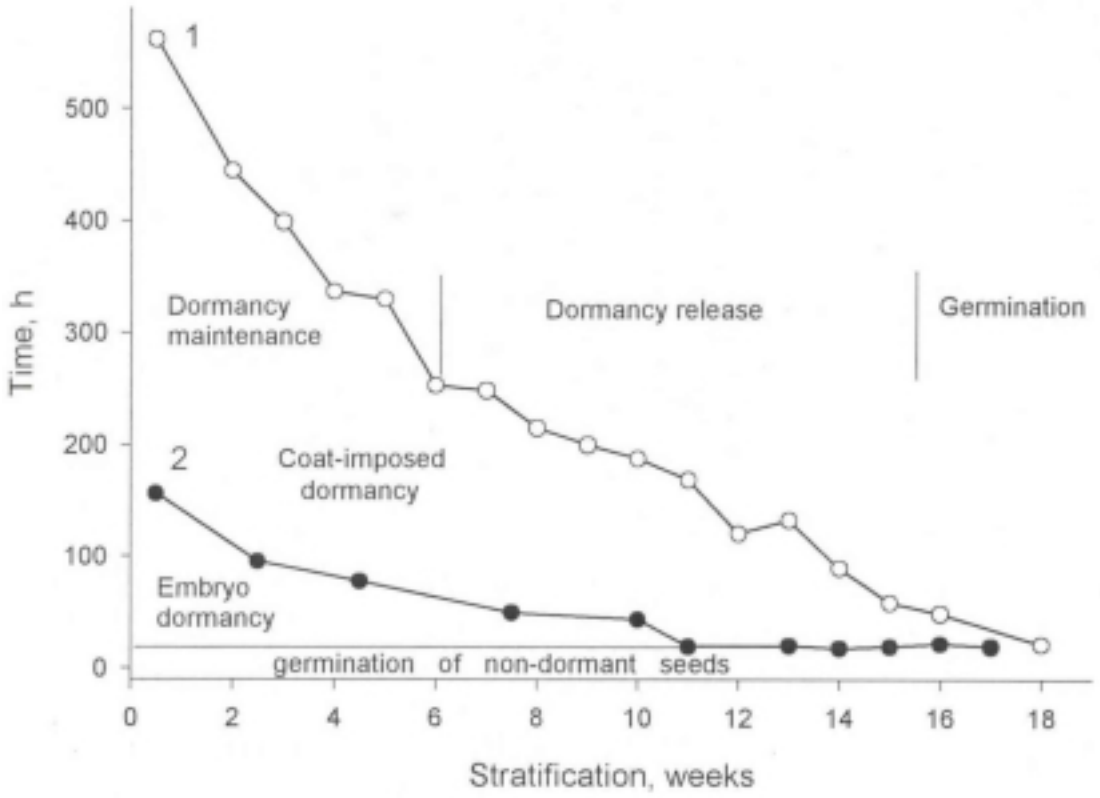

Figure 2. Contribution of embryo and coat-imposed dormancy to dormant state of horse chestnut seeds (expressed as time of radicle emergence in 50% intact (1) and partially decoated (2) seeds.

Dormant seeds are sensitive to ABA (Table 1). The inhibitory effect of 10^{-4} M ABA was retained up to the end of stratification time and indicated the presence of ABA receptors in the embryo axes. Despite their presence,

Table 1. Effect of ABA on germinability of partially-decoated dormant horse chestnut seeds.

Stratification Time (weeks)	Time of radicle emergence in 50% seeds, h			
	H_2O	ABA, 10^{-6}M	ABA, 10^{-5}M	ABA, 10^{-4}M
1	156	> 200	> 380	> 400
3	95	190	> 200	> 200
5	77	100	170	> 200
8	50	65	96	190
10	43	40	96	190
12	35	-	90	>140
15	19	22	62	70
17	21	24	30	50
19	19	20	20	28

10^{-5} M ABA did not inhibit the germination of non-dormant seeds, whereas 10^{-6} M ABA exerted no effect after week 8. Apparently, the embryo axes of dormant seeds developed the capacity of ABA cobjugation during dormancy, resulting in complete inactivation of 10^{-6} M ABA after week 8 and of 10^{-5} M ABA after week 17.

The content of endogenous ABA in seed axes (Fig. 3B) peaked at weeks 7-11, when embryo dormancy decline was discontinued for some weeks (Fig. 2). Apparently this rise in free ABA level occurring by deconjugation of preformed bound ABA prevented the too early termination of embryo dormancy, thus providing prolongation of seed dormancy.

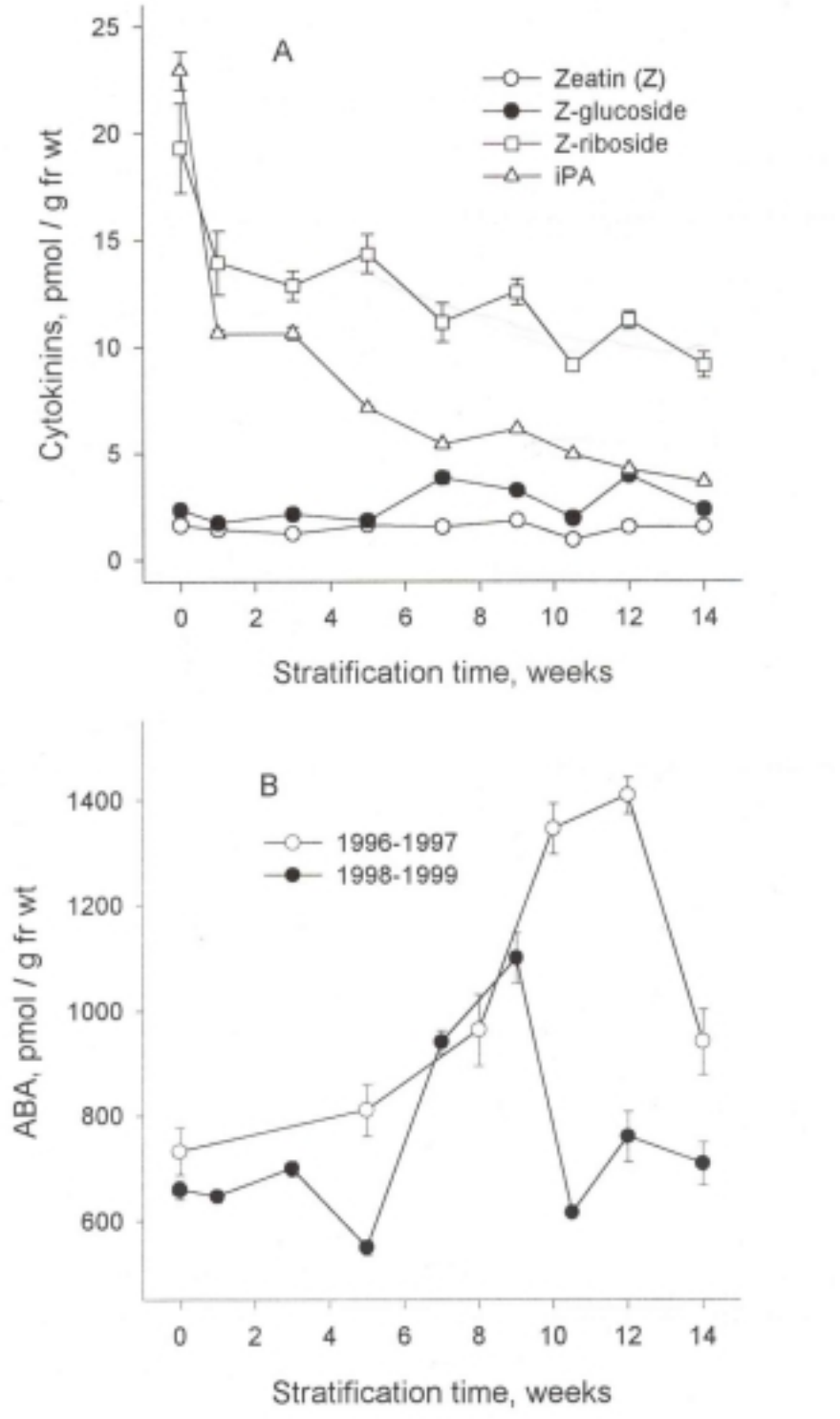

Figure 3. Endogenous cytokinins (A) and ABA (B) in the embryo axes of dormant horse chestnut seeds.

The dormancy of horse chestnut seeds can be broken only by cytokinins, both synthetic (benzylaminopurine and its riboside) and natural (isopentenyladenine and dihydrozeatin). Table 2 shows that all cytokinins accelerate visible germination of partially-decoated seeds. The effect of natural cytokinins is limited to the time of embryo dormancy; no acceleration occurs after week 10. The content of zeatin riboside and especially of isopentenyladenine in seed axes (Fig. 3A) gradually decreased during stratification while the levels of free zeatin and its glucoside remained unchanged. Therefore, in the horse chestnut seeds, dormancy maintenance and release are hormone-controlled events; ABA prolongs the dormancy state and prevents too early a loss of dormancy whereas cytokinins promote dormancy loss.

Table 2. Effect of cytokinins on germinability of partially-decoated dormant horse chestnut seeds.

Stratification time, weeks	Time of radicle emergence in 50 % seeds, h			
	H_2O	BAP ($2x10^{-4}$ M)	DHZ (10^{-5} M)	iPA (10^{-5} M)
0	143	86	34	30
2	100	75	40	30
4	65	65	32	25
6	43	45	30	22
8	44	45	28	22
10	36	40	19	21
11	19	30	22	20
13	20	30	20	19

However, the seeds that have already lost their dormancy were unable to germinate if they were unable to take up more water. The water content in the embryo axes was maintained at the level of 62-64% fr wt and gradually rose to 66-67% by the end of dormancy. We exposed such seeds to 30% polyethylene glycol (m.w. 6000) solution, which allowed no further water inflow (Fig. 4). These seeds were unable to germinate. When this solution was replaced with water, the axes elevated their water content up to 74% fr wt, i.e. the level at which growth of axial cells started, and radicle emergence occurred.

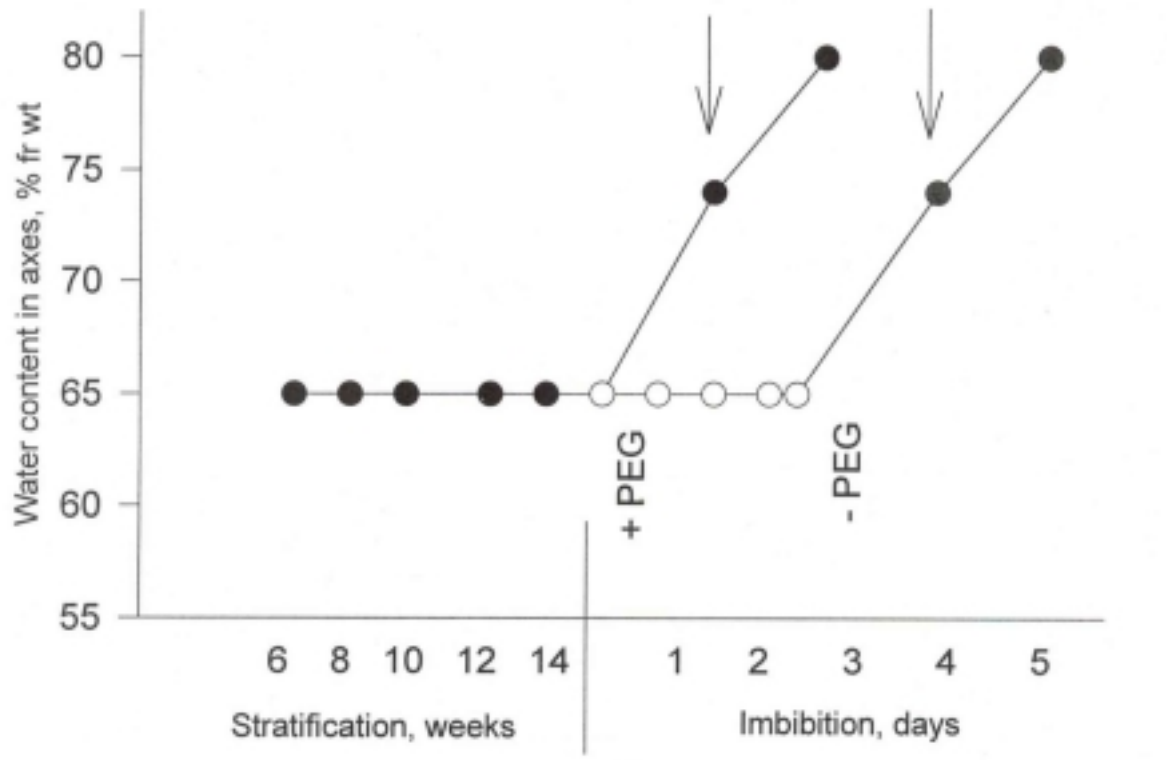

Figure 4. Delayed germination of non-dormant horse chestnut seeds treated with polyethyleneglycol (PEG-6000). Arrows indicate visible germination.

In the horse chestnut seeds, growth initiates by cell elongation only. The mitoses appear in the radicle tip when the axis (hypocotyl and radicle) is about 3-3.5 cm long (Obroucheva 1999). Cell elongation begins in the

hypocotyl, which extends up to almost 2 cm, and then in basal radicle cells. Prior to elongation commencement, some processes preparing its initiation begin to operate in the axes. Firstly, the endogenous osmotica accumulated (Fig. 5), namely glucose and fructose (at the expense of sucrose) as well as K^+ ions. Secondly, the acidification of cell walls in embryo axes (measured as Δ pH of the ambient solution) started (Obroucheva and Antipova, 2000) leading to cell wall loosening. Taken together, these processes provide additional water inflow, cell vacuolation, and elongation. Thus, early germination *per se* is a water-driven event.

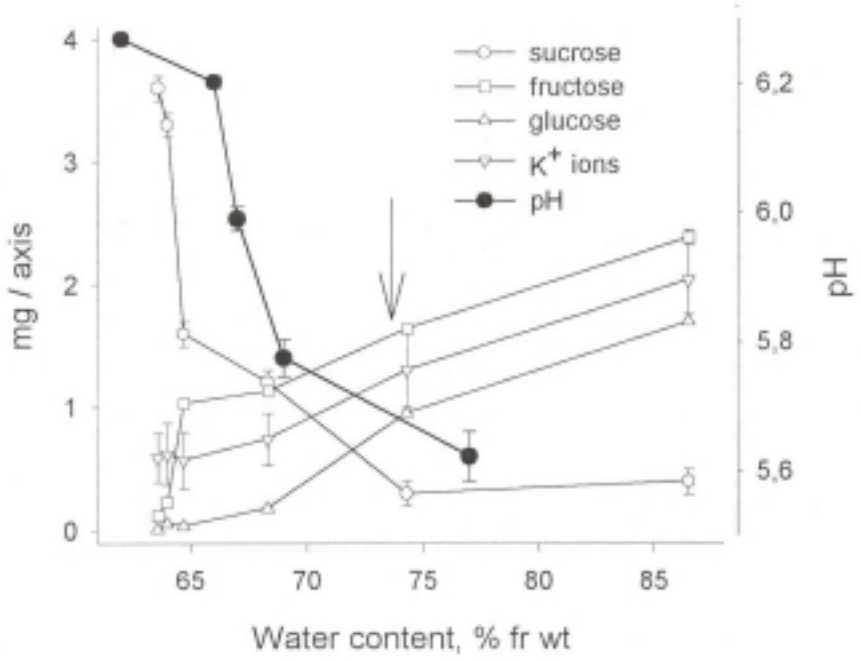

Figure 5. Accumulation of osmotic solutes and acidification of ambient solution in the embryo axes of imbibing horse chestnut seeds. The arrow indicates radicle emergence of 50% seeds.

Discussion

Although germination follows seed dormancy, the nature and regulation of these events differ. Seed dormancy is aimed at the protection of the mature embryo and its survival under unfavorable conditions, whereas germination focuses on rapid initial extension of embryo organs, primarily to provide sufficient water supply and penetration to the soil surface for light.

Dormancy is maintained by ABA and relieved by cytokinins in the horse chestnut seeds; cytokinins are not a common dormancy-breaking hormone. Hence, the dormant state is controlled by two hormones, whereas initial germination is regulated only by hydration. Subsequent organ growth is closely related to *de novo* phytohormone production by seedlings (Obroucheva 1999).

The initial germination of horse chestnut seeds, as in other non-dormant seeds, is triggered by entering water (Obroucheva and Antipova 2000). It results from accumulation of osmotic solutes and acidification of cell walls resulting in further water inflow necessary for the initiation of cell elongation in embryo axes. The unique property of horse chestnut seeds is germination only by cell elongation, providing rapid growth.

Conclusions

The recalcitrant nature of horse chestnut seeds manifests itself in high water content of seed axial organs and deep dormancy, the main component of which is coat-imposed dormancy. The latter prevents water loss by seeds and ensures seed survival at dormancy. The insufficient seed coat permeability to water continues to delay the germination even after termination of embryo dormancy and determines the end of the dormant state, permitting rapid seed hydration leading to germination.

Acknowledgment

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Coated Scots Pine Seeds Makes Autumn Direct Seeding Possible

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Abstract

Scots pine (*Pinus sylvestris* L.) seeds are unsuitable for autumn direct seeding as they readily permit the entry of moisture which causes seed mortality due to a low tolerance for freezing temperatures at high moisture contents. In the present study it was hypothesized that if the moisture content of Scots pine seeds could be kept under a critical level during the winter months with low freezing temperatures, seed germination the following spring could be accomplished. To ensure different levels of water uptake delay, experiments were conducted with different thickness (2.0, 5.0, and 20.0% Ethocel™ (ethyl cellulose) coated on Scots pine seeds. Moisture content before snowfall and seedling emergence were analyzed in field experiments at two sites during 1999-2000 (uncoated and 2.0% weight gain) and at three sites during 2000-2001(uncoated and 2.0, 5.0 and 20.0% weight gain) in northern Sweden. The analyzed factors were site, coating thickness, seedbed treatment (whether microsite preparation or not) and seedtime (autumn and spring). Coated seeds had lower moisture content (23.4%) before snowfall and higher seedling emergence (19.0%) than uncoated seeds (37.8% and 1.0% respectively), regardless of site or seedbed preparation. Samples on the three dryer and mesic sites had lower moisture content (21.8%) before snowfall and higher seedling emergence (23.0%) compared to the two moist sites (39.8% and 0.4% respectively). On the average, moisture contents and seedling emergence of seedbed treated plots were higher (39.8%, 23%) than non-seedbed treated plots (21.8%, 0.9%). Autumn direct seeded sites had a 10% increase in seedling emergence compared to spring. The coating thickness with the best seedling emergence (19.8%) on the average for all sites after autumn seeding, was 2.0% weight gain. On the three typical seeding sites this coating thickness entailed a seeding emergence of 33.7%, 30.0% and 14.1% after autumn seeding while the control values were only 12.3%, 0.0% and 3.2%, respectively. In conclusion, Ethocel™ (ethyl cellulose) reduced water uptake in Scots pine seeds, which allowed for an increased seedling emergence after autumn direct seeding. The results support the hypothesis that seed moisture content during the winter months is a determining factor for seedling emergence after autumn direct seeding of Scots pine.

Keywords: Direct seeding, moisture content, Scots pine, seed coating, seedling emergence, water uptake

Cone - Seed Biometry and Germination Ecophysiology in *Pinus nigra* from Several Greek Provenances

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Introduction

The European black pine (*Pinus nigra* Arn.) is a native species in Europe, Africa and Asia. Its range is circum-Mediterranean and extends from Spain and Morocco to Cyprus, eastern Turkey and Crimea. Since it is a very widespread species, its ecotypic variation has led taxonomists to recognize and classify the species using subspecies and varieties, which often overlap and create confusion. Our study is focused on the different populations of *Pinus nigra* in Greece which all belong to the subspecies *pallasiana* (Strid 1989). The variations among the populations are studied on two levels: morphological and ecophysiological.

Materials and Methods

In order to perform this study pine cones were collected from 9 different indigenous populations all over Greece. All populations were growing at a high altitude (over 1000 m) and they were isolated from other populations, constituting 'virtual island, mountainous refugia'.

Figure 1. Location of the *Pinus nigra* populations used as cone and seed sources for the present study (numbers correspond to the populations described in detail in Table 1).

A total of about 100 cones of each population were collected and transferred to the laboratory. 40 of the cones had their length, width and weight measured a day after their collection. All of the contained seeds for each of the 100 cones were extracted (by mechanical removal of the scales). Then morphometric parameters of the seeds were measured (seed length, seed width, seed wing morphology and biometry, seed weight, seed coat contribution, sound seed percentage etc), and the seeds of the extra cones of each area were germinated under a broad range of constant temperatures, in the dark or under white light.

All cones of *Pinus nigra* were randomly selected among mature trees of each population. No more than 2 cones

were collected from a single tree. All cones were apparently healthy and well developed. Within a day from their collection, they were transferred to the laboratory. All cone size-related variables (length, width and weight) were measured within a short period thereafter. Subsequently, all seeds of each cone were extracted and an additional number of seed-related variables were measured (length, width, wing morphology and biometry, seed weight, seed coat contribution, sound seed percentage etc). Finally, the seeds were stored at laboratory conditions until used in germination experiments.

Table 1. Maturation year, altitude and location of each population sampled for its cones and seeds.

Site Number	Maturation year	Altitude (m)	Area	Mountain
1	2001	840	Dadia	Sapka
2	2001	1490	Metsovo	Hasia
3	2001	1035	Parnassos	Parnassos
4	2001	1095	Euboea	Ksiro
5	2001	1115	Derveni	Mavro
6	2001	1250	Killini	Killini
7	2001	1430	Taygetos	Taygetos
8	2001	1233	Parnonas	Parnonas
9	2001	1276	Samos Island	Karvounis
10	2000	1000	Thasos Island	Ipsarion
11	2000	na	Kastoria	Vernon
12	1999	210	Elassona	Olympus
13	1999	na	Sperxeiada	Oiti
14	2001	650	Pyrgos	Foloi

Germination experiments took place in controlled chambers (temperature and light conditions) in temperatures ranging from 5 ºC to 25 ºC. The experiments at 5 ºC were conducted in a cold chamber (Sanyo Medicool), while those at 10 –25 ºC in germination cabinets (Model BK 5060 EL, W.C. Heraeus, Hanau, Germany). A programmable bench of alternating light and dark conditions was also used (Model GB48, Conviron, Controlled Environments, Winnipeg, Canada) for the simulation of average mid April conditions (Athens Airport Meteorological Station). All the measurements with seeds imbibing in the dark were carried out under a dim, green safe light.

Additional germination experiments were conducted with seeds provided by the Reforestation Division of the Greek Ministry of Agriculture.

Results and Discussion

The measurements revealed differences among the populations sampled in all the morphometric characteristics Figures 2 and 3). These differences spanned to several levels: differences among populations, among individuals in a population, among cones within an individual tree and even within the cone (among seeds).

To determine the significance of these differences we used a statistical approach (nested Anova), which revealed statistically important differences in the morphometric characteristics of some populations (mainly populations 1 and 4) and the majority of the samples tested.

Figure 2 summarizes the results on cone measurement for all the population sampled. Although there exist some differences among the values of cone weight, the differences among the respective values in cone length and width are comparatively minor. Most importantly the ratio cone length / cone width seems to remain quite constant among the different populations.

The seed characteristics differ much more among the populations tested, than those of the cones. As shown in Figure 3 the average number of seeds contained in each cone as well as the average seed weight for each individual population are quite different among the populations tested.

The low number of seeds in population 2 is due to the fact that the cones were collected slightly open (thus a portion of seeds might have already been dispersed) but mostly because of the high degree of insect infection, which greatly diminished the number of sound seeds extracted per cone. For populations 10-14, no standard error bars are given for seed weight, since the seeds were weighted in groups of 20.

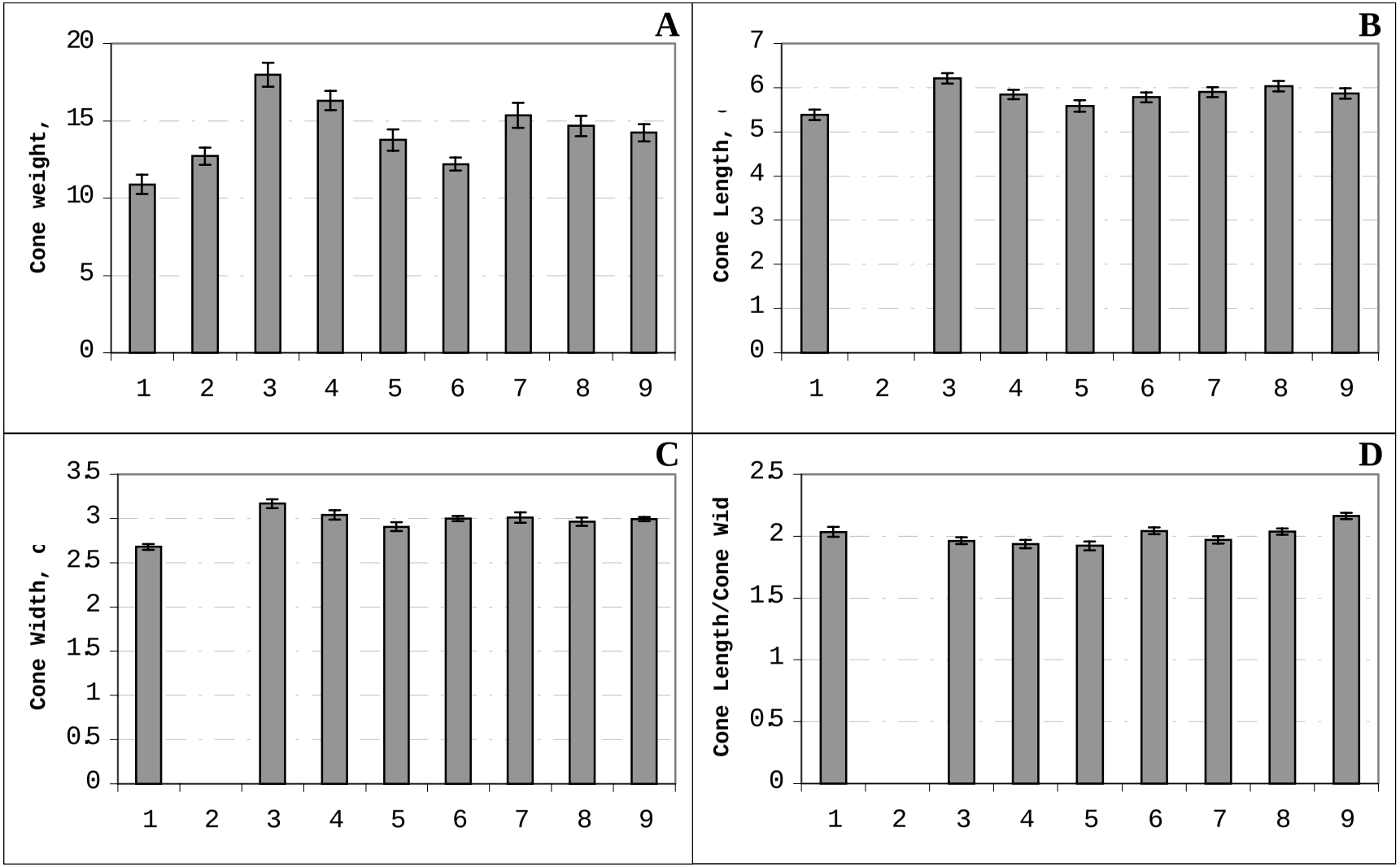

Figure 2. Average cone weights (A), cone lengths (B), cone widths (C) and ratios of cone length / cone width (D) for each of the 9 populations sampled. The cones from population 2 were slightly opened at the time of gathering, thus no width values were measured. Vertical lines over each bar represent ± SE values.

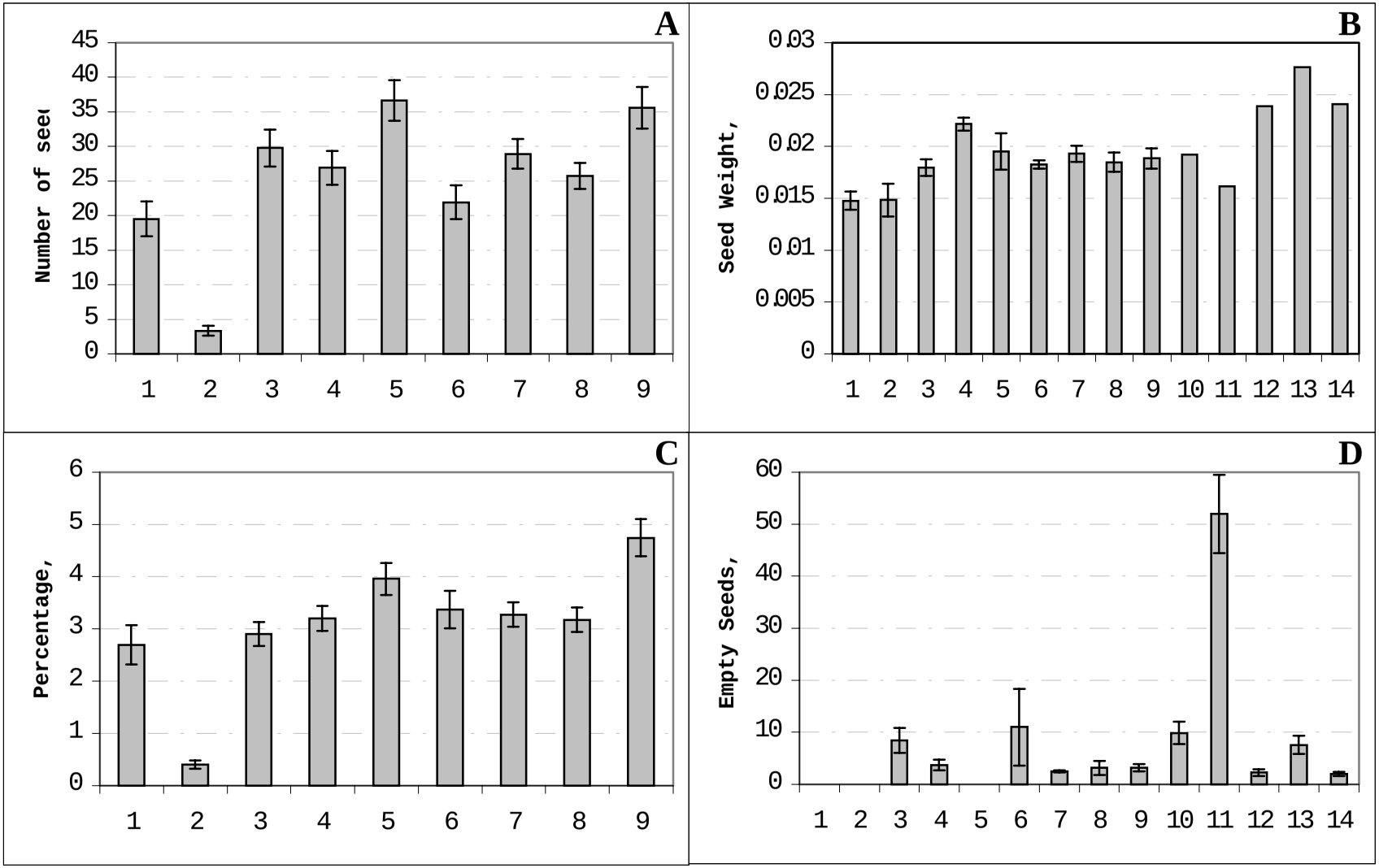

Figure 3. Average numbers of seeds extracted per cone (A), average seed weight (B), total seed weight as percentage of the respective, total cone weight (C) and average portion (%) of empty seeds per cone (D) for each one of the 9 populations sampled. Vertical lines over each bar represent ± SE values.

As shown in figure 3C, the average percentage of the seed portion within a single cone (on a weight basis) differs considerably among particular populations but it ranges at rather low levels (3-4%).

In figure 3D percentage of empty seeds for each population is illustrated. This percentage usually ranges between 5 and 10% with the exception of population 11 (over 50%). Germination experiments were not conducted with seeds from populations 1, 2 and 5 thus in those provenances, the empty seed percentage was not estimated.

The number of cotyledons for large samples of seedlings (grown for an initial period from germinated seeds in the germination experiments) was estimated and is presented in Figure 4. As shown in the figure, although there exist differences among the populations, the average number of cotyledons is always between 7 and 8.

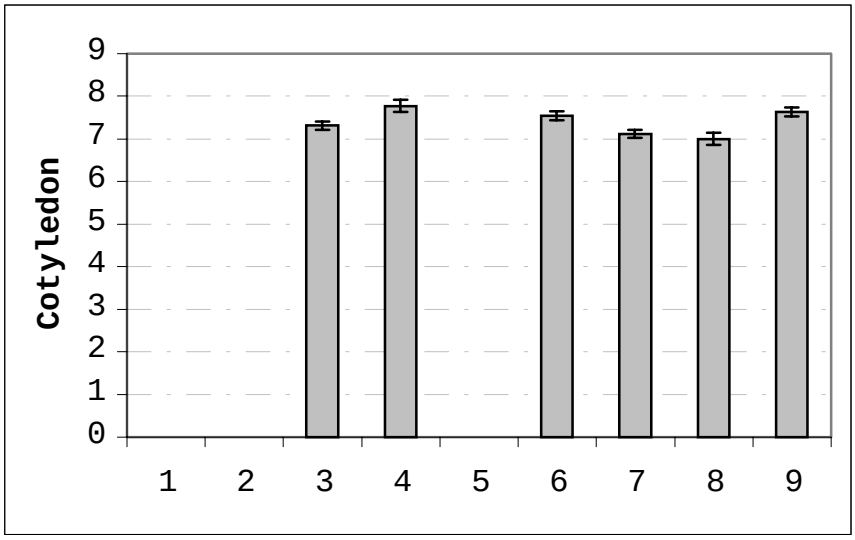

Figure 4. Average number of cotyledons of *Pinus nigra* seedlings from each population (measurements were not carried out with populations 1, 2 and 5). Vertical lines over each bar represent ± SE values.

Figure 5 shows typical germination time courses for 2 seedlots, over a wide range of experimental conditions tested. Similar germination results were obtained in the experiments with seeds from each one of the populations tested (populations 10-14).

Figure 6 shows typical curves of final germination as a function of constant temperature for each one of the seed populations available. As we can see most of the populations achieve highest germination at temperatures 15 and 20 °C while significant variation is observed at the suboptimal temperatures 5, 10 and 25 °C.

From the germination tests that followed (temperature range tested: 5 – 25 ºC), a relatively constant pattern (with minor variations) emerged from all the seed populations tested (populations 1-9). The germination maxima are found at the 'Mediterranean' temperatures 15 and 20 ºC, while light results over dark gives us 10-20% higher germination.

In addition, extended germination experiments were conducted with seeds provided by the Ministry of Agriculture. Constant and alternating temperatures were used. The temperatures used ranged from 5 to 25 ºC, while an additional, alternating temperatures regime was applied, simulating the conditions prevailing naturally in mid April (according to the average climatic values of the years 1955-1987 for M.S. Ellinikon, Athens Airport). The germination results were not much different than the ones mentioned before. Once again best germination results were obtained at temperatures 15 and 20 ºC. The seeds tested under light reached significantly higher (10-20%) germination percentages compared to those imbibing under dark conditions.

In Figure 7, final germination percentages are pooled for all populations and for each temperature tested. It is quite clear that highest germination is equally achieved at temperatures 15 and 20 °C, while the time required for germination is much less at temperatures 20 and 25 °C as indicated by the T_{50} values in the same figure. At 20 °C, white light shows a significant promotion of germinability (from 70 to 90%) while at the same time a considerable reduction of T_{50} is also obtained.

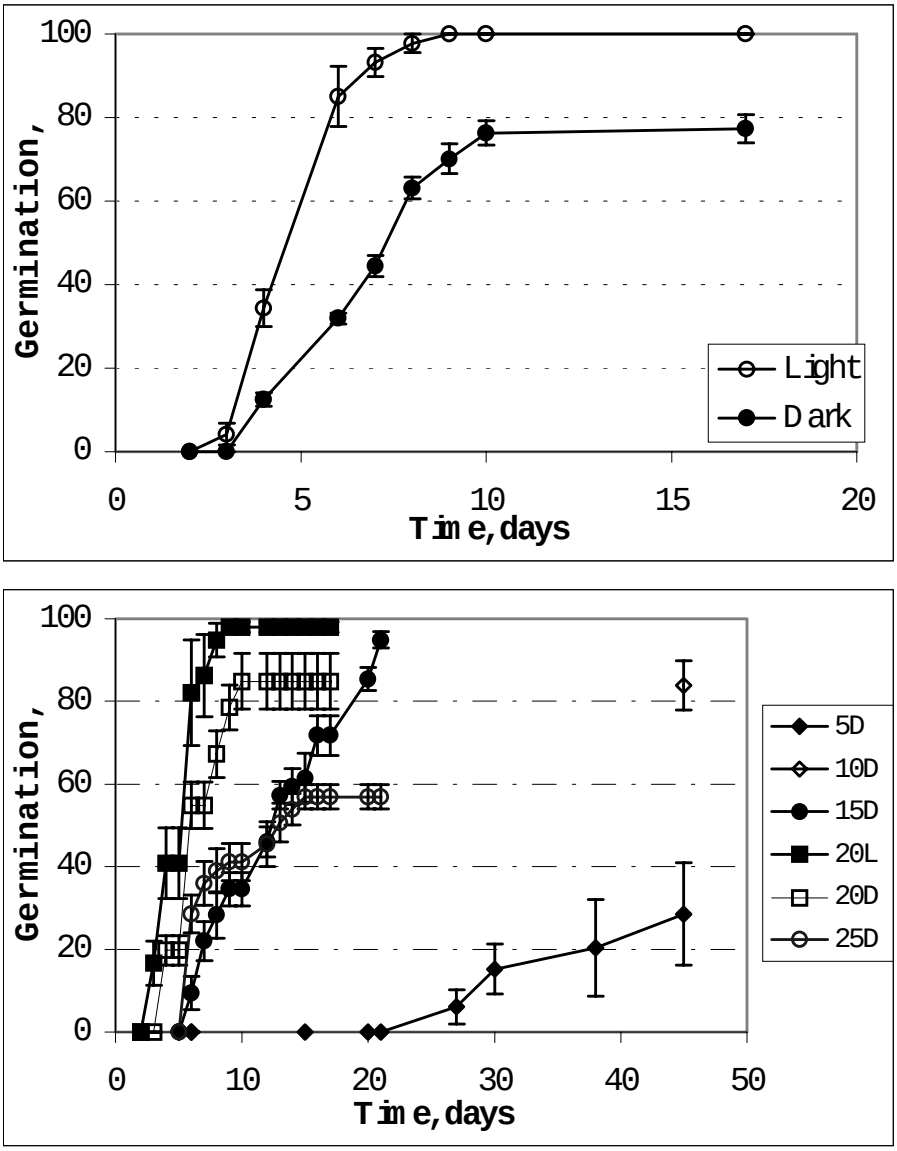

Figure 5. Typical time courses of seed germination of *P. nigra*. The diagram on top illustrates both the enhancement of germination rate and the partial increase of germinability induced by diurnal white light at 20 °C (seedlot provenance: Parnassos Mountain). The diagram below shows the time courses of seed germination under a range of temperature and light conditions (seedlot provenance: Samos Island, 2001). 5D-25D: 5-25 ºC in the dark. 20L: 20 ºC under continuous light. Germination of seeds at 10 ºC (darkness) was measured only once, 45 days after onset of imbibition. Vertical lines on each point represent ± SE values.

In conclusion, *P. nigra* seed germination was quite fast in a wide temperature rate (also in agreement with populations of other origins, eg. Boydak 1984, Paci, 1989; Skordilis and Thanos 1997), indicating that seed germination in nature will take place in spring, soon after dispersal. In contrast to the dry summer conditions prevailing in *P. halepensis* and *P. brutia* distribution areas, water availability in the mountainous habitats ensures the successful survival of the spring germinating *P. nigra* seedlings. On the other hand, *P. nigra* seeds are not adapted to fire at all, as already known from several previous works (Trabaud and Campant. 1991; Skordilis and Thanos 1997; Escudero et al. 1999). In regard to the role of light: phytochrome is long ago known to mediate

germination (Orlandini and Malcoste 1972) but its mediation seems to affect a relatively small proportion (20%) of seed population.

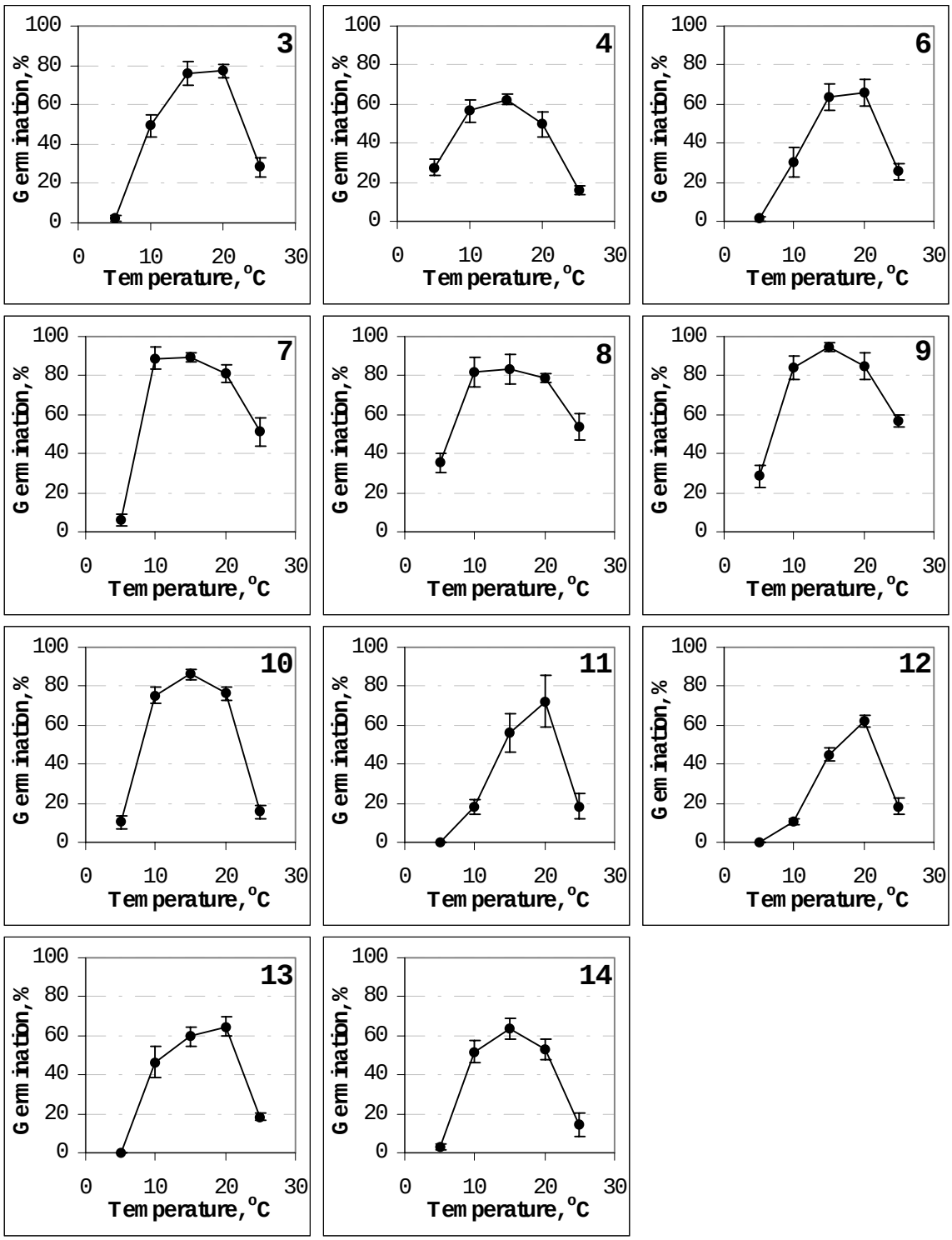

Figure 6. Final germination in the dark as a function of constant temperatures (5 - 25 °C) for each particular seedlot (numbers at the right top of each graph correspond to the provenance numbers of Table 1). Vertical lines on each point represent ± SE values.

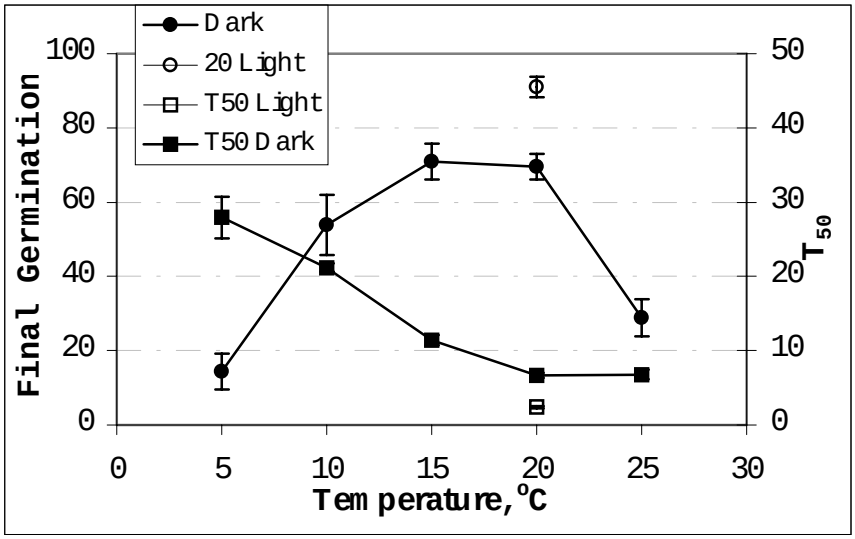

Figure 7. Final germination percentage for each temperature tested; average values from all populations tested. Average T_{50} values are also pooled for each temperature. Vertical lines on each point represent ± SE values.

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Knowing Propagation of the Mediterranean Trees and Shrubs from Seed to Preserve their Biodiversity

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Abstract

Nearly 27% of the Italian territory, mainly located in areas with Mediterranean climate and vegetation, is threatened by processes of soil degradation, erosion or desertification. Mediterranean vegetation needs particular attention: the role of plant cover is essential for mitigating desertification processes because vegetation and connectivity of 'green areas' strongly condition the quality and evolution of soil. The Mediterranean flora is well described from a botanical point of view but much less is known about their natural and artificial regeneration. This lack of knowledge is particularly serious because it represents a limit for multipurpose afforestation, restoration and reclamation and explains the fact that plantings are often carried out employing a narrow range of species which are easy to grow in the nursery. This practice greatly reduces levels of biodiversity. Up to 70% of Mediterranean trees and shrubs growing in Italy are threatened in at least one of the 21 Italian Regions. Lack of knowledge in their propagation is related with seed storage (Mediterranean oaks), dormancy removal in seeds dispersed by birds or small mammals (*Ruscus aculeatus*, *Smilax aspera*, *Lonicera* spp., etc.), dormancy removal in seeds showing the morphophysiological type often associated with secondary dormancy (*Daphne* spp., *Rhus* spp., etc.), germination response of Mediterranean species to smoke, heat and fire exposure (*Erica* spp., *Cistus* spp., etc.) and growing techniques able to preserve biodiversity.

Seasonal and Chilling Effects on Germination of Norway Spruce (*Picea abies* (L.) H. Karsten) and Scots Pine (*Pinus sylvestris* L.) Seeds

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Abstract

The objective of the study was to determine the effects of season when germination tests are done and chilling on germination energy (rate) and germination capacity of Norway spruce and Scots pine seeds. Four replicates of 100, non-chilled and chilled (3 weeks at 3-5°C) seeds of four seedlots of Norway spruce and three seedlots of Scots pine were germinated every 3 weeks from March 1997 to February 1998. The germination tests were done according to the ISTA Rules (1996). A statistically significant decline occurred in germination energy during the summer months (namely in July and August) for both chilled and non-chilled seeds of both species. However, no similar change was detected in germination capacity. Germination energy appeared to fluctuate the most in the seeds with no chilling. The speed of seed germination (germination energy) of both tree species was positively stimulated by chilling while germination capacity was either the same for chilled and non-chilled seeds of Norway spruce or even lower for chilled Scots pine seeds. Generally, Norway spruce seeds appeared to be more influenced by time and random fluctuations than Scots pine seeds. A significant interaction was detected for time in the case of spruce and pine seedlots (all seedlots combined), indicating different time patterns in these two species. Overall time fluctuations and the effect of tree species (or origin) accounted for less than 50 % of total experimental variance. This could indicate the effects of inherent germination variability and random fluctuations.

Keywords: biorhythms, conifer seeds, germination capacity, germination rate, pre-chilling

Introduction

Several authors (e.g. Schmidt 1930; Baldwin 1935; Mamonov et al. 1986; Barnett et Mamonov 1989) have reported a fluctuation in the germination capacity of stored conifer seeds throughout the year. This phenomenon has been called seasonal periodicity, or biorhythms (Barnett and Mamonov 1989). The objective of the germination test is to determine the maximum germination potential of a seed lot, which can then be used to compare the quality of different lots and also estimate the field planting value (ISTA Rules 1996). Standardised tests such as the ISTA Rules for Seed Testing have been used worldwide to obtain valid information for determining germination capacity. In the Czech Republic, seeds of many tree species, especially conifers, have been stored for many years at the central Tree Seed Plant in Tyniste nad Orlici. Germination of both fresh seeds (just after processing) and stored seeds has been tested each year at the ISTA laboratory at Uherske Hradiste. In the past, stored conifer seeds such as Norway spruce (*Picea abies* (L.) H. Karsten) and Scots pine (*Pinus sylvestris* L.) were tested mostly from autumn (September) through spring (April–May) and nearly no tests were conducted during the summer. However, since the 1990s increasing numbers of such tests have been done during the summer (e.g.. in July and August) and an increase of fresh (viable, but not germinating) seeds at the end of the germination tests was observed (Procházková 1996). The present experiment aims to determine the possible effects of season of testing, and chilling, on the germination speed and capacity of Norway spruce and Scots pine seeds, especially when re-testing stored seeds.

Materials and Methods

Four replicates each of 100 non-chilled and chilled (3 weeks at 3-5°C) seeds of four seedlots of Norway spruce and three seedlots of Scots pine were germinated every 3 weeks from March, 1997, to February, 1998. For seeds, whose germination test started on July 22, chilling was carried out for 35 days only. Seeds of both species were collected in 1995 (Table 1) and stored at 4°C in plastic containers until they were used. The germination tests were done at alternating temperatures (30/20°C for 8/16 hours) according the ISTA Rules (1996). Germinated seeds were counted daily except on weekends and holidays. After 21 days all non-germinated seeds were cut and classified as dead, fresh or empty. For each of the treatments (non-chilled and chilled seeds) the following parameters were calculated: the date of each germination test, germination energy (at 7 days), germination capacity (at 21 days), and germination value (Czabator 1962). The percentage data for germinated seeds required simultaneous application of different statistical procedures that allowed both quantitative and qualitative comparisons to be made. Where necessary, selected extreme points (up to two per variable and time unit) were

excluded in order to obtain a more normal distribution of the data. Evaluation of germination parameter time profiles utilized the two-way ANOVA models (random models) after preliminary tests of normality and homogeneity of variance. The ANOVA models were used to assess the significance of both random experimental factors (season of the year, treatment or tree species) and their interactions. The null hypothesis for an interaction between any two combined factors was that the response of germination did not differ among specific levels of one factor depending upon the particular level of the second factor (Anderson and McLean 1974). Tukey´s multiple-range test or standard two-sample t-test were applied subsequently for detailed comparison of differences among variants using P = 0.05 as the limit of probability (Zar 1984). Final conclusions were made based on the results the Kruskal-Wallis analysis followed by Mann-Whitney test for non-parametric alternatives (Zar 1984). The effect of each experimental treatment in the models was evaluated on the basis of significance level of the global ANOVA F test and the so-called Variance ratio, which estimates the proportion of total variability that belongs to the effect of the evaluated type of treatment. The ratio was computed from common ANOVA tables as $[SS_{Treatment} / SS_{Total}] \times 100$. The germination rate and capacity for the same seedlots were compared using a two-sample t-test for dependent parameters. Interrelationships among variables were assessed using Pearson linear correlation coefficients (SPSS 1990; Zar 1984).

Table 1. Provenances of Norway spruce and Scots pine seeds used in the studies.

Species	Seedlot number	Collection year	Natural forest region	Seed-collection zone	Stand certification number
Norway spruce	8260/96/OZ	1995	13	South Bohemia	B-SM-349-13-8-KT
	8540/96/OZ	1995	13	South Bohemia	B-SM-13-8-KT
	8065/96/OZ	1995	1	Ore Mountains	B-SM-12-1-7-CV
	8542/96/OZ	1995	27	East Sudeten Mountains	A-SM-0-27-8-UO
Scots pine	8019/95/OZ	1995	17	East Bohemia	A-BO-2-17-1-HK
	8064/96/OZ	1995	17	East Bohemia	A-BO-581-17-1-RK
	8022/95/OZ	1995	6	West Bohemia	S-BO-95-6-2-PS

Results

A statistically significant decline in germination energy was evident during the summer months (namely in July and August) for both chilled and non-chilled seeds of both tree species; however, no similar change was detected in germination capacity (Figures 1 and 2, Table 2). Combining the data for all seedlots of both tree species revealed variability low enough to allow statistical comparisons to be made. Germination capacity was the least significant parameter – it showed nearly identical values throughout the entire year (Figure 1, Table 2). Germination energy seemed to fluctuate the most, especially in the non-chilled treatments. Tables 3 and 4 summarize the original statistical values and the levels of differences for both species. Differences were evaluated within time intervals that revealed statistical homogeneity for the parameters. The tables clearly show the quantitative differences in germination energy and mainly germination value between Norway spruce and Scots pine seeds. Conversely, germination capacity values were rather similar.

The speed of seed germination (germination energy) of both tree species was positively stimulated by chilling while germination capacity was either the same for chilled and non-chilled seeds of Norway spruce or even lower for chilled pine seeds (Tables 3 and 4). Higher germination capacity of chilled Scots pine seeds occurred only during July and August germination test periods (Table 4).

Generally, spruce seeds were more influenced by time and random fluctuations than pine seeds (Figure 2). A significant interaction with time was detected in the case of spruce and pine seedlots that indicated different time patterns for these two species. Both time fluctuations and the effect of tree species (or origin) combined accounted for less than 50 % of the total experimental variance (Table 2). Figure 3 documents the correlation pattern between germination energy and germination capacity for identical seedlots. The main hypothesis was: To what extent is the germination capacity dependent on the germination energy? The rather non-significant dependence was detected for spruce non-chilled seedlots due to time-related changes of the germination energy data. Both spruce and pine seedlots revealed significant correlation patterns; however again, least significant relations were found in non-chilled treatments. Highly significant interrelationship found in chilled treatments,

Table 2. ANOVA table summarizing the effect of the main experimental treatments on germination energy, germination capacity and germination value in chilled and non-chilled seeds.

Experimental treatments	Variance ratio in % and significance level of F test (in parentheses) [1]					
	Germination energy		Germination capacity		Germination value	
	Chilled seeds	*Non-chilled seeds*	*Chilled seeds*	*Non-chilled seeds*	*Chilled seeds*	*Non-chilled seeds*
Effects within Norway spruce seedlots						
Norway spruce (S; 4 seedlots)	39.65 (0.001)	12.15 (0.001)	38.12 (0.001)	23.44 (0.001)	35.62 (0.001)	25.41 (0.001)
Time (T; 12 months)	17.38 (0.001)	30.81 (0.001)	10.57 (0.101)	15.41 (0.012)	19.91 (0.024)	32.36 (0.001)
Interaction T x S	6.58 (0.541)	5.49 (0.871)	6.55 (0.369)	8.18 (0.315)	7.29 (0.363)	5.29 (0.472)
Effects within Scots pine seedlots						
Scots pine (P; 3 seedlots)	19.69 (0.001)	9.27 (0.001)	16.05 (0.001)	28.41 (0.001)	15.17 (0.001)	15.28 (0.001)
Time (T; 12 months)	16.37 (0.008)	35.54 (0.001)	10.89 (0.041)	11.16 (0.035)	29.86 (0.001)	31.31 (0.001)
Interaction T x P	5.44 (0.358)	6.27 (0.284)	6.71 (0.365)	10.27 (0.196)	8.55 (0.264)	5.24 (0.458)
Summarized comparison of trends for Scots pine and Norway spruce [2]						
Tree species (TS) [2]	23.42 (0.001)	43.07 (0.001)	17.48 (0.105)	30.59 (0.001)	42.61 (0.001)	41.52 (0.001)
Time (T; 12 months)	5.76 (0.751)	14.44 (0.001)	12.59 (0.001)	10.05 (0.072)	4.89 (0.786)	14.69 (0.001)
Interaction T x TS	8.96 (0.001)	2.55 (0.010)	5.54 (0.001)	3.45 (0.010)	8.23 (0.001)	1.73 (0.393)

[1] Variance ratio represents an estimate of the proportion of total variability that belongs to the effect of the evaluated type of treatment. The ratio is computed from common ANOVA table as ($SS_{treatment}$ / SS_{total}) x 100.

[2] The effect of tree species (TS) represents two distinguishable items: Scots pine and Norway spruce. Scots pine and Norway spruce seedlots were combined in two groups in order to assess general difference between species.

Table 3. Comparative, monthly germination of chilled and non-chilled Norway spruce seedlots based on monthly germination data[1]

Estimates evaluated within specified groups of months: January (I) to November (XI)

	I - II	III	IV - VI	VII	VIII	X - XI
Germination energy						
Chilled	66.21 (1.24)	65.81 (1.93)	67.64 (1.19)	59.75 (4.02)	49.50 (4.14)	64.00 (1.15)
Non-chilled	36.39 (2.26)	42.88 (2.54)	32.33 (2.36)	14.13 (1.58)	3.37 (0.73)	25.00 (2.54)
p level [2]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Germination capacity						
Chilled	71.56 (1.33)	68.63 (2.35)	72.96 (1.16)	72.69 (2.36)	69.31 (2.97)	72.97 (0.79)
Non-chilled	71.26 (0.92)	75.63 (1.33)	75.59 (2.41)	61.06 (2.37)	67.63 (2.49)	73.84 (1.05)
p level [2]	0.858	0.001	0.112	0.009	0.665	0.505
Germination value						
Chilled	21.14 (0.89)	20.63 (0.95)	35.50 (1.32)	27.29 (2.39)	21.75 (3.36)	31.61 (1.05)
Non-chilled	31.01 (1.02)	34.26 (2.85)	22.06 (1.75)	11.91 (0.95)	11.54 (0.823)	21.49 (0.807)
p level [2]	0.005	0.006	0.001	0.001	0.032	0.007

[1] Data represent mean estimates supplied by corresponding standard errors; four independent experimental runs with four Norway spruce seedlots were realized each month

[2] Significance level of t-test for comparison of two independent seedlots

Table 4. Comparative, monthly germination chilled and non-chilled Scots pine seedlots based on monthly germination data[1]

Estimates evaluated within specified groups of months: January (I) to November (XI)

	I - II	III	IV - VI	VII	VIII	X - XI
Germination energy						
Chilled	71.50 (1.75)	68.42 (3.03)	76.07 (1.05)	83.50 (1.96)	79.75 (1.84)	80.35 (2.89)
Non-chilled	64.41 (1.28)	52.17 (3.35)	66.94 (1.47)	55.42 (3.94)	40.08 (3.95)	62.21 (1.76)
p level [2]	0.005	0.003	0.001	< 0.001	< 0.001	< 0.001
Germination capacity						
Chilled	75.19 (2.74)	68.92 (3.01)	79.73 (0.97)	87.75 (1.77)	86.25 (1.29)	85.00 (1.84)
Non-chilled	86.33 (1.75)	79.42 (1.75)	84.83 (1.64)	78.67 (3.94)	78.00 (2.77)	87.00 (0.64)
p level [2]	0.008	0.007	0.019	0.017	0.013	0.085
Germination value						
Chilled	58.19 (3.71)	52.29 (4.66)	60.12 (164)	83.52 (7.24)	47.19 (6.48)	63.18 (1.82)
Non-chilled	34.46 (070)	27.14 (2.42)	34.94 (0.77)	27.92 (2.59)	23.49 (1.69)	34.08 (2.11)
p level [2]	< 0.001	< 0.001	< 0.001	< 0.001	0.019	< 0.001

[1] Data represent mean estimates supplied by corresponding standard errors; four independent experimental runs with three Scots pine seedlots were realized each month

[2] Significance level of t-test for comparison of two independent seedlots

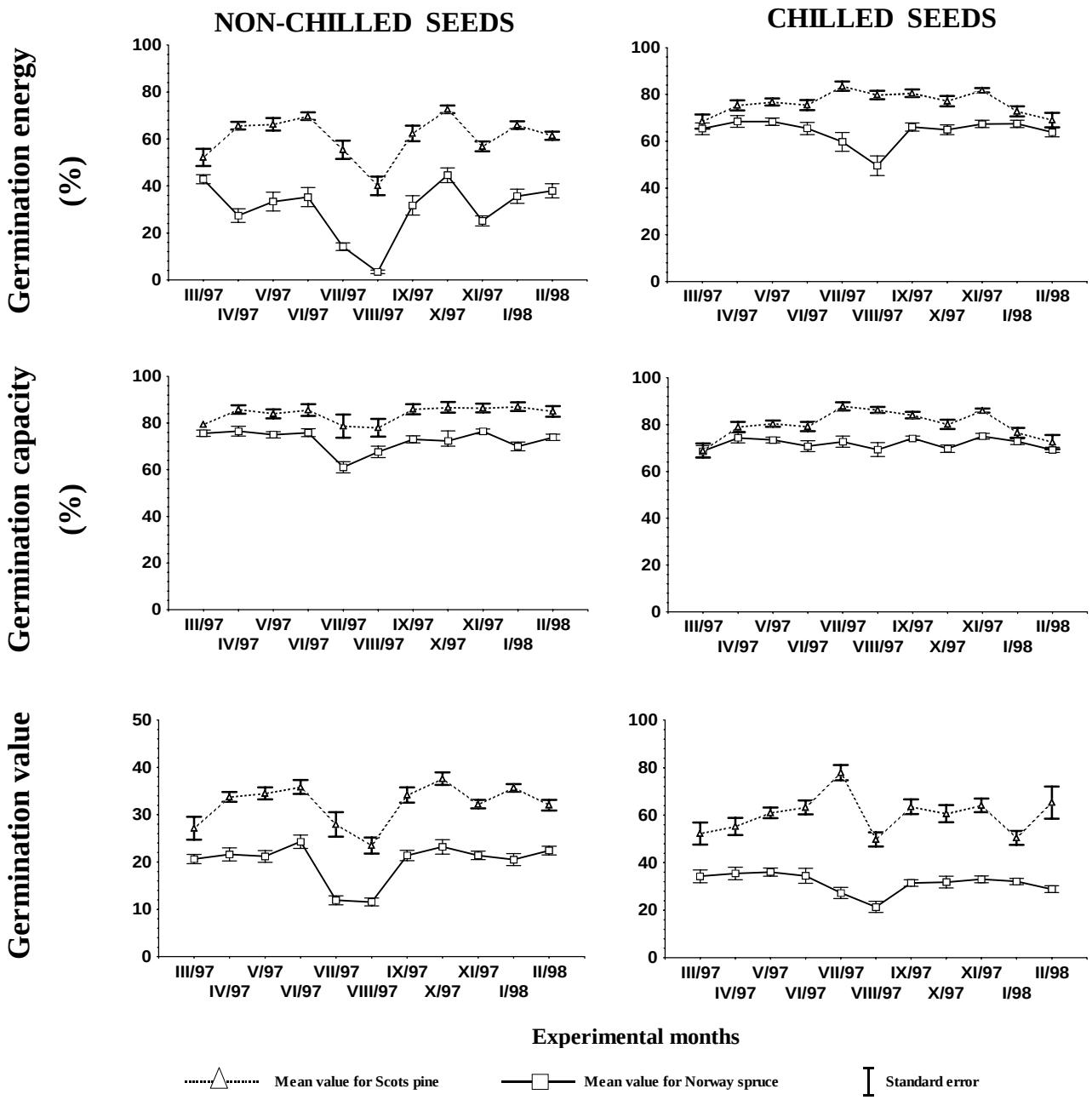

Figure 1. Seasonal fluctuation of germination energy and germination capacity of chilled and non-chilled seeds of Norway Spruce and Scots pine.

predominantly for pine seedlots, confirm consistent time profile changes of pine seedlots described earlier. This could indicate a significant influence of inherent germination variability and random fluctuations.

Discussion

Seasonal changes in seed germination have been reported for several conifer species. Baldwin (1935) found seasonal variability in germination of red spruce (*Picea rubens* Sarg.) seeds with a peak in late winter and early spring. For Scots pine seeds Schmidt (1930) described two peaks of germination (maximum germination capacity) - in spring and early autumn, and a considerable germination decrease in summer while for Norway spruce seeds he reported only a spring peak without any significant decrease during the summer. These differences correspond to the differences in ecological site requirements of the two species. Norway spruce grows on wetter sites where the conditions for seed germination are more favourable even during summer while Scots pine grows on dry sites with adverse summer conditions for germination (Schmidt 1930). Rehackova (1954) also reported two peaks of germination energy and germination capacity for Scots pine - in spring (March, April) and early autumn (September) but also in July. For European larch seeds she observed maximum

germination in April and June. For both species the lowest germination occurred in August. However, Rehackova did not consider these changes to be a reliable indicator of periodicity. Mamonov et al. (1986) carried out germination tests of fresh and stored Scots pine, Norway spruce and Siberian larch (*Larix siberica* Ledel.) seeds for several years and confirmed distinct germination variability for all three species. Also, they reported an increase for fresh seeds during a subsequent period of lower physiological activity. Barnet and Mamonov (1989) based on the results with monthly germination tests of longleaf pine (*Pinus palustris* Mill.) and slash pine (*Pinus elliottii* Engelm.) and earlier results of Mamonov (Mamonov et al. 1986) have supported the concept of biorhythms in conifer seed germination and concluded that i) biorhythms are more likely to be found in seeds of non-dormant species than in dormant species, ii) stored seeds show more seasonal fluctuation than freshly collected seeds, iii) variation in biorhythm differs from one year to another, and iv) germination peaks occur in early spring and lows occur in late summer. The data in the present work confirmed the concept of seasonal fluctuation of seed germination and showed that the lowest germination energy of both of the tree species tested occurs in July and August, while the variation in germination capacity within a year is not significant. Chilling stimulated the speed of seed germination (germination energy) of both tree species while germination capacity was either the same for chilled and non-chilled seeds of Norway spruce or even lower for chilled pine seeds. Our results also reaffirmed the findings of Schmidt (1930) about a significant influence of inherent germination variability and random fluctuations of each tested tree species.

Conclusions

Based on the results it can be stated that:

i) the germination capacity of stored Norway spruce and Scots pine seeds fluctuates within the year, but the variation is not significant;

ii) however, a statistically significant decline in germination energy was evident during the summer months (namely in July and August) for both chilled and non-chilled seeds of both tree species;

iii) chilling stimulated the speed of seed germination (germination energy) of both tree species while germination capacity was either the same for chilled and non-chilled seeds of Norway spruce or even lower for chilled Scots pine seeds, and

iv) spruce seeds were more influenced by time and random fluctuations than pine seeds and this could indicate a significant influence of inherent germination variability and random fluctuations.

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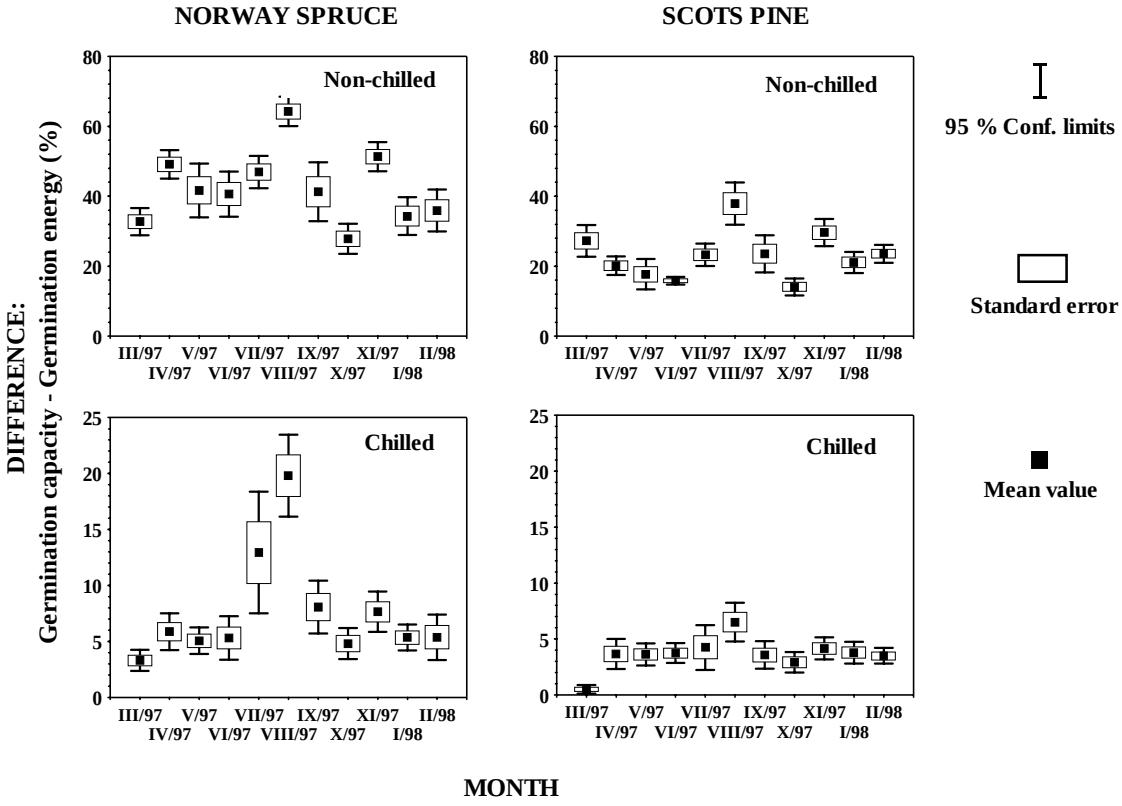

Figure 2. Comparison of germination energy and germination capacity of chilled and non-chilled seeds of Norway spruce and Scots pine.

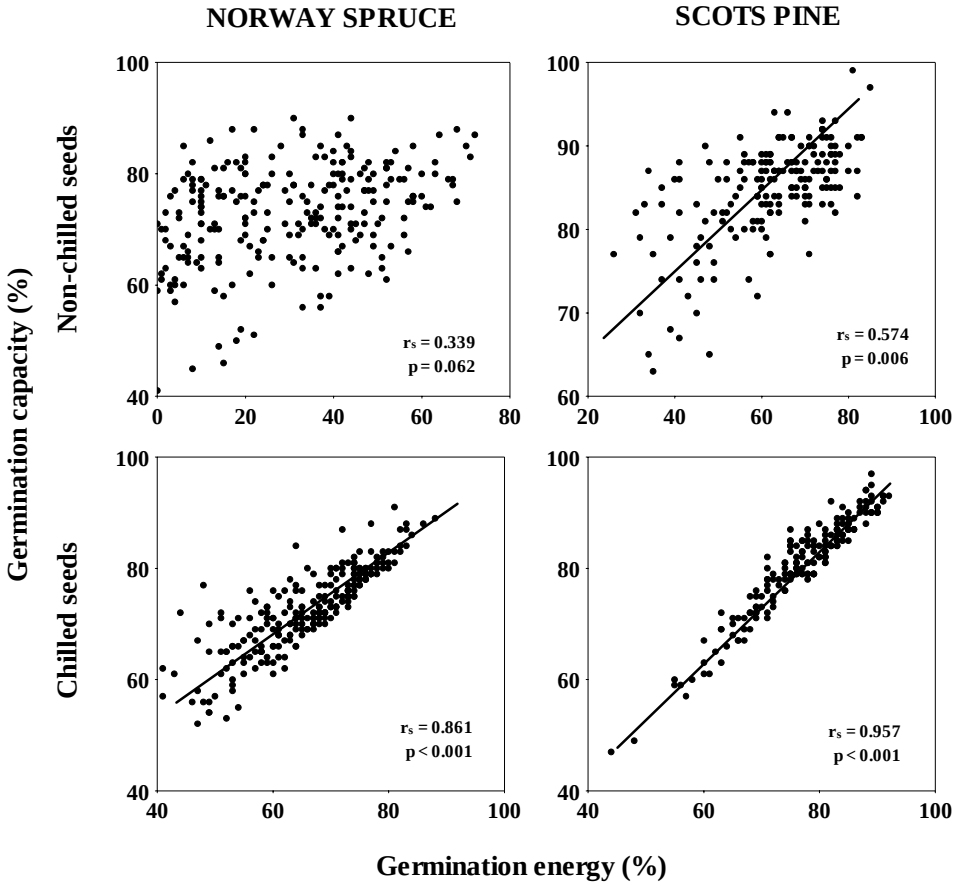

Figure 3. Correlation between germination energy and germination capacity of chilled and non-chilled seeds of Norway spruce and Scots pine.

Physiological and Biochemical Changes During Desiccation of Recalcitrant Arecanut (*Areca catechu* L.) Seed

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Abstract

The critical moisture content for safe storage of recalcitrant arecanut seed is 32.8%. It was found that electrical conductivity of seed leachate was negatively correlated with the seed quality and increased from 0.189 to 0.665 dSm^{-1} during desiccation. Free sugar content in seed leachate was also increased. The protein and phenol content were reduced from 17.4 to 13.2 mg g^{-1} and 3.1 to 2.1 mg g^{-1}, respectively during desiccation. The fat content was maximum (12.1 percent) in fresh seeds but was reduced and converted to free fatty acid during desiccation. The lipid peroxidation value increased from 0.208 to 0.710 (OD value) with the reduction in seed moisture content. The activities of scavenging enzymes such as catalase (1.75 to 0.73 µg H_2O_2 g^{-1} min^{-1}) and peroxidase (0.324 to 0.037 OD g^{-1} min^{-1}) were reduced with increased moisture loss. Other enzymes, *viz.* α-amylase and polyphenol oxidase, were also reduced during desiccation. It is concluded that the desiccation of arecanut seed leads to the disturbance of its internal mechanisms, which ultimately a leads to loss in viability.

Keywords: Arecanut, biochemical changes, desiccation sensitive, viability loss

Introduction

Arecanut (*Areca catechu* L.), also known as betelnut, is an important commercial plantation crop of India and belongs to the family Arecaceae. India is the largest producer of arecanut and accounts for 85 percent of world's output. The other countries in which it is grown are Bangladesh and Sri Lanka, Malaysia, Indonesia, Philippines and some of the Pacific Islands. The hard dried endosperm of ripe and unripe seed, called `nut', is chewed as a narcotic and outrivals chewing gum in popularity on a world basis. It may be chewed alone or as a constituent, along with leaves of *Piper betel*, slaked lime and chewing tobacco. Arecanut seed contains alkaloids like arecaine, arecoline, arecaidine, guvacoline, guvacine and chlonine. These compounds have pharmacological properties including actions on intestinal helminths and parasympathetic system (Mujumdar *et al.* 1979).

Arecanut is propagated through seed, and the seed has been classified as `recalcitrant' (Raja 2001). Roberts (1973) clarified the situation by introducing the terms 'orthodox' and 'recalcitrant' to describe the storage behaviour of seeds. He referred to seeds obeying Harrington's rule, i.e. seeds, which can tolerate desiccation and freezing temperatures, as orthodox. The many other types of seed, which are readily killed by desiccation if the moisture content falls below a critical value and cannot tolerate freezing temperatures, were classified as recalcitrant. In addition, it is very difficult to maintain the quality of recalcitrant seeds during storage, as the seeds themselves are variable in their moisture, size and variability (Chin 1989). There is a lack in understanding of basic mechanisms and behaviour in recalcitrant group of seeds. Hence, a study on seed longevity, and on the physiological and biochemical changes during desiccation in arecanut has been undertaken.

Materials and Methods

Desiccation

Fully matured uniform size areca seed nuts were collected from the 45 years old plantation and were spread in a sterilized plastic tray and kept under ambient conditions with free air circulation to facilitate desiccation. Critical moisture content, physiological and biochemical changes were estimated during desiccation at two-day intervals up to 24 days.

Physiological and biochemical changes

Seed moisture content and germination

Seed moisture content was estimated after cutting the nuts into small pieces and drying at 105°C for 16 ± 1 h in a hot air oven (ISTA 1999). The germination test was carried out in sand medium with 100 seeds for each

replication at 25 ± 2°C and 95 ± 2 percent relative humidity. At the end of 90 days (Nagwekar *et al.* 1997), the number of normal seedlings was counted and the germination percent was calculated (ISTA 1999).

Leachates

The dehusked seeds were soaked in 50 ml of distilled water for 20 h and the electrical conductivity of seed leachates was measured with an electrode possessing a cell constant of one and expressed in dSm^{-1} (Presley 1958). The seed leachates from electrical conductivity were used to determine the free sugars by the addition of Nelson's arseno molybdate reagent. The intensity of colour was measured at 620 nm (Somogyi 1952).

Total soluble protein

One gram of finely powdered seed material was homogenized with 10 ml of phosphate buffer at pH 7.0 and centrifuged at 3000 rpm. One ml of supernatant was taken in a test tube, the volume was made up to 5 ml and 5 ml of copper reagent was added. After 10 min, 0.5 ml of folin-ciocalteau (phenol reagent) reagent was added and colour intensity was read at 660 nm (Lowry *et al.* 1951).

Total phenols

One gram of seed sample was ground in 10 times the volume of 80 per cent ethanol and the solution was centrifuged at 3000 rpm for 10 min. From the supernatant, one ml of solution was pipetted out and the volume was made up to three ml with distilled water. To that 0.5 ml of folin-ciocalteau reagent was added. After three min, two ml of 20 per cent Na_2CO_3 solution was added, mixed well and the sample was placed in a boiling water bath for one min. After cooling, the absorbance was measured at 650 nm (Malik and Singh 1980).

Fat and fatty acid

One gram of ground seed material was packed in a filter paper, placed in the Soxhlet extraction apparatus and the fat was extracted with petroleum ether for 3h without interaction by gentle heating (AOAC 1960). To assess the free fatty acid, one gram of fat was dissolved in 50 ml of neutral solvent comprised of 25 ml petroleum ether and 25 ml 95 percent alcohol. Then the contents were added with one ml of phenolphthalein indicator and titrated against 0.1 N potassium hydroxide (Cox and Pearson 1962). The lipid peroxide formation was also studied by the thiobarbituric acid (TBA) colour reaction method (Bernheim *et al.* 1948).

Enzymes

To find out the α-amylase activity, finely ground seeds were homogenized in 1.8 ml of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuged at 2000 rpm for 10 min. Then the reaction was started in the mixture consisted of one ml of 0.067 percent starch solution and 0.5 ml of enzyme extract. The reaction was stopped after 10 min of incubation at 25°C by the addition of one ml iodine HCl solution and change in the colour was measured at 620 nm (Paul *et al.* 1970).

For catalase, finely ground seeds were homogenized in 0.066 M sodium phosphate buffer (pH 6.8) and centrifuged at 2000 rpm for 10 min. The reaction mixture consisted of 5 ml of phosphate buffer (pH 6.8), 4 ml of 0.3 N hydrogen peroxide (substrate) and 0.5 ml of enzyme extract. The reaction was stopped after 15 min of incubation, by the addition of 10 ml of 2N H_2SO_4. The blank was maintained for each set in which 0.5 ml of enzyme extract was added after the addition of 2N H_2SO_4. The contents were titrated against 0.1 N $KmnO_4$ and titre values were noted. The difference between the titre values gave the volume of permanganate equivalent to enzyme activity (Povolotskaya and Sedenka 1956).

The peroxidase activity was measured in 500 mg of seeds homogenized in 5 ml of 0.25 M tris buffer (pH 6.0) and centrifuged at 10,000 rpm for 10 min at 5°C. The enzyme activity was measured by using pyrogallol as the substrate (Malik and Singh 1980).

For polyphenol oxidase activity, 500 mg of finely powdered seeds in each sample were homogenized in five ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 10 min at 4°C. The reaction mixture consisted of 0.5 ml of enzyme extract, 0.2 ml of 0.01 M catechol (substrate) and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). After adding the substrate, the absorbance at 495 nm was measured every 30 seconds (Esterbaner *et al.* 1977).

Results and Discussion

The important property of recalcitrant seeds is their high moisture content, even after they have been shed from the mother plant. Unlike orthodox seeds, they do not undergo maturation drying. These recalcitrant seeds generally have high moisture content, ranging from 30 to 70 percent (Chin 1989). In the present study, the freshly collected arecanut seed had 50.1 percent moisture content. However, unlike orthodox seeds, they are highly intolerant of further desiccation which makes them recalcitrant (Das and Ray 1985; Nagwekar *et al.* 1997).

In recalcitrant seeds, decline in viability occurs abruptly below a certain moisture level, which is called " Critical Moisture Content (CMC) " (Poulsen and Eriksen 1992) or "Lowest Safe Moisture Content (LSMC)" (Tompsett 1986) which is defined as the moisture content below which freshly collected seeds died when the seed lot is dried. In the present study, it was found that the reduction in germination from 100 percent to 85 percent was gradual up to 14 days of desiccation. After that, there was a marked reduction in germination from 85 percent to 60 percent with mean seed moisture content of 32.8 percent at 14 days after desiccation. Below this moisture level, a rapid reduction in germination occurred which resulted in complete loss of viability with seed moisture content of 17.7 percent (Table 1). This abrupt reduction in viability was also reported by Poulsen and Eriksen (1992) in *Quercus robur***,** in lychee and longan (Fu *et al.* 1990) and in jack (Bhattacharyya and Basu 1992). It was found that the seed moisture content below 32.8 percent caused a rapid reduction in germination, and it could be fixed as the lowest safe moisture content for arecanut.

Table 1. Effect of desiccation on physiological and biochemical changes in recalcitrant arecanut seed.

Period of desiccation (days)	Seed moisture content (%)	Germination (%)	Electrical conductivity (dSm^{-1})	Free sugars (mg $seed^{-1}$)	Protein (mg g^{-1})	Phenol (mg g^{-1})
0	50.1	100 (90.00)	0.189	12.6	17.4	3.1
2	47.2	95 (80.78)	0.265	12.6	17.1	3.1
4	44.9	90 (71.56)	0.320	13.0	16.3	2.9
6	38.2	90 (71.56)	0.322	14.0	15.9	2.7
8	37.3	90 (71.56)	0.343	14.0	15.2	2.6
10	36.7	90 (71.56)	0.378	14.0	15.0	2.4
12	35.1	85 (67.50)	0.510	53.2	14.7	2.5
14	32.8	85 (67.50)	0.512	59.8	14.0	2.5
16	31.8	60 (50.77)	0.532	61.5	13.7	2.4
18	27.8	35 (36.22)	0.553	63.7	13.5	2.3
20	24.9	35 (36.22)	0.556	65.8	13.3	2.3
22	24.8	20 (26.56)	0.638	65.1	13.2	2.2
24	17.7	0 (0.00)	0.665	67.6	13.2	2.1
SEd	2.55	4.28	0.03	5.8	0.36	0.07
CD (P=0.05)	5.52	9.26	0.06	12.6	0.79	0.17

(Values in parentheses indicate arc sine values)

In the event that the germination response is known to be protracted, there is a need to assess growth potential along with biochemical quality; this is particularly important for seeds with hard endocarps, like the palms (Ouedraogo *et al.* 1995). In arecanut, the electrical conductivity of solute leakage was minimum (0.189 dSm^{-1}) in the freshly collected seeds and it increased slowly during desiccation. Free sugar content in the seed leachate also exhibited an increasing trend from 12.6 to 67.6 mg during desiccation (Table 1). When the moisture content was reduced below the critical level, it increased rapidly due to loss of membrane integrity and nuclear disintegration. The dehydration-induced deterioration of the cell membrane in recalcitrant seeds was indicated by

high increase in leakage of solutes like sugar, amino acid, phenol and phosphates (Chin and Roberts 1980; Oleveira and Valio 1992). Heydecker (1972) suggested that weakening of cell membrane was the cause of leaching metabolite like electrolytes and other cell soluble compounds into the imbibing medium. Also the membrane conductance depends upon the concentration of the carrier for electrolytes in the membrane, the density of the membrane surface charge, and the quantity of permanent low and the ionic strength on both the sides of the membrane (Mclanghlin *et al.* 1970).

Protein synthesis was found to be affected by desiccation in arecanut seeds. Protein reserves in the seed decreased from 17.4 mg g^{-1} to 13.2 mg g^{-1} with moisture loss (Table 1). The seed vigour is more closely related to the integrity of the protein synthesizing system than to the protein content of the seed (Abdul-Baki 1980). Szezotka (1975) also reported that both decreased protein synthesis and oxygen uptake are parallel to the decline in germination of *Quercus robur* and *Q. borealis.* A similar result was observed in the present investigation. It was found that the desiccation of developing seeds was characterized by the accumulation of a particular set of m RNA and related proteins called 'Late Embryogenic Abundant (LEA) proteins' in the desiccated state (Blackman *et al.* 1991). Further, Farrant *et al.* (1993) reported that *Avicennia marina* seeds do not produce LEA proteins, which supported the suggestion that production of such proteins might facilitate desiccation tolerance. Farrent *et al.* (1992) reported that no such new proteins are produced during the late stages of development in the highly desiccation–sensitive seeds of *Avicennia marina*.

Phenols are aromatic compounds which include an array of components like tannins, flavonoids etc. The seeds of many tropical plants contain high concentrations of phenolic compounds and phenolic oxidases. These compounds are normally compartmentalized within cells. On desiccation, the cell membranes are damaged and the phenolic compounds are released. They are then oxidized and protein–phenol complexes are formed leading to loss of enzyme activity (Loomis and Battaile 1966). In the present investigation, reduction in phenol content during desiccation was evident due to the destruction of the tanniferous cells and release of phenolic acids to the surrounding cells.

Table 2. Effect of desiccation on physiological and biochemical changes in recalcitrant arecanut seed.

Period of desiccation (days)	Fat (%)	Free fatty acid (g oleic acid g^{-1} seed)	Lipid peroxidation (OD value)	α- Amylase (µg maltose mg^{-1} min^{-1})	Polyphenol oxidase (OD g^{-1} min^{-1})
0	12.1	0.012	0.208	0.061	0.090
2	11.8	0.020	0.234	0.060	0.090
4	10.8	0.020	0.257	0.058	0.099
6	10.6	0.027	0.303	0.048	0.094
8	10.6	0.035	0.450	0.043	0.074
10	10.0	0.039	0.571	0.040	0.060
12	9.9	0.051	0.654	0.011	0.056
14	8.6	0.063	0.656	0.011	0.050
16	8.4	0.063	0.679	0.010	0.044
18	8.1	0.067	0.689	0.007	0.039
20	8.1	0.086	0.694	0.007	0.030
22	8.1	0.098	0.699	0.005	0.029
24	7.8	0.106	0.710	0.004	0.022
SEd	0.10	0.005	0.04	0.006	0.009
CD (P=0.05)	0.21	0.012	0.10	0.013	0.020

Arecanut seed has 12.1 percent fat when it was fresh, and fat decreased with desiccation (Table 2). Clatterbuck and Bonner (1985) observed a steady decrease in crude fats of recalcitrant seeds of *Quercus robur* and the degradation of fat leads to an accumulation of free fatty acids (Georgi *et al.* 1983). A steady increase in free fatty

acids due to desiccation of arecanut seeds was also observed (Table 2). It might be that the oxidation of fat contributed to the accumulation of free fatty acids. Free fatty acids in the seeds are subjected to slow and constant attack by oxygen resulting in the production of hydrogen peroxide and other oxygenated free fatty acids and free radicals. Due to the accumulated oxygenated fatty acids, death occurs through the destruction of the respiratory pathway by the toxic aldehydes and free radicals formed by peroxide and epoxide decomposition (Harrington 1973).

Millard reaction, in which lipo-protein cell membranes are degraded by free radical induced lipid peroxidation reaction, has been suggested to be a basic reason of senescence and ageing (Koostra and Harrington 1969). The present study also evidenced the increased lipid peroxidation value from 0.208 to 0.710 during desiccation of seed (Table 2). Chaitanya and Naithani (1994) had observed increased lipid peroxidation in association with loss of viability in the recalcitrant seeds of *Shorea robusta*. Lipid peroxidation is a cause of membrane deterioration during desiccation of recalcitrant seeds and such damage would result in loss of semipermeability, increase in free fatty acids, and accumulation of thiobarbituric acid-reactive substances such as melondialdehyde (Senaratna and McKersie 1986). The mechanism by which water loss can generate free radicals remains under investigation. However, according to Leprince *et al.* (1994), mitochondria might be the primary source of electrons leading to the production of stable free radicals in desiccation intolerant radicles.

Generally in the seeds, the multiple protective mechanisms exist against the highly reactive oxygen radicals. They involve free radical and peroxide-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Ouedraogo *et al.* 1995). Recalcitrant seeds (or their embryos) do appear to possess antioxidant mechanisms (Hendry *et al.* 1992). However, these protective mechanisms may become impaired under conditions of water stress (Smith and Berjak 1995). Certainly, they are ineffectual in terms of protecting against desiccation damage. In the present study, the antioxidant enzymes like peroxidase and the catalase activities were increased with mild stress and thereafter a sharp decline in their activity was evident (Figure 1). This indicates that oxygen free radicals are continuously produced in the seeds. When exposed to stress they produce the enzymes in large amounts to eliminate the free radicals. As they exceed the eliminating ability, the seed system is damaged. The peroxidase enzyme is also involved in the dehydrogenation of a large number of organic compounds like phenols and aromatic amines. The destruction of the enzyme can lead to the accumulation of toxic substance in the seed. Szezotka (1974) observed a very high amylolytic enzyme activity at the beginning of storage in *Quercus robur* and *Q. borealis*. However, it quickly declined to non-detectable levels by eight months. A similar result was observed in arecanut in that the α-amylase activity was maximum (0.061 µg maltose mg^{-1} min^{-1}) in the fresh seeds. It then decreased with moisture loss to very minimum level of 0.004 µg maltose mg^{-1} min^{-1} (Table 2).

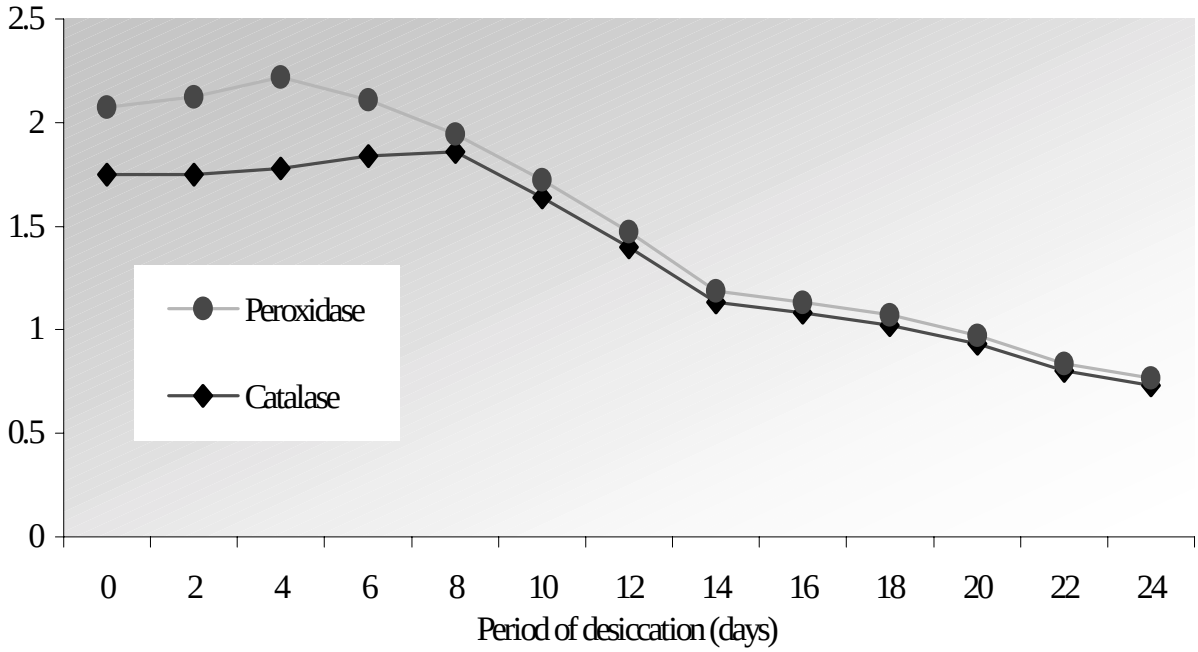

Figure 1. Effect of desiccation on catalase (µg H_2O_2 g^{-1} min^{-1}) and peroxidase (OD g^{-1} min^{-1}) activity in arecanut.

Polyphenol oxidase, another enzyme capable of degrading H_2O_2 and using as an electron acceptor, is capable of catalyzing the same type of reactions as peroxidase (Kahn 1983). In the present study, arecanut seeds retained active metabolism and thus an enhanced level of polyphenol oxidase activity (0.099 OD g^{-1} min^{-1}) during the initial drying period (Table 2). The suppression of polyphenol oxidase activity during later desiccation might be due to loss of moisture content associated with the seed viability. Nkang *et al.* (2000) also found the same result in seeds of *Telfairia occidentalis*. Clegg (1979) has suggested that the structured water in the seed be involved in ensuring the precise functioning of these multi-enzyme systems. All metabolic activities probably do take place in structured water. Loss of structured water results in the disruption of metabolism. In the case of orthodox seeds, this presumably does not occur, as shown by their tolerance to desiccation. But in recalcitrant seeds, the situation is quite different (Berjak *et al.* 1984).

Conclusion

The result of the experiment indicates that the desiccation of arecanut seed leads to the disturbance of its internal mechanisms and biochemical losses, which ultimately a lead to loss in viability.

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Effect of Wax Coating on Storage of Recalcitrant Arecanut (*Areca catechu* L.) Seeds

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Abstract

The wax-coated arecanut seeds stored in a gunny bag under ambient conditions successfully extended the storage period up to 50 days with 60 percent germination. Uncoated seeds stored in ambient condition lose their viability completely within 24 days. Generally in arecanut seeds the speed of germination, seedling vigour and vigour index values were decreased with increased storage periods. However, the reduction was less in wax-coated seeds stored in a gunny bag. Electrical conductivity, free sugar, and lipid peroxidation values increased during storage of arecanut seeds, but the wax-coated seeds stored in a gunny bag recorded minimum increase compared with other treatments.

Keywords: Arecanut, recalcitrant seed, storage, wax coating

Introduction

Arecanut (*Areca catechu* L.) or betelnut, is an important commercial plantation crop of India and accounts for 85 percent of world's output. It is mainly propagated through seed; the seed has been classified as `recalcitrant' (Raja 2001). Recalcitrance can be defined as a deviation from the normal behaviour. It is very difficult to store these seeds for a long period as they lose viability when desiccated. These seeds are also very sensitive to freezing temperatures. Seeds are living things and as such must be handled with great care whether they are intended primarily for planting or as a genetic resource. Reports indicate that the recalcitrant seeds can only be stored for short periods, a few months to a year. So, any improvement of short-term storage will be valuable and will ease the problem of field collection and transportation to gene banks.

Maintaining high seed moisture content and storing seeds in the containers that allow some gaseous exchange is important to preserve the viability. This method, involving the storage of seeds in various gases or in sealed containers or by waxing, has had some success (Chin, 1989). In the present study, it is necessary to store the recalcitrant arecanut seeds for short term transportation to the nurseries or gene banks without loss of moisture from the seed. Hence, a study was conducted to reduce the moisture loss by coating the seeds with wax and storing in different conditions.

Materials and Methods

The fully matured uniform size areca fruits, also called ‘seed nuts’, were collected and coated uniformly with a thin layer of paraffin wax (melting point 58-60°C) boiled in a vessel. The coating process was carried out rapidly to avoid heat injuries. The wax coated and uncoated fruits were packed in different containers and stored under ambient condition as follows:

T_1 - Uncoated seeds in open condition (control)
T_2 - Uncoated seeds in a gunny bag
T_3 - Uncoated seeds in a polythene bag (350 gauge)
T_4 - Wax coated seeds in open condition
T_5 - Wax coated seeds in a gunny bag
T_6 - Wax coated seeds in a polythene bag (350 gauge)

The samples were drawn at five-day intervals, and the quality characters were assessed after removing the wax from the seed. The germination test was conducted in sand medium with 100 seeds for each replication at 25 ± 2°C and 95 ± 2 percent relative humidity (ISTA 1999). At the end of 90 days (Nagwekar *et al.* 1997), the number of normal seedlings was counted and germination percentage was calculated. The seedling vigour parameters such as speed of germination (Maguire 1962) and vigour index [germination percentage x seedling length (cm)] (Abdul-Baki and Anderson 1973) were also measured during the viability assessment. In addition,

the biochemical changes such as electrical conductivity (Presley 1958), free sugar (Somogyi 1952) and lipid peroxidation (Bernheim *et al.* 1948) levels of the differently-stored seeds were observed in each period of evaluation.

Results and Discussion

Recalcitrant seeds are called such because of the difficulties in handling and storage. These seeds do not tolerate desiccation and low temperatures, both pre-requisites for optimal storage and hence it is necessary to store these seeds without incurring moisture loss. In the present investigation, it was observed that coating seeds with wax had a significant effect during the storage. Regarding the seed moisture content, the uncoated and wax coated seeds stored in a polythene bag recorded maximum moisture content irrespective of storage periods (Table 1). This is perhaps due to the slow moisture loss in polythene bag-stored seeds. Polythene bags of 350 gauge were used in the present study. These bags are moisture impervious and vapour pervious (Bhattacharyya and Basu 1992). Similar superiority in conserving the seed moisture was reported by Oliveira and Valio (1992) in *Hancornia* and Madhusudhanan and Babu (1994) in nutmeg.

Wills *et al.* (1981) stated that the wax formulations on fruits generally inhibit senescence. For example, the most successful storage method for cocoa could be storing the seeds within pods coated with paraffin wax. In the present investigation, similar results were observed when Arecanut seeds were coated with paraffin wax. The seeds coated with paraffin wax and stored in a gunny bag recorded maximum germination (60 percent) even after 50 days of storage (Table 1). Friend (1964) stored cocoa seeds within pods coated with paraffin wax. Litchi seeds retained within their fruits and treated with benomyl (0.05%) and wax emulsion (6%) and sealed in polythene bags maintained 42 percent viability for 24 days (Ray and Sharma 1987). The viability retained due to wax coating might be due to a reduced rate of respiration and moisture loss. Shivarama Reddy (1987) reported similar result in coffee seeds and stored the seed up to 300 days with 49 percent germination. Chin (1989) stated that this type of controlled atmosphere storage would have much practical application in the storage of recalcitrant seeds. Coating had little effect on internal CO_2, O_2 and C_2H_4 levels and a great effect on reducing water loss (Hagenmaier and Baker 1993; Amarante 1998). Conversly, Umboh (1987) reported that the coating of seeds with substances such as paraffin wax might be expected to restrict oxygen access to the seed and reduce storage life in *Shorea javanica.*

In the present investigation, wax coated seeds stored in a gunny bag registered maximum speed of germination and vigour index up to 50 days of storage followed by wax coated seeds stored in open condition (Table 2). Thus, the thin film of wax layer on the seeds helped minimize the respiratory rates, resulting in significantly higher germination percent and seedling vigour. Ray and Sharma (1987) reported similar results in litchi and Shivarama Reddy (1987) in coffee seeds.

Even though uncoated and wax coated seeds stored in polythene bag registered maximum moisture content, they lose their viability and vigour rapidly. The reason might be due to the increased toxic gases and moisture contents released from the seed. Tompsett (1992) stated that ventilation of recalcitrant seed is needed to remove the excess toxic gases and to prevent ‘anoxia’.

Electrical conductivity and free sugar content in seed leachate were lower in wax coated seeds stored in a gunny bag followed by wax coated seeds stored in open condition (Table 3). The reason might be due to the slower loss of moisture in wax coated seeds when compared with uncoated seeds. In addition, the values were increased with the advanced storage periods. Chin *et al.* (1981) suggested that the reduction in moisture content could cause a loss of membrane integrity and nuclear disintegration. It promotes leaching of free sugars, amino acids, inorganic phosphorus in *Shorea robusta* (Nautiyal and Purohit 1985), in *Artocarpus heterophyllus* (Bhattacharyya and Basu 1992), and in *Camellia sinensis* (Berjak *et al.* 1993).

Drying of desiccation sensitive seeds induced damage in cell membranes that could not be repaired after imbibition and finally resulted in loss of viability (Kundu and Kachari 2000). Copeland and McDonald (1995) have suggested the depletion of food reserves and accumulations of toxic substances are two of the causes of seed deterioration. Farrant *et al.* (1988) reported that highly recalcitrant seeds lose viability with sudden loss of water, gradual deterioration of cellular organization, and breakdown of cellular storage components. Similar occurrences in arecanut seeds cannot be ruled out.

In the present investigation, a lower level of lipid peroxidation was observed in the seeds coated with wax and stored in a gunny bag (Table 4). The reason might be due to the retention of moisture during storage of wax coated seeds. Uncoated seeds registered the maximum lipid peroxidation value, related positively with loss of

moisture content. Nkang *et al.* (2000) observed similar results with desiccation of *Telfairia occidentalis* seeds. Free radical activity has been associated with viability loss in several recalcitrant seeds during desiccation (Finch- Savage *et al.* 1994; Chaitanya and Naithani 1994). Various authors (Senaratna and McKersie 1986; Li and Sun 1999) have supported the idea that lipid peroxidation is a cause of membrane deterioration during desiccation and that the damage would result in loss of semipermeability, an increase in free fatty acids, and an accumulation of thiobarbituric acid-reactive substances such as melondialdehyde. Results obtained by Senaratna *et al.* (1987) with microsomal membranes seem to show that free radicals induce desertification of membrane phospholipids rather than change in fatty acid saturation.

Conclusion

The results revealed that the wax coating of seeds is one method of controlled storage for recalcitrant seeds and therefore, it could be used as a short-term storage method for transit of arecanut seeds.

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Table 1. Effect of wax coating and containers on seed moisture content and germination during storage in arecanut.

Treatments / Periods	Moisture content (%)							Germination (%)						
	T_1	T_2	T_3	T_4	T_5	T_6	Mean	T_1	T_2	T_3	T_4	T_5	T_6	Mean
(Days)														
0	48.1	48.1	48.1	48.1	48.1	48.1	48.1	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5	43.6	45.3	46.7	47.3	45.2	46.5	45.7	90 (71.56)	95 (80.78)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	98 (85.39)
10	40.2	45.1	44.6	44.8	41.8	46.3	43.8	60 (50.77)	95 (80.78)	95 (80.78)	100 (90.00)	100 (90.00)	100 (90.00)	92 (80.39)
15	27.1	44.7	45.3	44.1	41.6	43.8	41.1	50 (45.00)	95 (80.78)	95 (80.78)	95 (80.78)	100 (90.00)	100 (90.00)	89 (77.89)
20	20.7	42.1	45.0	43.0	41.2	43.0	39.2	20 (26.56)	95 (80.78)	85 (67.50)	90 (71.56)	100 (90.00)	100 (90.00)	82 (71.07)
25	17.0	36.6	43.7	42.9	41.1	42.9	37.4	0 (0.00)	85 (67.50)	80 (63.43)	85 (67.50)	95 (80.78)	70 (56.86)	69 (56.02)
30	13.0	29.4	43.8	42.2	40.9	42.8	35.3	0 (0.00)	70 (56.79)	55 (47.89)	60 (50.77)	95 (80.78)	20 (26.56)	50 (43.79)
35	11.9	18.0	42.6	29.4	39.3	40.0	30.2	0 (0.00)	10 (18.43)	35 (36.22)	40 (39.23)	90 (71.56)	15 (22.50)	32 (31.33)
40	11.8	15.7	42.6	27.7	35.4	40.7	29.0	0 (0.00)	0 (0.00)	20 (26.56)	35 (36.22)	90 (71.56)	0 (0.00)	24 (22.39)
45	10.6	15.5	42.4	19.9	34.4	40.1	27.1	0 (0.00)	0 (0.00)	10 (18.43)	15 (22.50)	60 (50.77)	0 (0.00)	14 (15.28)
50	10.5	12.0	42.4	15.6	34.5	40.8	25.9	0 (0.00)	0 (0.00)	0 (0.00)	10 (18.43)	60 (50.77)	0 (0.00)	12 (11.53)
55	9.1	10.6	41.4	14.8	31.1	42.8	25.0	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	35 (36.22)	0 (0.00)	6 (6.04)
Mean	22.0	30.2	44.0	35.0	40.0	42.7		27 (23.66)	54 (46.32)	56 (50.14)	61 (54.75)	85 (74.37)	50 (46.33)	
			T	P	T x P					T	P	T x P		
SEd			0.64	0.91	2.24					1.43	2.03	4.97		
CD (P=5%)			1.29	1.82	4.47					2.86	4.05	9.92		

(Values in parentheses indicate arc sine values)

Table 2. Effect of wax coating and containers on speed of germination and vigour index during storage in arecanut.

Treatments / Periods	Speed of germination							Vigour index						
	T_1	T_2	T_3	T_4	T_5	T_6	Mean	T_1	T_2	T_3	T_4	T_5	T_6	Mean
(Days)														
0	0.14	0.14	0.14	0.14	0.14	0.14	0.14	2935	2935	2935	2935	2935	2935	2935
5	0.14	0.16	0.14	0.14	0.14	0.14	0.14	2311	2528	2514	2522	2610	2424	2484
10	0.11	0.18	0.15	0.14	0.14	0.15	0.15	1721	2757	2747	2279	2800	2690	2499
15	0.09	0.18	0.18	0.16	0.17	0.18	0.16	1691	3058	3185	2901	3301	3162	2883
20	0.03	0.21	0.17	0.15	0.18	0.17	0.15	333	3384	2189	2556	3203	2548	2369
25	0.00	0.16	0.11	0.13	0.16	0.09	0.11	0	2620	2038	2399	2807	1524	1898
30	0.00	0.13	0.10	0.10	0.16	0.03	0.09	0	1937	1114	1670	2882	438	1340
35	0.00	0.01	0.07	0.07	0.15	0.02	0.05	0	151	425	857	2599	249	713
40	0.00	0.00	0.06	0.05	0.12	0.00	0.04	0	0	303	718	2483	0	584
45	0.00	0.00	0.02	0.02	0.11	0.00	0.03	0	0	214	260	1600	0	345
50	0.00	0.00	0.00	0.02	0.11	0.00	0.02	0	0	0	127	1465	0	265
55	0.00	0.00	0.00	0.00	0.06	0.00	0.01	0	0	0	0	807	0	134
Mean	0.04	0.10	0.10	0.09	0.14	0.08		749	1614	1472	1602	2457	1330	
			T	P	T x P					T	P	T x P		
SEd			0.0009	0.001	0.003					15.6	22.1	54.2		
CD (P=5%)			0.0018	0.002	0.006					31.2	44.1	108.2		

Table 3. Effect of wax coating and containers on electrical conductivity and free sugars during storage in arecanut.

Treatments / Periods	Electrical conductivity (dSm^{-1})							Free sugars (mg $seed^{-1}$)						
	T_1	T_2	T_3	T_4	T_5	T_6	Mean	T_1	T_2	T_3	T_4	T_5	T_6	Mean
(Days)														
0	0.108	0.108	0.108	0.108	0.108	0.108	0.108	7.2	7.2	7.2	7.2	7.2	7.2	7.2
5	0.227	0.131	0.141	0.144	0.158	0.119	0.153	15.8	8.4	14.5	10.9	7.4	12.3	11.5
10	0.280	0.230	0.169	0.191	0.193	0.185	0.208	25.6	17.5	17.3	20.7	8.1	12.6	16.9
15	0.448	0.392	0.201	0.341	0.264	0.205	0.308	43.4	25.6	21.4	34.3	8.8	17.5	25.1
20	0.585	0.393	0.291	0.443	0.280	0.257	0.375	43.1	26.3	22.1	35.0	8.8	17.9	25.5
25	0.593	0.395	0.414	0.464	0.375	0.299	0.423	52.5	28.4	26.3	38.2	9.1	22.8	29.5
30	0.603	0.437	0.432	0.481	0.424	0.353	0.455	55.7	33.3	30.9	41.0	9.8	27.7	33.0
35	0.610	0.464	0.454	0.490	0.454	0.529	0.500	61.6	37.1	32.9	42.0	10.5	25.2	39.4
40	0.849	0.674	0.578	0.534	0.462	0.601	0.616	73.2	56.4	55.0	43.4	37.1	57.8	53.8
45	0.866	0.687	0.574	0.554	0.473	0.624	0.629	90.0	103.6	78.1	76.7	70.7	100.5	86.6
50	0.877	0.703	0.574	0.598	0.489	0.682	0.654	136.0	116.2	112.0	89.3	99.8	134.8	114.7
55	0.897	0.739	0.607	0.606	0.516	0.742	0.684	146.7	144.7	142.8	120.8	120.4	153.7	138.2
Mean	0.578	0.446	0.378	0.413	0.350	0.392		62.5	50.4	46.7	46.6	33.1	51.4	
			T	P	T x P					T	P	T x P		
SEd			0.006	0.009	0.02					1.06	1.50	3.68		
CD(P=5%)			0.013	0.018	0.04					2.11	2.99	7.34		

Table 4. Effect of wax coating and containers on lipid peroxidation during storage in arecanut.

Treatments / Periods (Days)	Lipid peroxidation (OD value)						
	T_1	T_2	T_3	T_4	T_5	T_6	Mean
0	0.115	0.115	0.115	0.115	0.115	0.115	0.115
5	0.164	0.144	0.118	0.120	0.118	0.119	0.130
10	0.305	0.255	0.154	0.184	0.176	0.201	0.212
15	0.405	0.258	0.206	0.208	0.190	0.256	0.254
20	0.732	0.507	0.288	0.279	0.214	0.272	0.382
25	0.797	0.603	0.314	0.357	0.290	0.317	0.446
30	0.940	0.699	0.406	0.401	0.328	0.405	0.530
35	1.037	0.948	0.501	0.475	0.447	0.432	0.640
40	1.115	1.033	0.633	0.533	0.459	0.482	0.709
45	1.153	1.084	0.707	0.540	0.543	0.572	0.766
50	1.214	1.177	0.851	0.651	0.648	0.601	0.857
55	1.226	1.199	0.915	0.714	0.689	0.673	0.902
Mean	0.767	0.668	0.434	0.381	0.351	0.370	
		T	P	T x P			
SEd		0.007	0.01	0.02			
CD(P=5%)		0.014	0.02	0.05			

Association of Seed-Borne Microflora and their Management in Recalcitrant Arecanut (*Areca catechu* L.) Seed

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Abstract

The composition of the microflora on and in recalcitrant arecanut seeds has been found to narrow with increasing storage period. The fungal species *viz.*, *Fusarium* spp., *Penicillium* spp., *Aspergillus flavus, A. niger, Rhizopus stolonifer, Trichoderma* spp. and *Botryodiplodia theobromae* were found to be associated with arecanut seed. Generally, the infection in seed could be prevented or minimized by seed treatments. In this case, the present study showed that the association of *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus stolonifer* and *Botryodiplodia theobromae* in the untreated control increased from 7 to 21 days after incubation. However, the incidence of the above organisms was found to be lower in nuts treated with *Pseudomonas fluorescens* @ 10 g kg^{-1} followed by carbendazim or thiram @ 2g kg^{-1} at all periods.

Keywords: Arecanut, management, recalcitrant seed, seed-borne microflora

Introduction

Arecanut (*Areca catechu* L.) is an important commercial plantation crop mainly propagated through seed. The seed has been classified as `recalcitrant' (Raja 2001). Most of the recalcitrant seeds are from the moist warm tropical forest environment. Therefore, the relatively high moisture contents and temperatures that tropical recalcitrant behaviour demands also favour profuse growth of pathogenic (and nonpathogenic) microorganisms (Bonner 1995). There can be no doubt in the mind of any investigator working on desiccation–sensitive (recalcitrant) seeds that microorganisms, more particularly fungi, play a significant role in post harvest deterioration (Berjak 1995). Hence, the present study has been undertaken to find out the pathogens associated with recalcitrant seed storage.

Materials and Methods

Freshly collected arecanut seeds of apparently healthy nature were given the following treatments:

T_0 - Untreated control

T_1 - Carbendazim @ 2 g kg^{-1} of seed

T_2 - Thiram @ 2 g kg^{-1} of seed

T_3 - *Pseudomonas fluorescens* @ 10 g kg^{-1} of seed

T_4 - *Trichoderma harzianum* @ 4 g kg^{-1} of seed

Treated seeds were packed separately in a gunny bag and stored at ambient conditions of temperature (28 ± 2°C) and relative humidity (75 ± 2%). Treated seeds were assessed at weekly intervals for the association of seed-borne microflora by the seed wash technique. Five seeds per replication were washed in 50 ml of sterile distilled water under aseptic conditions. A ten ml sample of seed wash was diluted to 10^{-4}. One ml of the 10^{-4} dilution was transferred to a sterile petri plate. To it, 15 ml of potato dextrose agar (PDA) was poured and incubated for three days at room temperature under 12 h of alternate cycles of near ultra violet (NUV) light and darkness. The various microflora associated with the seed were identified under stereozoom microscope. The seed-borne microflora were assessed at intervals of 7, 14 and 21 days after incubation.

Results and Discussion

The experimental results indicated that fungal species viz., *Fusarium* spp., *Penicillium* spp., *Aspergillus flavus, A. niger, Rhizopus stolonifer, Trichoderma* spp. and *Botryodiplodia theobromae* were found to be associated with different days of incubation. Anon. (1961) has reported a number of fungi from the husk and kernel of the areca seed nuts, which include *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp**.,** *Diplodia* spp., *Mucor* spp. and

Thielaviopsis spp. Some of the bacteria associated are *Phomopsis heteronema* (Butler and Bisby 1931) and *Colletotrichum gloeosporioides* (Saraswathy *et al.* 1977). Nambiar and Nair (1970) stated that during dropping of the bunches on the ground, the nut surface is mechanically injured by abrasion, and these abraded areas serve as entry points for the microorganisms. In such nuts, microorganisms like *Aspergillus niger, A. flavus, Botryodiplodia theobromae* and *Rhizopus* spp. were identified. Several other workers also found a number of microorganisms including, *Aspergillus niger arecae* (Lal and Chandra 1953), *Subramanella arecae* (Srivastava *et al.* 1962), *Aspergillus chevalieri* (Anon. 1971), *Aspergillus* spp. and *Penicillium* spp. (Koti Reddy *et al.* 1978) in arecanut.

The infection in arecanut can be prevented or minimized by eliminating soil contact at the time of harvest (Nambiar and Koti Reddy 1979). In the present investigation, association of *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus stolonifer* and *Botryodiplodia theobromae* in the untreated control was found to increase from 7 to 21 days after incubation. Mycock and Berjak (1990) reported that the composition of the microflora on and in recalcitrant seeds has been found to narrow with increasing storage period. In arecanut, the incidence of above organisms was found to be lower in nuts treated with *Pseudomonas fluorescens* @ 10 g kg^{-1} followed by carbendazim or thiram @ 2g kg^{-1} at all periods. Association of *Fusarium* spp., *B. theobromae, Penicillium* spp., *Aspergillus* spp. and *R. stolonifer* in the untreated control was found to increase from 7 to 21 days after incubation. Also the association of *Trichoderma* spp. ranged from 0 to 31 cfu x 10^4 ml^{-1} of seeds incubated up to 21 days, irrespective of various treatments.

Among the various treatments, the lowest association of *Trichoderma* spp. (0.0) was observed in the control and carbendazim treated seeds, whereas the highest of 31 x 10^4 cfu ml^{-1} was estimated in the *T. harzianum* treated seeds. The seeds treated with *P. fluorescens* recorded 9, 4 x 10^4 cfu ml^{-1} of *A. flavus* and *A. niger* at 21 days after incubation, respectively. The rest of the fungal flora including the seed-borne pathogen *B. theobromae* was completely suppressed after 21 days of incubation. Similarly, carbendazim and thiram were equally effective in reducing the association of fungal microbes 21 days after incubation.

In arecanut**,** *B. theobromae* was reduced to an extent of 72.7 percent over the control in the seeds treated with *T. harzianum* whereas a 100 percent reduction was observed with carbendazim, thiram and *P. fluorescens*-treated seeds. Anon. (1962) recorded minimum percentage of infection with fungicidal treatments and among the fungicides, blitox was found to be effective in arecanut. Several other workers have studied the effectiveness of seed treatment with fungicides viz., King and Roberts (1982) and Hor (1988) in cocoa. Similarly, seed treatment with *Pseudomonas fluorescens* was effective against the seed microflora in bean (Alstrom 1991), in tobacco (Maurhofer *et al.* 1994), and in rice (Vidhyasekaran *et al.* 1997).

Conclusion

The observations indicated that a number of microbes are associated with the recalcitrant seeds. The high moisture content of the seed favours microbe incidence. These microbial incidences in recalcitrant seeds can be controlled to a certain extent by proper seed treatments.

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Table 1. Association of seed-borne microflora and their management in arecanut

Seed-borne microflora (cfux10^4 ml^{-1})	7 days after incubation					14 days after incubation					21 days after incubation				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
Fusarium spp.	11.0	0.0	0.0	0.0	0.0	14.0	0.0	2.5	0.0	4.0	15.0	0.0	3.0	0.0	3.0
Penicillium spp.	15.0	3.0	5.0	0.0	3.0	15.0	4.0	4.0	0.0	4.0	17.0	5.0	5.0	0.0	5.0
Aspergillus flavus	16.0	3.5	4.0	5.5	8.5	17.5	4.5	5.5	8.0	9.0	18.5	6.0	5.0	9.0	7.0
Aspergillus niger	21.5	6.0	6.0	4.5	10.0	27.0	6.0	7.0	5.0	11.0	29.0	6.0	9.0	4.0	11.0
Rhizopus stolonifer	11.0	0.0	0.0	0.0	2.0	13.0	0.0	0.0	0.0	3.0	15.0	0.0	0.0	0.0	4.5
Trichoderma spp.	0.0	0.0	2.0	0.0	23.0	3.0	0.0	0.0	0.0	25.0	5.5	0.0	0.0	0.0	31.0
Botryodiplodia theobromae	6.5	0.0	1.0	0.0	0.0	10.0	0.0	0.0	0.0	1.5	11.0	0.0	0.0	0.0	3.0
			T	M	T x M			T	M	T x M			T	M	T x M
SEd			0.29	0.35	0.77			0.33	0.39	0.88			0.38	0.45	1.01
CD (P=5%)			0.59	0.70	1.57			0.67	0.80	1.78			0.77	0.91	2.04

Variation in Fruit Characteristics of Teak According to Various Sources

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Abstract

Teak seed germination is a serious problem. Preliminary observations on teak seed indicate that germination behaviour varies according to their provenance. Seeds from some provenances are easy to germinate while some from other provenances are difficult. Most of the reports indicate that germination inhibitors, such as thick pericarp and hard impermeable endocarp, in addition to seed emptiness, are factors affecting seed germination.

The present study was undertaken to observe the variations existing in the fruit characteristics from ten different sources in Madhya Pradesh, India. Variation was observed in colour, fruit size, thickness of mesocarp/endocarp, emptiness, floating fruit percent, water uptake, number of kernels per 100 fruit, seed germination, fruit leachate's colour, and pretreatment intensity. Significant variation has been observed in fruit mesocarp in terms of its hardness and thickness. The present study mainly emphasized the structural and morphological variations among the different sources. The distinct nature of the mesocarp may be a significant factor for the intensity of any pretreatment. Teak fruit showed a lot of variation, which can be attributed to the different sources of their origin and not linked to the experimental site conditions.

Key words: mesocarp-endocarp, water uptake, seed source, variation, pretreatment

Introduction

Teak is known as one of the most important timber plantation species in the tropics. The teak forests of Madhya Pradesh (M.P.), India, are distributed over a wide range and cannot be considered since a uniform type as the quality, density and composition changes from place to place and often within a short distance. In M.P., teak appears to be luxuriant where rainfall is above 1270mm. It thrives best in warm and moist climates although it also occurs over areas of low annual rainfall below 508mm. (Dubey 1967). The productivity of planting stock is below expectation. Seed dormancy is one of the biggest obstacles for producing large amounts of planting stock. Teak fruit requires special treatment before sowing because of special characteristics. The present study deals with the sort of variation according to site conditions and is mainly demonstrated at the populations/provenance level. Variation in morphological characteristics such as leaf size, colour, bark type, branch size and branching habit, and flowering and fruiting have been observed from provenance trials (Bor 1939). Variation in seed dormancy is also often much higher within individuals. This has been demonstrated most clearly in connection with provenance trials (Keiding 1985). Discovering a satisfactory pretreatment for teak seeds, especially from less moist or from dry areas, has been almost a century old problem. This paper deals with the sort of variations that exist in teak fruit.

Materials and Methods

Fruit collection

Fruit collection was done in the months of February and March, 2000 from different sources of M.P. India, i.e. Dewas, Nepanagar-Khandwa Division, Jhabua (forest type dry teak 5A/C2b), Seoni, Jagdalpur, Raipur, Jagmandal-Mandla Division Khari-Mandla Division (forest type semi moist teak 3Bb/C1a), and Bori-Hoshangabad (forest type moist teak 3B/C1b). Fruits of Burma teak (origin Burma teak) were collected in May 2000 from Mandla.

Fruit characteristics

Fruit colour, texture of mesocarp, thickness of mesocarp and endocarp were observed. Ten fruits were cut transversely. Endocarp was measured at two different radii from the central axis, including locules. The measurements were averaged for the final value. Mesocarp thickness was also observed at two different radii for each source. The length-width of 100 x 3 fruits was determined using a vernier calliper (West Germany). Also, 100gms x 3 fruits from each source were inspected for holes on their mesocarp, an indication of a fruit borer attack.

In addition, 100 x 3 fruits were cut using a nut cutter and the completely empty fruit and kernel percentage was calculated for each source. Then 100 x 3fruits (their inflated calyx removed properly) were immersed in water, nearly doubling the seed volume and after 24 hours number of floating fruits were calculated. After counting, floating fruits were cut to observe interior emptiness. The correlation between floating fruit and fruit emptiness was studied.

Water uptake

Fresh teak drupes were cut with a seed nut cutter, and the initial moisture percent of 100 x 3 seeds was measured using a MA30 Sartorius Moisture Balance. The instrument was set in fully automatic drying mode. Temperature setting was done at 130° C, timer setting 0.0 and range 0-100. The sample was placed on the dish, and the lid was closed. The moisture determination procedure ended automatically when a significant weight loss was no longer detected. Teak drupes of different sources were soaked in water for 24 hours after which the drupes were cut, seeds (kernels) were extracted, and seed moisture of 100 x 3 seeds was recorded.

Optical density

Twenty-five fruits were soaked in 100 ml of double distilled water for 24 hours. Fruits were separated through a fine iron sieve. The optical density of fruit leachates was measured at 620mμ using a Photoelectric Colorimeter, Model AE-11N**.**

Germination test

From each seed source, 100 x 4 kernels were placed on moist blotter paper in petri dishes and kept at 30±2° C in a seed germinator. Germinants were recorded up to 28 days.

Pretreatment

Fruits were mixed with a thick slurry (equal volume of the fruit) of fresh cow dung and tied in a black polythene sheet supported with a pipe (diameter 2 cm.) for aeration. This was kept at room temperature (average temperature was minimum 19.5°C, maximum 36.42 °C) and observed until the softening of the mesocarp. After washing, samples were rubbed between two rough surfaces to remove the mesocarp, washed again, and air-dried.

Results

Fruit characteristics of the ten seed sources are shown in Table 1. Variation in fruit colour was observed. Some fruits were shiny golden, some were dull brown, greyish golden, light brown and dull muddy. Mesocarp texture also varied from source to source in term of hardness. It was observed that mesocarp tissue was very hard in Seoni and Raipur, hard in Nepanagar, Jagmandal, Jhabua and Dewas, and corky in Khari, Bori and Jagdalpur. Mesocarp was soft corky in Mandla (Burma teak seed source).

Mesocarp thickness ranged from 1.4 mm to 4.5 mm and endocarp thickness from 3.8 to 4.3 mm. There was variation in fruit size among various sources, but when fruits of the same size from different sources were cut, it was found that endocarp and mesocarp thickness ratio varied from source to source (Fig. 1).

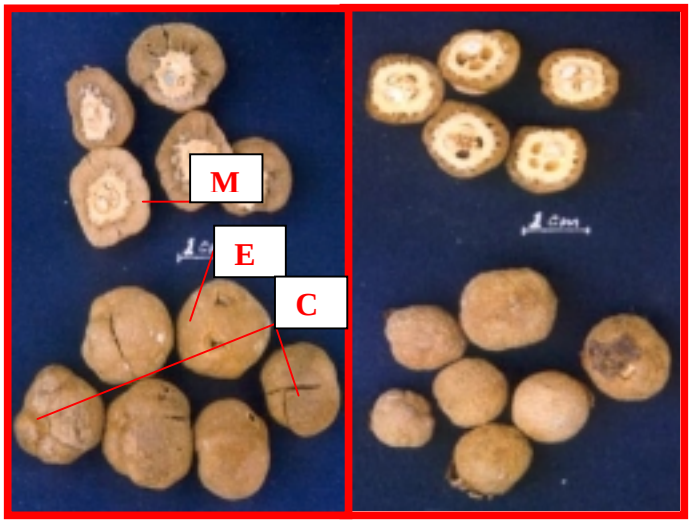
Thickness of mesocarp was observed as a specific character of Burma teak fruit. Mesocarp was much thicker than endocarp in the case of Mandla (origin Burma teak), which was a distinguishable characteristic of these fruits. On the other hand, narrow endocarp was observed in most of the cases i.e. Seoni, Raipur, Nepanagar, Khari, Bori, Dewas, Jhabua, and Jagmandal. An uncommon feature was the is 3-4 longitudinal cracks in the mesocarp (Fig. 1) observed in 35% of the Mandla (Burma teak) fruits, which is noted to be a specific character not seen in local teak fruit of M.P.

The average fruit size ranged from 10.14 to16.95 mm across. Number of fruits per liter ranged from 355 to 951, which demonstrated the great variation in fruit size by seed source. Fruits with borer damage on the mesocarp were observed in all the seed sources, ranging from 2.62 to 32.39% of the fruits.

When teak fruits were immersed in water, all fruits floated initially. Some fruits sank after few hours; when these fruits were cut open and examined, it was found that they were not empty. It was concluded that empty as well as full seed floated. After 24 hours, 44 to 76% of the fruits were still floating. The exception was that only 4% in Burma origin teak seed floated, whereas the emptiness percentage of various other sources ranged from 15 to

Dewas | Nepanagar | Jhabua | Seoni

Jagdalpur | Raipur | Jagmandal | Khari (Mandla)



Mandla (Burma Teak) | Bori (Hoshangabad)

Abbreviations used

C - Crack in mesocarp
E - Endocarp
L - Loculae
M - Mesocarp
PL - Placental axis
S - Seed
T.S. - Transverse section of fruit

Figure 1. Variation in fruit size, colour, thickness of mesocarp and endocarp followed by their sources.

52%. There is a positive correlation between floating and empty fruits. Correlation is significant at the 0.05 level, but the value of r = +0.348 which is significantly less than 1. This indicates that there was not a very strong positive correlation. The value of r should be greater than 0.5. It may be that this was a chance correlation.

Table 1. Fruit characteristics of teak of different ten sources.

S. No.	Name of seed source / Fruit characteristcs	Dewas	Nepanagar	Jhabua	Seoni	Jagdalpur	Raipur	Jagmandal (Mandla)	Khari (Mandla)	*Burma teak (Collected from Mandla)	Bori (Hoshangabad)
1	Teak forest type	Dry teak 5A/ C2b	Dry teak 5A/ C2b	Dry teak 5A/ C2b	Semi moist teak 3B/ C1a	Semi moist teak 3B/ C1a	Semi moist teak 3B/ C1a	Semi moist teak 3B/ C1a	Semi moist teak 3B/ C1a	Semi moist teak 3B/ C1a	Moist teak 3B/ C1b
2	Fruit colour	dull golden yellow	shiny golden yellow	golden yellow	golden yellow	light brown	shiny golden yellow	dull golden yellow	greyish golden yellow	dull muddy golden	light brown
3	Texture of mesocarp	hard	hard	hard	very hard	corky	very hard	hard	corky	soft corky	corky
4	Thickness of mesocarp/ endocarp in mm.	1.80/ 4.20	1.65/ 3.8	1.57/ 4.35	1.42/ 4.30	1.95/ 4.0	1.90/ 3.95	1.55/ 3.85	1.65/ 4.10	4.50/ 3.50	1.55/ 3.87
5	***Mesocarp/ endocarp ratio	1: 2.38^{bc}	1: 2.32^{bc}	1: 3.17^{c}	1: 3.0^{c}	1: 2.08^{b}	1: 2.08^{b}	1: 2.5^{bc}	1: 2.5^{bc}	1:0.78^{a}	1: 2.5^{bc}
6	%of fruit showing borer attack	5.15	8.24	2.62	9.15	9.17	16.67	26.22	7.42	32.39	8.06
7	Width/Length in mm	10.42/ 10.22	10.14/ 10.75	10.64/ 10.47	10.68/ 10.34	11.70/ 11.30	10.47/ 10.25	10.52/ 9.74	11.4/. 9.30	16.95/ 13.25	10.42/ 10.32
8	No. of fruits per liter	872	733	877	826	710	723	816	893	355	951
9	**Floating fruits after 24 hours in %	69	76	50	47	75	70	53	44	4	63
10	**Fruits bearing no seeds in %	32	31	26	39	37	32	25	15	31	52
11	Seed germination %	38	46	13	31	26	30	27	67	31	25
12	Initial seed moisture in %	8.34 (±0.89)	9.04 (±0.87)	8.79 (±1.01)	9.11 (±1.02)	8.66 (±1.59)	10.43 (±1.08)	8.93 (±0.63)	9.19 (±0.62)	12.05 (±1.17)	9.73 (±1.79)
13	Seed moisture % after 24 hours fruit imbibition	26.68 (±1.08)	28.61 (±0.69)	26.05 (±1.25)	27.75 (±2.01)	28.67 (±1.37)	28.86 (±0.79)	28.79(±2. 35)	27.39 ±1.54)	36.67 (±1.55)	27.96 (±1.74)
14	***Optical density of fruit leachates	0.27^{a}	0.50^{ef}	0.52^{f}	0.34^{bc}	0.40^{d}	0.32^{b}	0.33^{b}	0.37^{cd}	0.32^{b}	0.42^{e}
15	***No. of days taken during cow-dung slurry treatment	7^{ab}	8^{b}	8^{b}	6^{a}	12^{d}	15^{e}	10^{c}	8^{b}	18^{f}	12^{d}

* 3-4 cracks in mesocarp were observed in 35% of fruits.

** Correlation coefficient r = 0.348.

*** Means within rows followed by the same letters are not significantly different from each other at the 5% level, by the F-LDS test.

Values given in parentheses are standard deviation.

Teak fruit is a quadra-locular drupe but there were very few fruits which contain 4 seeds (kernels). Some fruits contain 3 kernels, some 2, some only one and some had no kernels. The highest percentage of emptiness was observed in Bori fruits.

Water uptake by teak seed is shown in Table 1. Initial moisture percent of teak seed (kernel) ranged from 8.34 to12.05%. After 24 hours of imbibition of fruit, seed moisture from 26.05 to36.67% was recorded. Increase in seed moisture, when they are encased in their pericarp, indicated the presence of water in the locule of the fruit.

Colour difference in fruit leachates of various sources was observed, i.e. light brown to dark brown, ranging in optical density from 0.27 to 0.58. Number of days recorded during the cow dung slurry treatment was 6-18.

Seed germination of freshly collected fruits of various sources was observed after cutting the drupes. Germination percentage of seed ranged from 13 to 67%, showing great variation. Highest germination i.e. 67%, was recorded for the Khari seed source.

Discussion

There are considerable variations in fruit characteristics among the various seed sources as well as within the crop of the same source. Teak provenances at the FAO-Danish Forest Tree Centre at Humlebaek gave an average of 2062 fruit per kilogram with variation from 1070 to 3467 fruit per kilogram. The diameter of fruit was highly variable for the different provenances. Variability in morphological characters, fruit diameter, shape, colour, outer surface, thickness of mesocarp and seed size were studied for the clonal seed orchards (Gogate 1993). During observation, major differences between Burma teak, which has been collected from Mandla, and local teak of M.P. were noted in terms of its size, softness of mesocarp and thickness of mesocarp. The distinguishing features of all the seed sources are shown in Fig. 1.

These variations indicate that method of pretreatment has been developed locally based on the experiences over a number of years with seed from more or less the same source. However, because of the great variation in average seed size and thickness and hardness of mesocarp from different sources, it is very difficult to state the exact limits of how seeds should be differently treated. The present study indicated that there is size variation among the sources even within the crop of the same source but texture of mesocarp and mesocarp/endocarp ratio, and fruit colour was not as varying. Fruit emptiness also varied from source to source, and it was not restricted to floating fruit. Empty fruits (fruits without seed) as well as seed bearing fruits were observed floating even after 24 hours.

Eidmann (1933) concluded that larger diameter is mainly caused by the spongy mesocarp. In the present study, the larger diameter of Burma fruit, which was collected from Mandla, was due to thickness of the mesocarp, but the big fruit of various sources of local M.P. teak showed that the larger area of fruit is covered by stony endocarp rather than by mesocarp. Mesocarp and endocarp ratio varied from source to source even in the same size fruit. According to Beekman (1949), fruit of Indian origin (Godavari, Madhya Pradedh, and Malabar) have an extremely thin mesocarp and are smaller in size compared to Burmese and Indonesian seeds. Teak seeds from different provenances in India, both from dry and moist localities, have exhibited large variation in the capacity of and time taken for germination and responiveness to pretreatment. Pretreatment and germination media requirements of teak seeds vary with the provenance (Madan Gopal *et al*. 1972). Gupta and Pattanath (1975) also advocated that the pretreatment requirements must vary from one seed source to another, since seed from different sources behave differently. Most of the reports reveal that impermeable woody endocarp and felty mesocarp were the only obstacles to seed germination, but the present study clearly proved that the teak fruit was highly permeable to water. Teak fruit structure gives the appearance of being highly permeable (Mackenzie and Jones 1998). There is no evidence of existing physical dormancy in teak (Rajput and Tiwari 2001). Treatment intensity seems related to the thickness of mesocarp. A higher number of days i.e. 18, are taken by Burma teak fruit having thicker mesocarp. On the other hand, 6 days were taken by Seoni teak fruit during treatment, and these fruit have narrower mesocarp. According to Pattanath (1980), different seed sources require different pretreatments. Most of the conventional methods have the drawback that a large proportion of the seeds is rendered useless by deterioration, which may be attributed to the permeable nature of the pericarp.

Conclusions

The lack of uniformity in fruit characteristics is a very big problem in teak fruit pretreatment. The complex nature of the fruit is one of the major problems when dealing with teak seed in the nursery. A general prescription for pretreatment is difficult because of the variation in fruit structure; these fruit features and characteristics account for nursery practices, especially pretreatment intensity. During the present observations, fruits of Burma teak, Jagmandal and Khari collected from the same locality have great variation. Variation between fruit characteristics of same localities but of different origin demonstrates the strong hereditary character of teak. Provenance trials of teak suggest that genetic differences exist between sources within

provenances. There is a need for evolving a more dependable and efficient method of raising seedlings by solving the problems of germination of teak seed. It is imperative to examine the fruit structure of various sources. Structural variation in teak fruit should be considered along with the factors which affect germination such as light, temperature, media and humidity. Even after decades of research on teak seed, there are still many problems requiring future research.

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Quercus rotundifolia Germination Related to Forest Fires: The Effect of High Temperatures, Ash, Smoke and Charcoal

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Abstract

The geographical distribution of *Quercus rotundifolia* covers a large area of the Mediterranean Basin. This species occupies the continental, subcontinental or coastal areas of Spain, but always under climatic conditions determined by warm and dry summers. It is precisely in the Mediterranean Basin that fire has been a very frequent ecological factor for a long time, both when produced naturally or through use by man.

In this work we have proposed the study of the effects of fire factors on the germination of *Q. rotundifolia*. We have analysed the effect of the high temperatures, ash, charcoal, and smoke, on germination. For this, we have performed a germination test over a five month period in which the following treatments were tested: Control, 60ºC-5min, 60ºC-15min, 90ºC-5min, 110ºC-5min, 150ºC-5min, a dilution of ashes, ashes added directly, charcoal and smoke applied during 5, 10 and 15 minutes.

We have found that the rate of germination in this species, in absence of any treatment, is very high (close to 100%) and that, in almost all the treatments applied, the germination rate was above 84%. A strong inhibition in germination is detected only when the applied temperatures are very high (150ºC-5min). This is probably because the heat kills most of the seeds.

Introduction

The populations of species of the genus *Quercus* were the dominant component of the sclerophyllous forests that at one time dominated vast areas of the Mediterranean Region (Herrera 1995). Within the Iberian Peninsular, the populations of *Quercus rotundifolia* (also known as *Quercus ilex* subesp *ballota*, Rico-Hernández 1992) are found in continental, subcontinental, and coastal zones with Mediterranean climates. In the rest of the world, the geographic distribution of this species is centred in the area of the Mediterranean Basin (S.S. A.A. 1999). In Spain *Q. rotundifolia* is normally found forming part of the "dehesa" (i.e. open forest with an herbaceous layer occasionally mixed with scrub). The "dehesa" is used as traditional grazing for cattle and sheep and the scrub is burnt frequently in small patches.

The individuals of this species are sclerophyllous and perennial trees that are very resistant to the summer droughts and the low temperatures of the continental winters. The substratum on which they grow is very varied, from granite and slate to sedimentary soils (Rico Hernández 1992).

In the Mediterranean Basin, fire has been an element used by man since the Neolithic for agricultural management and game preservation (Trabaud and Casal 1989). Today, forest fires continue to be frequent in this zone and for this reason, there is an interest in understanding the germination behaviour of this species with relation to fire.

Therefore, we have centred this study on the observation of the effects of the high temperatures, ash, smoke and charcoal on the germination response of the seeds from *Q. rotundifolia*.

Materials and Methods

The biological materials used for this study were acorns from *Quercus rotundifolia*, collected directly from the mother trees in Ricobayo (province of Zamora, Spain) in October 2000. The type of soil on which the population developed was a sandy and acidic soil, with predominance of Cambisols on granite bedrock. The climate is Mediterranean, with an average annual rainfall of 660mm that decreases during the summer. Between the months of May and September, the rainfall is only 23% of the annual value (Fernández-Santos et al. 1999).

The seeds were maintained at 4ºC from their collection in October 2000 until the experiment was begun in December 2000. The treatments applied were Control, 60ºC-5min, 60ºC-15min, 90ºC-5min, 110ºC-5min 150ºC-5min, Dilution of Ash, Ash, Charcoal, Smoke-5min, Smoke-10min and Smoke-15min. The thermal treatments

were applied by introducing the seeds into a forced-air stove for the fixed time. In the Ash Dilution treatment, 120ml of a 5g/L dilution of ash in distilled water was added to the seeds. The Ash treatment was performed by adding 0.168g of *Q. rotundifolia* ash to each of the incubation trays. This amount of ash was selected taking into account the surface of the trays and the fact that in a real fire, 8g of ash/m^2 was registered (Soto et al. 1997). The Charcoal treatment was applied by incubating the seeds of each tray in the presence of 1 g of crushed holm-oak charcoal. The Smoke treatment was achieved by exposing the seeds during 5, 10, or 15 minutes to a smoke saturated atmosphere. The smoke was produced by burning slender branches of *Q. rotundifolia* and the methodology was that of Casal et al. (2001), based on De Lange & Boucher (1990) and Baxter *et al.* (1994).

The seeds were incubated in plastic trays with a surface area of 210cm^2 containing perlite to favour humidification. Six replicas of 25 seeds each were prepared for each treatment. Incubation of the seeds was begun on the 26-12-2000 and lasted 5 months. Germination was checked weekly and the percentage germination rate, the time required for 50% of total germination (T_{50}), and the germination/time distribution ratio was calculated from the data obtained.

Both the germination rate and the T_{50} were subjected to Variance Analysis to detect whether there are significant differences between the treatments. In those cases in which significant differences were detected the Tukey test was applied to detect which treatments were responsible for the differences.

Results

Germination rates

In general, the germination rates obtained in *Q. rotundifolia* are very high, close to 100% (Figure1). A rate of 97.33% was obtained in Control and values of over 90% were obtained in the treatments of 60ºC-15min, 90ºC-5min, Charcoal, Diluted Ash, Smoke-5min, Smoke-10min and Smoke-15min. There is another group of treatments with germination rates of between 80 and 90%. These treatments are 60ºC-5min, 110ºC-5min and Ash. Finally, the treatment of 150ºC-5min obtained the lowest value, with a germination rate of only 14.66%.

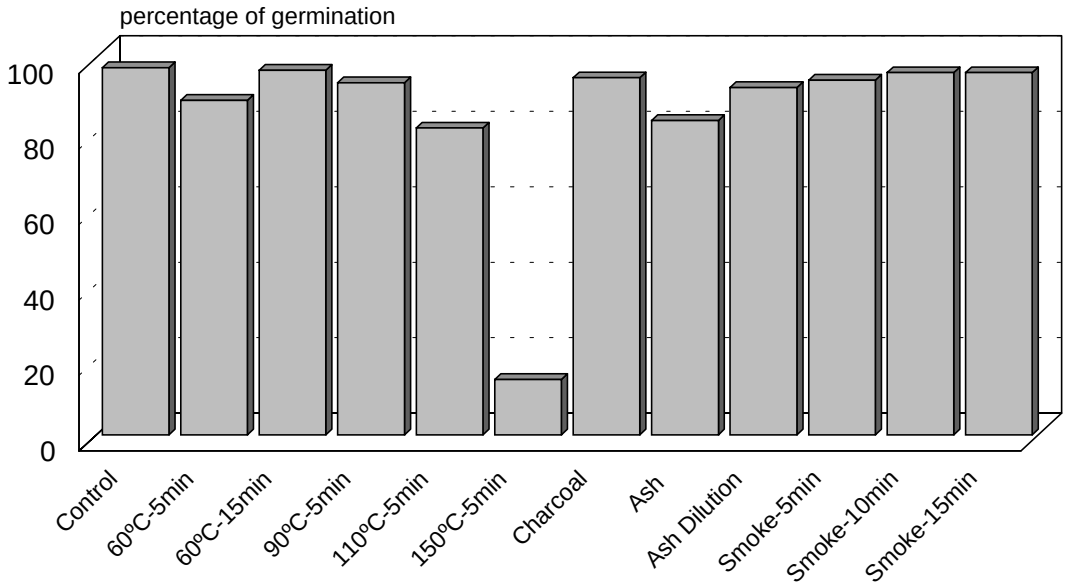

Figure 1: Percentage of germination corresponding to each of the treatments applied.

The ANOVA applied to the germination data showed highly significant differences between the treatments ($p<0.0001$) and the Tukey test indicated that this is due to the treatment of 150ºC-5min, the results of which are different to all the rest. The differences that were observed between the treatments with germination values above 90% and those above 80% are not significant. Therefore, only the thermal treatments at very high temperatures affect the germination of the seeds from *Q. rotundifolia*, inhibiting them.

T50

The average time required to reach 50% of total germinations (T50) varies between treatments. From 21 and 24 days in 150ºC-5min and Smoke-10min respectively to 57 days in Charcoal (Figure 2). The treatments that best accelerate germination are 150ºC-5min, Smoke-10min, 60ºC-15min and Smoke-15min with T50 values of between 21 and 28 days. Control, 60ºC-5min, Dilution of Ash, and Smoke-5min, have T50 values of between 30 and 40 days. The rest of the treatments have T50 values of over 40 days.

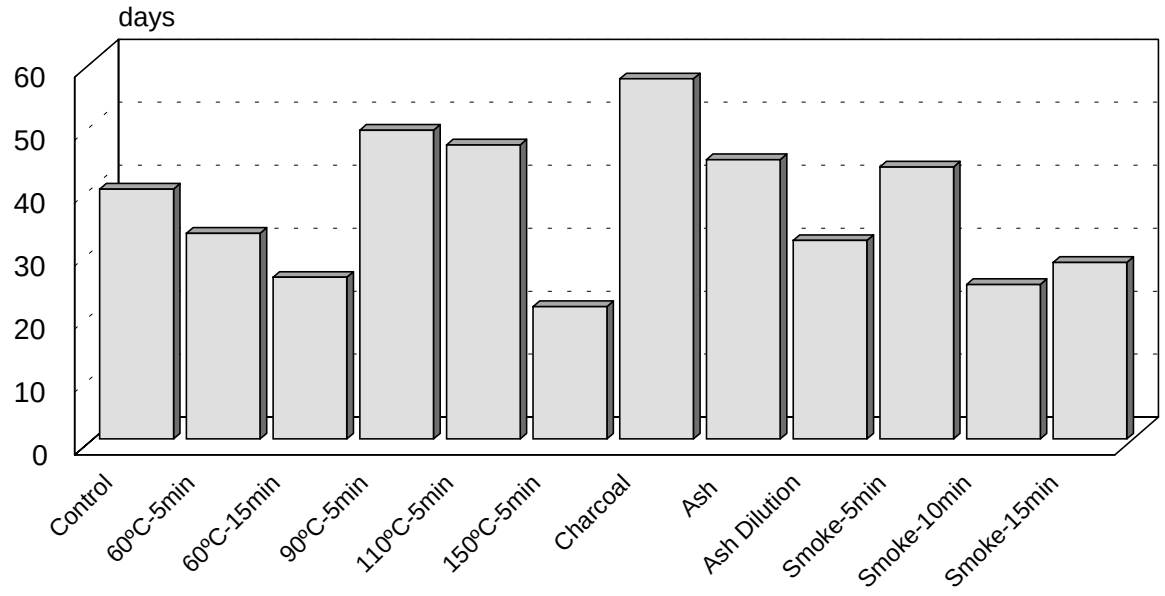

Figure 2: Average time required to reach 50% of germination with respect to the total amount germinated (T50) corresponding to each of the treatments applied.

After applying ANOVA to the T50 data, we proved that there are highly significant differences between the treatments ($p<0.0001$) and that, according to the Tukey test, they are due to three groups of treatments that are different to each other. The first of these groups is formed by 60ºC-15min, 60ºC-5min, Smoke-10min, Smoke-15min and Dilution of Ash. The second group is formed by Smoke-15, Ash and 110ºC-5min and the third group corresponds to the Charcoal treatment alone. The Control and 150ºC-5min treatments are in an intermediate position between the first and second group and are only significantly different to Charcoal. The treatment of 90ºC-5min is only significantly different to the first group.

Temporal distribution of germination

The temporal distribution of germination of *Q. rotundifolia* varies greatly depending on the treatment applied. This can be seen in Figure 3 and Figure 4. In the Control treatment (Figure 3), germination has been distributed over the whole incubation period of 5 months, but not in a constant manner. Two important peaks can be seen, during the first and second month in one case and during the fourth month in the other. The treatment of 60ºC-5min (Figure 3) behaves similarly, but the second peak is much less important than the first. In the 90ºC –5min and 110ºC-5min treatments, germination is concentrated in the first four months and there are practically no new germinations in the last month. The treatment of 150ºC-5min shows almost no germinations and these are reduced to the second months of incubation. The treatments of 60ºC-15min, Ash, Smoke-5, Smoke-10 and Smoke-15 (Figure 4) have in common a marked peak of germination during the first two months, but the germinations produced in the last three months contribute very little to the final germination rate. Finally, two treatments show a more or less constant distribution of germination without obvious peaks. These are Charcoal and Smoke-5.

Discussion

In this study, we have proven that *Q. rotundifolia* seeds have a germination capacity, in many cases, of close to 100%. From this, two facts can be deduced. Firstly, that the viability of the seeds after maturing is very high and secondly, that they lack any type of dormancy or latency. On the other hand, we have also proven that of all the

fire factors analysed (high temperatures, ash, smoke, and charcoal), only high temperatures affect the germination rate. This effect is inhibitory and is only produced at very high temperatures.

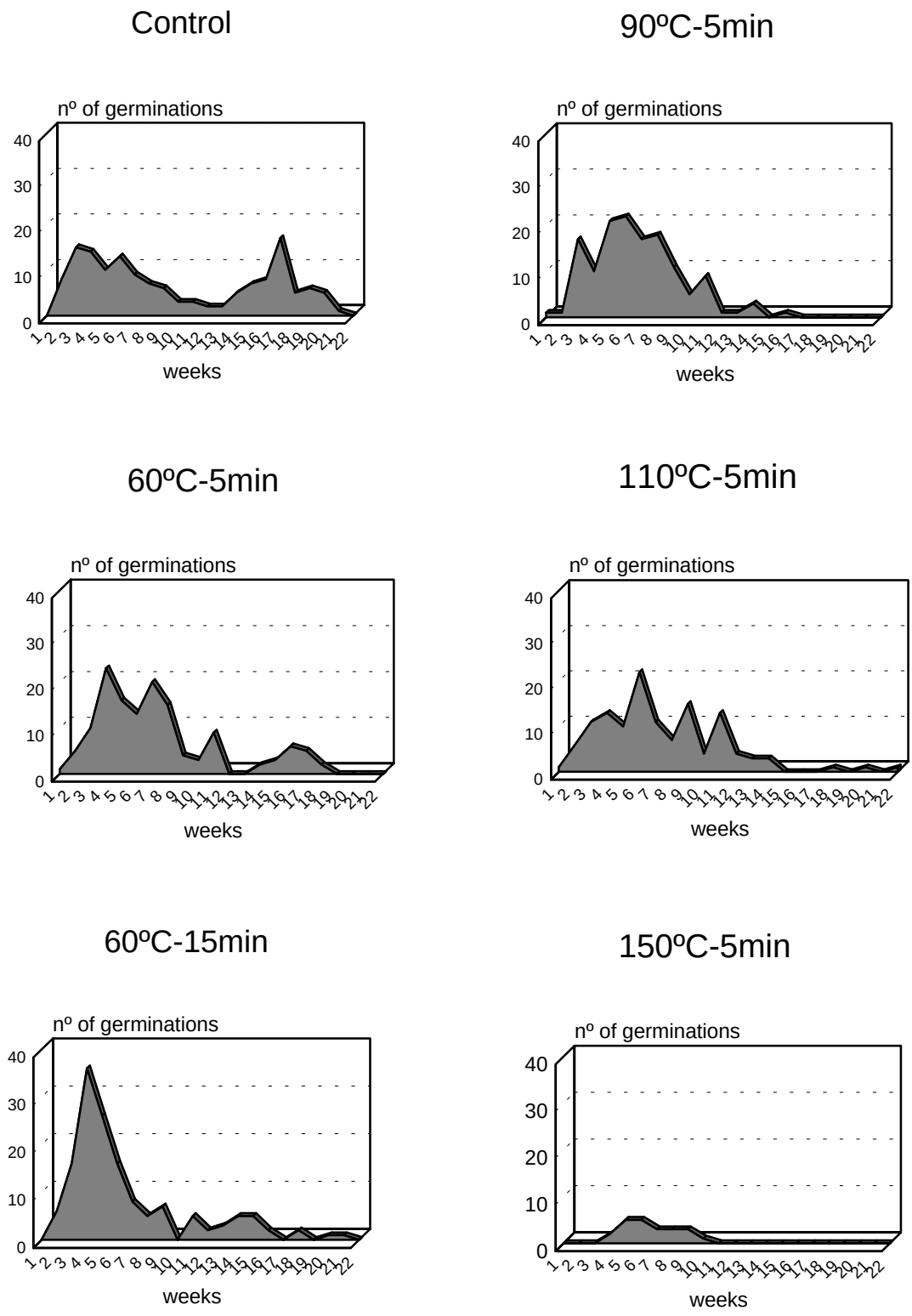

Figure 3: Temporal distribution of *Quercus rotundifolia* in Control and five thermic treatments.

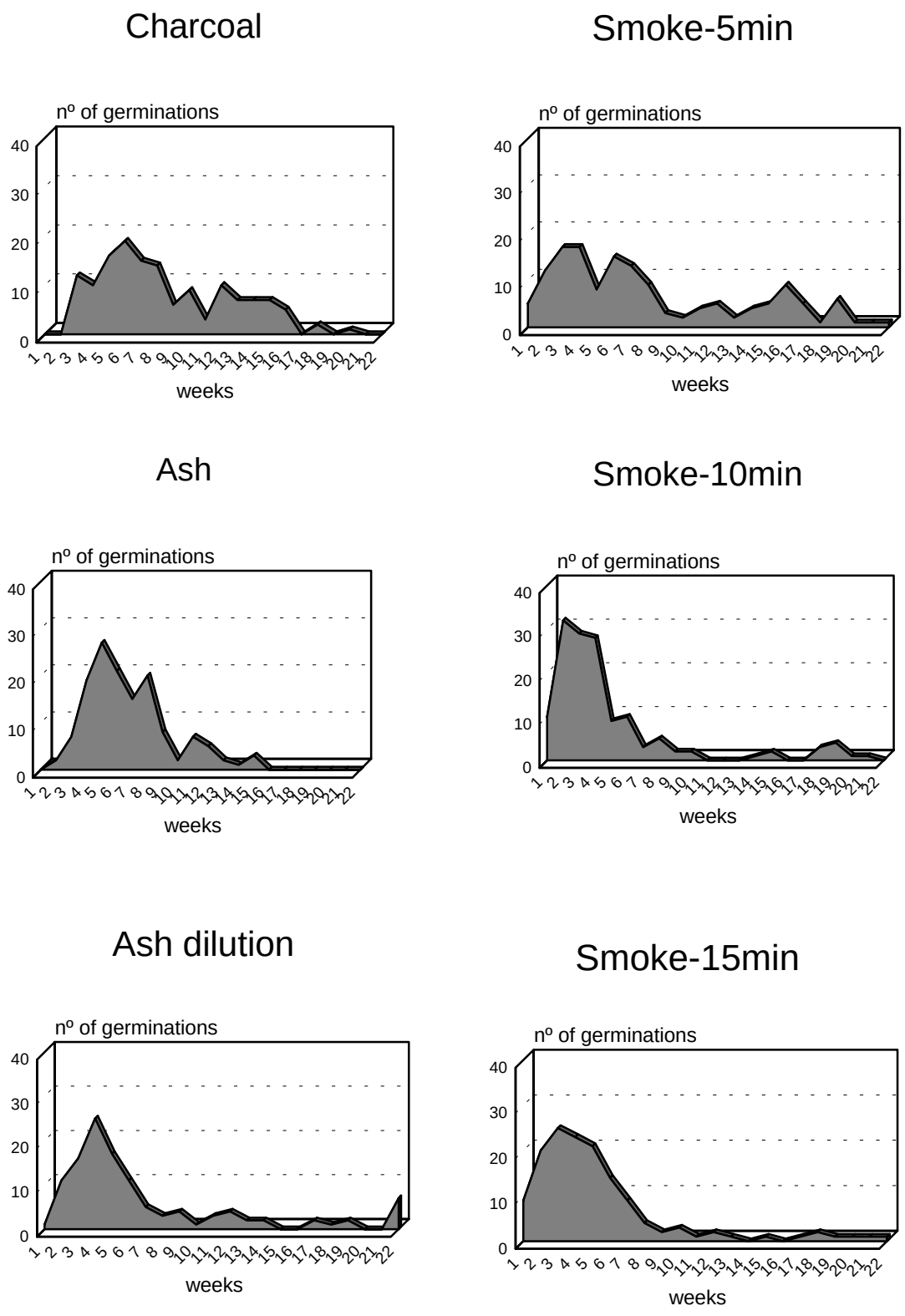

Figure 4: Temporal distribution of *Quercus rotundifolia* in several treatments of ash, smoke and charcoal.

Other authors have studied these factors in other species and have obtained results that vary greatly depending on the species studied. Keeley (1987) found that if high temperatures were maintained for a long time, they inhibited the germination of *Quercus dumosa*. However, we ourselves, in a previous study (Reyes and Casal 1993), did not find significant effects of high temperatures on the germination of Quercus *robur* and *Quercus*

pyrenaica. Keeley (1987) also found that in some species, high temperatures increased germination, in others, the response was not modified, and in others, it decreased. This same behaviour has been detected in many species from different parts of the world by various authors (Añorbe-Urmeneta et al. 1990, Tárrega et al. 1992, Thanos et al. 1992, Gónzalez-Rabanal y Casal 1995, Keith 1997, Herranz et al. 1998, Reyes et al. 2000).

The action of ash on germination has been not been studied in depth. Even then, Thomas & Wein (1990), Ne'eman et al. (1993), Gónzalez-Rabanal & Casal (1995), Reyes & Casal (1998) and Reyes et al. (2000) found that the ash either does not affect germination or inhibits it.

Keeley & Bond (1997) and Enright & Kintrup (2001), among others, found in North American and Australian species that the charcoal also produced very different effects on germination according to the species studied.

The effect of smoke on germination has been tested in a large number of species from South Africa, Australia and U.S.A. Thus, Brown (1993), Brown et al. (1993), Brown & van Staden (1997), Enright & Kintrup (2001), Keeley & Bond (1997) have detected very high degrees of stimulation of the germination rate in many species and nothing in others.

With respect to the delay in germination, we have proven that smoke and not very high, but persistent temperatures (60ºC-15min) greatly accelerate germination without reducing the final rate. In addition, in these treatments the temporal distribution of germination is concentrated within a very short period. This behaviour could be an advantage in the occupation of space and the availability of resources. In the genus *Quercus,* the development of the seedlings is extremely rapid and they acquire a considerable biomass in a very short time (Reyes 1996). It is also true that the seedlings have large cotyledons from which they can feed for a long time (various months) if the conditions of the medium are not adequate and thus, the initial advantage that could be gained by early germination would be of less importance.

According to Keeley (1998), the species of the genus *Quercus* found in the chaparral can be classified as disturbance-free recruitment species, i.e. the recruitment of new plants does not depend on the production of a perturbation (e.g. fire). We have proven that fire does not increase the germination rate of *Q. rotundifolia* in any case (high temperatures, ash, smoke, of charcoal) and therefore the recruitment of new individuals is independent of fire. This is in agreement with the conclusions of Keeley (1998) about the regenerative strategies of species of the genus *Quercus*.

Acknowledgments

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Germination Behaviour of *Picea abies*: Effects of Incubation Temperature, Light, Collection Time and Tree on Germination Capacity and Rate

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Abstract

The effects of constant incubation temperatures of 12°C and 21°C on germination of *Picea abies* ((L.) Karst.) seeds collected in October and the following January from the same six trees were tested in the light and in darkness.

Only about 8 % of the seeds geminated in the dark at 12°C. Light-requirement had been induced in the seeds already by the beginning of October. At the same suboptimal incubation temperature but in the light, seeds germinated well. Germination capacity (GC) was 95 % for the autumn-collected, and 88 % for the winter-collected seeds. The effect of interaction between light and collection time on GC was statistically significant at 12°C, as well as the effect of light alone. There were, however, no statistically significant differences in GC between the trees. At the optimum germination temperature of 21°C GC was in darkness lower than in the light. The overall mean for seeds germinated in the light was 94 % and that for the dark-germinated 77 %. There were also some differences in GC between the trees, especially when the seeds germinated in the dark. The influence of cone-collection time became obvious only through interaction of light and tree.

Seeds germinated slowly at 12°C. Mean germination time (MGT) at the suboptimal temperature was on the average 20 days, when it at the optimal temperature was only 8 days. MGT slightly decreased, if seeds overwintered in natural conditions in cones. At the incubation temperature of 12°C MGT of the autumn-collected seeds was on the average 21 days and that of the winter-collected 19 days. At 21°C MGTs were 8 and 7 days accordingly. The decrease was notable especially when the seeds were incubated in the dark. Interaction of light and collection time was statistically significant at both incubation temperatures.

The results revealed that *Picea abies* seeds are light-requiring not only when germinating at suboptimal temperature, but to a certain amount also when germinating at optimal temperature. The changes that usually happen during overwintering in cones in natural conditions and/or during dry storage of seeds and which are seen as reduced germination in darkness, had taken place surprisingly early in the autumn. However, other ripening processes proceeded, as indicated by the increased germination rate especially in the dark.

Keywords: Norway spruce, germination capacity, germination rate

Seed Quality and Sucrose Synthase Activity Changes during Development and Maturation of *Pinus sylvestris* L. Seeds

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Abstract

To investigate the changes of sucrose synthase activity (SSA) and seed quality following seed development and maturation, SSA, seed quality, seed development and maturation parameters were measured using *Pinus sylvestris* L. seeds. Germination and SSA were assayed using untreated, primed and stratified seeds. Seed development and maturation parameters were assayed using untreated seeds. Following seed development and maturation, germination capacity (GC) increased (from 6 to 87%), and mean germination time (MGT) decreased (from 24 to 6.5days) rapidly during the period. SSA increased from 6 to 11 µmol sucrose/min·mg protein from August 21 to 25 when embryo growth and seed filling increased quickly, and decreased later when seed development slowed down. Priming and stratification increased seed quality in a certain extent. SSA was significantly related to both GC and MGT (R^2=0.79 respective 0.85). Thus, it can be concluded that seed quality increases following seed development and maturation, SSA increases following active seed development and tend to decrease when seed development slows down. SSA can be used as an indicator for quality of developing *Pinus sylvestris* seeds.

Keywords: Scots pine, seed development, seed maturation, seed quality, sucrose synthase activity

Assessment of Genetic Variability in Seed Characteristics of Various Ecotypes of Neem (*Azadirachta indica* A. Juss.)

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Abstract

Neem (*Azadirachta indica* A. Juss.), a multipurpose tree species native to Indian subcontinent, is used from time immemorial as timber, antiseptic, medicine, insecticide and also posseses sociocultural values. The present study was undertaken to explore genetic variability among seed characteristics of this species in its natural range. Seeds were collected from 43 sources that covered ten states of India.

There were significant variations among seed sources for seed morphometric traits (Seed length, width, 1000 seed weight. No. of seeds in 100 g), moisture content and germination percentage. Difference in traits mean and variances were found to be highly significant among the populations. Coefficient of Variation (C.V.) was highest for germination percentage (60.57%) and the least in seed width (7.46%) among sources. Simple correlation between geographical factors with traits displayed that moisture content as well as germination percentage had significant positive association with latitude and 1000 seed weight with altitude. Coefficient of correlation (r) studies among seed traits revealed that the seed length had significant positive association with seed width, 1000 seed weight and significant negative association with number of seeds/100 gram. The seed width showed significant positive relationship with 1000 seed weight, moisture content and germination percentage. The moisture content of the seed revealed positive significant association with germination percentage. The cluster analysis of variation for studied traits among population displayed the existence of five major groups. Their intra-cluster distances and group mean values for studied traits were also analysed. Genotypic and phenotypic path analysis concluded that moisture content and seed width directly contributed to germination percentage. The heritability in broad sense was highest for germination percentage (98.6 %) but Genetic Gain (G.G.) was observed to be maximum for moisture content (74.02%). The present study will be useful in the establishment of germplasm bank and for laying out provenance/ progeny trial for tree improvement programme of this species.

Keywords: Ecotype, Genetic variability, Seed source, Heritability, Path, Clustering, Germplasm

Introduction

The neem tree (*Azadirachta indica*) belongs to the family Meliaceae, is a multipurpose tree species native and widely planted throughout the tropics in semi arid and arid areas. The species is thought to have originated in Asam (India) and Myanmar, but it is not possible to determine the natural area of distribution since the tree has been domesticated for centuries. The origin of the imported seed is often not known, and it is thought that the genetic base may be narrow. In the 1920s, Ghana is the first country in Africa believed to have imported neem seed. Apart from its value as a timber tree, neem has antiseptic, medicinal, insecticidal properties and socio cultural values (Troup 1921; Radwanski and Wickens 1981; Kaul et al., 1990; Tewari, 1992 and Ketkar and Ketkar 1995). It is very well adapted to dry conditions. Neem thrives well in sites with low rainfall (130 mm per year). In sites with high rainfall (3000 mm per year to 4000 mm per year), it has failed completely (Schmutterer 1990). Neem performs well on a wide range of soil types. In its native environment, neem is found growing in mixed forests in association with *Acacia* and *Dalbergia* species. Provenance research has defined the genetic and environmental component of phenotypic variability between trees from different geographic origins (Callaham 1964 and Wright 1976). Many countries have high demand of seed for planting. Large losses of seed are incurred due to improper selection of trees, untimely collection of seeds, age of trees, crown and environmental conditions. The present investigation was carried out to study the nature and pattern of variation among seed sources for seed parameters in *Azadirachta indica* at Forest Research Institute, U.P., Kanpur, India.

Material and Methods

Selection of C.P.Ts.

Forty-three seed sources representing the entire distributional range of this species in India, with a considerable

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geographic isolation (latitude, longitude, altitude and rainfall) between each seed source were selected from ten states of India (Table 1). Five to ten trees were selected within each seed source. The selected trees were located minimum 100 m. apart to avoid narrowing down the genetic variations.

Table 1. Geographical distribution of different seed source of *Azadirachta indica*.

Seed Sources	Latitude ^{0} N	Longitude ^{0} E	Altitude (m)	Rainfall (mm)
Kanpur(T_1)	26.28	80.21	126	880
Mathura(T_2)	27.30	77.41	175	750
Mirzapur(T_3)	25.09	82.35	141	683
Etawah(T_4)	26.46	79.02	157	762
Haldwani(T_5)	29.10	79.60	256	1500
Mau(T_6)	26.80	77.10	128	835
Lalitpur(T_7)	24.02	78.25	211	854
Jhansi(T_8)	25.26	78.35	251	1000
Varanasi(T_9)	25.30	83.00	76	1113
Bareilly(T_{10})	28.21	79.25	173	1068
Gorakhpur(T_{11})	26.45	83.22	120	1100
Behraich(T_{12})	27.35	81.36	124	1170
Gonda(T_{13})	27.08	81.56	110	1294
Pilibhit(T_{14})	28.38	79.48	190	1210
Ramnagar(T_{15})	29.13	81.10	380	1500
Lucknow(T_{16})	26.00	80.54	111	993
Raipur(T_{17})	21.14	81.38	280	1309
Jabalpur(T_{18})	23.10	79.59	400	1358
Balaghat(T_{19})	21.48	80.16	290	1573
Pinjore(T_{20})	29.10	75.43	236	486
Rohtak(T_{21})	28.54	76.34	178	567
Hissar(T_{22})	29.10	75.46	250	714
Kaithal(T_{23})	29.48	76.28	270	764
Barwala(T_{24})	29.22	75.55	255	715
Bijapur(T_{25})	16.58	75.43	594	585
Dharwad(T_{26})	15.25	75.38	650	663
Bhuvneshwar(T_{27})	20.14	85.50	90	1165
Daspalla(T_{28})	20.12	84.42	195	1231
Ludhiana(T_{29})	30.54	75.28	220	578
Pune(T_{30})	18.32	73.52	559	714
Chittoor(T_{31})	13.12	79.07	415	678
Coimbatore(T_{32})	11.17	77.58	360	916
Tiruvanamalai(T_{33})	12.20	79.10	200	778
Karure(T_{34})	11.07	78.02	240	965
Erode(T_{35})	11.31	77.44	230	834
Namakkal(T_{36})	11.18	78.10	210	867
Cuddalore(T_{37})	12.55	79.05	68	952
Kanyakumari(T_{38})	8.03	77.25	79	1560
Aruppukhottai(T_{39})	9.18	78.45	110	875
Chennai(T_{40})	13.05	80.17	102	1260
Tirunelveli(T_{41})	8.10	77.44	107	898
Krishnagiri(T_{42})	12.32	78.14	508	786
Pondicherry(T_{43})	11.75	79.45	214	1054

Seed studies

Ten samples with 200 undamaged freshly collected and depulped drupes from each seed lot were randomly obtained. Maximum length and width were measured to study the variation in seed size.

Seed weight

Fresh seed weight at extraction of ten samples with 100 seeds each was determined for each seed source

following ISTA 1993 rules. The weight of 1000 seeds was calculated for each source.

Moisture percentage

The moisture content of the seeds was determined by the method described by Willan 1985. One hundred seeds in three replications were dried in an oven for 17 hours at 103^0 C (low constant temperature oven method). The moisture percentage was calculated using the formula:

$$\text{Moisture \%} = \frac{\text{Final weight - Initial weight}}{\text{Final weight}} \text{ x } 100$$

Germination percentage

For each of the CPT, three hundred (300) seeds replicated three times were germinated at $30 \pm 2\ ^0$C and 90% humidity in the seed germinator for 15 days. A seed was considered to be germinated when radicle length reach 1 cm or equal to the length of seed (ISTA 1993). The total number of seeds that germinated after 15 days was taken as the final count. The plumule and radical lengths of 10 germinated seeds of each CPT of all the seed sources were also measured.

Statistical analysis

The data obtained on each character was subjected to statistical analysis (Snedecor & Cochran 1967, Panse & Sukhatme 1985 and Singh & Chaudhary 1985). Genetic parameters were estimated (Burton & Devane 1953 and Johnson et al. 1955).

Results

Table 2 shows the analysis of variance for the different morphological traits and germination percentage of the various seed sources of neem. All the six characters studied are significantly different for the seed sources examined in this study. The character studied were length of seed, width of seed, 1000 seed weight, seeds in 100 g, moisture percentage and germination percentage. The maximum seed length recorded was 1.55 cm in T_{41} seed source followed by 1.54 cm for seed sources T_{14} and T_{36} while the lowest seed length value of 1.08 cm was in obtained in T_4 seed source. The maximum seed width was obtained in T_{13} (0.75 cm) while the lowest seed width value was observed in T_{38} sources (0.58 cm). The highest 1000 seed weight was recorded for T_{15} source (255.55 g) while the lowest was in T_1 seed source (139.38 g). Seeds from T_{19} seed source have the highest seeds per 100 g (779.20) while the lowest was from T_{10} sources (225.67). The maximum moisture and germination percentage (54.30% and 92.60%, respectively) was recorded for seed source T_4 while the lowest moisture percentage (5.89%) was observed for T_{41} and germination percentage (3.33) in T_{28}.

Correlation analysis

Table 3 presents the simple correlation (r) between the studied traits and geographical factors. From the table it is evident that latitude has high significant positive association with moisture and germination percentage while with length of seed it has significant negative relationship. Altitude has significant positive relationship with 1000 seed weight. Longitude and rainfall have no clear linear relationship with any of the six characters studied. Correlation among the seed traits studied is presented in Table 4. Length of seed has a highly significant positive correlation with width of seed at genotypic, phenotypic and at environment levels. Length and width of seed have significant positive relationship with 1000 seed weight at genotypic and phenotypic levels. Length of seed has significant negative association with seeds in 100 g at all the three levels i.e. rg, rp & re, while with moisture percentage it has significant positive correlation at environment level (re) and negatively associated at genotypic level (rg). Seed width has shown significant positive association with moisture and germination percentage at both phenotypic and genotypic levels. The seeds in 100 g have significant negative correlation with germination of seed. However, moisture content of seeds has highly significant and positive association with germination percentage at all the three levels.

Path analysis

Tables 5 and 6 present genotypic and phenotypic path using germination percentage as dependent trait. Moisture percentage has direct effect on germination percentage with genotypic and phenotypic correlation coefficients of 0.877 and 0.852, respectively, but the direct effect is negative or negligible. In such situations, the indirect cause

Table 2. Analysis of variance for the different morphometrical seed traits and germination percentage for various seed sources of Neem.

Seed Source	Length (cm.)	Width (cm.)	1000 seed wt. (gm)	No. of seeds in 100 g	Moisture %	Germination %
Kanpur (T_1)	1.44	0.72	139.33	535.00	35.52	64.67
Mathura(T_2)	1.28	0.71	145.00	687.33	47.20	88.30
Mirzapur(T_3)	1.51	0.77	157.67	537.00	50.60	81.23
Etawah(T_4)	1.09	0.60	171.67	661.33	54.30	92.60
Haldwani(T_5)	1.50	0.71	190.00	419.67	46.90	86.33
Mau(T_6)	1.17	0.69	148.33	578.67	48.64	53.67
Lalitpur(T_7)	1.39	0.64	165.67	379.33	42.78	53.55
Jhansi(T_8)	1.30	0.63	165.33	406.00	38.34	63.03
Varanasi(T_9)	1.35	0.63	162.67	313.67	44.47	61.78
Bareilly(T_{10})	1.33	0.63	141.33	225.67	53.00	61.67
Gorakhpur(T_{11})	1.34	0.60	147.66	316.33	48.34	71.35
Behraich(T_{12})	1.22	0.67	158.73	770.33	22.34	20.55
Gonda(T_{13})	1.27	0.75	213.73	519.67	37.00	44.00
Pilibhit(T_{14})	1.54	0.71	215.33	411.67	41.43	85.00
Ramnagar(T_{15})	1.48	0.72	255.55	431.00	52.48	90.00
Lucknow(T_{16})	1.28	0.63	168.00	281.67	32.30	68.57
Raipur(T_{17})	1.31	0.61	161.67	426.33	27.63	25.33
Jabalpur(T_{18})	1.26	0.71	178.33	536.33	31.18	45.90
Balaghat(T_{19})	1.19	0.64	189.00	779.00	25.00	29.95
Pinjore(T_{20})	1.34	0.74	195.00	434.33	43.96	62.93
Rohtak(T_{21})	1.25	0.65	155.00	547.67	30.20	40.24
Hissar(T_{22})	1.19	0.69	180.00	572.00	28.25	45.97
Kaithal(T_{23})	1.24	0.58	161.00	612.33	9.95	16.00
Barwala(T_{24})	1.47	0.61	191.00	471.67	23.49	43.62
Bijapur(T_{25})	1.48	0.69	197.00	526.00	46.97	77.98
Dharwad(T_{26})	1.34	0.66	202.67	583.33	48.22	82.44
Bhuvneshwar(T_{27})	1.39	0.73	191.67	470.00	23.08	39.85
Daspalla(T_{28})	1.28	0.66	150.00	654.00	10.08	3.33
Ludhiana(T_{29})	1.50	0.67	206.67	509.67	7.89	6.67
Pune(T_{30})	1.30	0.64	168.00	360.33	18.41	31.62
Chittoor(T_{31})	1.48	0.63	205.00	481.67	18.17	30.38
Coimbatore(T_{32})	1.32	0.65	175.33	310.00	13.46	64.05
Tiruvanamalai(T_{33})	1.33	0.64	170.00	571.67	21.03	20.90
Karure(T_{34})	1.36	0.60	167.33	590.00	10.09	14.47
Erode(T_{35})	1.34	0.67	180.00	539.33	14.33	17.12
Namakkal(T_{36})	1.54	0.65	181.67	480.67	7.49	11.55
Cuddalore(T_{37})	1.42	0.66	203.33	481.67	6.18	17.34
Kanyakumari(T_{38})	1.33	0.58	173.00	582.67	9.94	30.42
Aruppukhottai(T_{39})	1.41	0.69	200.00	464.67	9.90	16.20
Chennai(T_{40})	1.49	0.68	158.33	455.67	11.77	19.47
Tirunelveli(T_{41})	1.55	0.67	208.33	560.33	5.89	8.90
Krishnagiri(T_{42})	1.39	0.72	210.00	520.33	6.92	17.48
Pondicherry(T_{43})	1.53	0.68	253.33	430.00	10.83	19.50
F- test	**	**	**	**	**	**
S.E.(d)±	0.026	0.014	5.715	17.354	1.753	2.241
C.D.at 5 % level	0.080	0.042	17.72	53.797	5.434	6.947

**Significant at 1% level

factors are to be considered simultaneously for selection. Another seed parameter which has pronounced effect on germination is seed width followed by 1000 seed weight. The effect determines how best the causal factors account for the variability of the dependent factor. It seems to vary with no significant correlations of seed length and 1000 seed weight at the both genotypic and phenotypic path level (Singh & Kakar, 1997).

Table 3. Simple correlation (r) between traits and geographical locations**.**

Sl. No.	Traits	Latitude ^{0}N	Longitude ^{0}E	Altitude (m)	Rainfall (mm)
1	Length of seed	-0.2945**	0.0251	0.0028	-0.0242
2	Width of seed	0.1607	0.1688	0.0003	-0.0218
3	1000 seed weight	-0.1820	-0.0940	0.2904**	0.1161
4	Seeds/1000 g	-0.0494	-0.0754	0.0019	-0.0129
5	Moisture %	0.6382**	0.1361	0.0307	0.0060
6	Germination %	0.5144**	0.0532	0.1178	-0.0029

** significant at 1 % level (p=0.01)

Table 4. Correlation coefficient among seed traits of *Azadirachta indica*.

Sl. No.	Traits		Length of seed	Width of seed	1000 seed wt.	Seeds/100 g	Moisture %	Germ %
1	Length of seed	rp rg re	-					
2	Width of seed	rp rg re	0.294** 0.278** 0.458**	-				
3	1000 seed wt.	rp rg re	0.466** 0.504** -0.074	0.317** 0.344** 0.035	-			
4	Seeds/100 g	rp rg re	-0.345** -0.353** -0.220*	0.059 0.036 0.013	-0.076 -0.076 -0.071	-		
5	Moisture %	rp rg re	-0.192 0.212* 0.283**	0.232* 0.245* 0.080	-0.140 -0.146 0.019	-0.180 -0.182 -0.085	-	
6	Germination %	rp rg re	-0.076 -0.083 0.132	0.246* 0.260* 0.083	-0.008 -0.013 0.198	-0.248* -0.252* -0.066	0.892** 0.899** 0.331**	

* significant at 5 % level (p=0.05)
** significant at 1 % level (p=0.01)

rp- Phenotypic level
rg- Genotypic level
re- Environmental level

Table 5. Path analysis of the genotypic correlation coefficients between germination percent and its contributing traits.

Traits	Length	Width	1000 seed weight (g)	No. of seeds in 100 g	Moisture percentage	Correlation coefficient with germination percentage
Seed length	0.027	-0.011	0.083	0.021	-0.189	-0.069
Seed width	0.007	-0.041	0.058	-0.004	0.245	0.265**
1000 seed wt.	0.014	-0.015	0.157	0.006	-0.080	0.082
No. of seeds in 100 g	-0.009	-0.003	-0.016	-0.059	-0.131	-0.218*
Moisture percentage	-0.006	-0.011	-0.014	0.009	0.899	0.877**

* significant at 5 % level
** significant at 1 % level
Diagonal values indicates direct effects
Residual effect = 0.1985

Table 6. Path analysis of the phenotypic correlation coefficients between germination percent and its contributing traits.

Traits	Length	Width	1000 seed weight (g)	No of seeds in 100 g	Moisture percentage	Correlation coefficient with germination %
Seed length	0.013	-0.006	0.075	0.024	-0.169	0.063
Seed width	0.004	-0.019	0.052	-0.004	0.217	0.250**
1000 seed wt.	0.006	0.007	0.151	0.007	-0.076	0.095
No. of seeds in 100 g	-0.004	-0.001	-0.016	-0.070	-0.123	0.214*
Moisture percentage	-0.002	-0.005	-0.013	0.010	0.862	0.852**

* significant at 5 % level
** significant at 1 % level
Diagonal values indicates direct effects
Residual effect = 0.2448

Genetic parameters estimates

Mean standard deviation (S.D.), coefficient of variation (C.V.), phenotypic coefficient of variation (P.C.V.), genotypic coefficient of variation (G.C.V.), heritability, genetic advance and genetic gain as percent of mean are presented in Table 7. The data revealed wide range of variability for traits under study. Standard Deviation (SD) and Coefficient of Variation (CV) for the studied traits namely length of seed, width of seed, 1000 seed weight, number of seeds in 100 g, moisture and germination percentage were calculated to be 0.11 and 8.09, 0.05 and 7.46, 28.53 and 15.34, 120.47 and 24.18, 16.29 and 57.66, 27.13 and 60.57, respectively. However, maximum coefficient of variation was observed in germination percentage (60.57) followed by moisture percentage (57.66) with the lowest obtained for the seed width.

Table 7. Estimates of genetic parameters of seed traits of *Azadirachta indica*.

Characters	Range		Mean	S.D.	C.V. (%)	PCV	GCV	H^2 (bs)	Genetic advance	Genetic gain
	Min.	Max.								
Seed length	1.09	1.55	1.36	0.11	8.09	8.58	8.26	92.8	0.22	16.18
Seed width	0.58	0.75	0.67	0.05	7.46	7.29	6.86	88.4	0.09	13.43
1000 seed wt.	139.33	255.55	181.29	28.53	15.74	16.98	16.64	96.0	61.40	33.86
No. of seeds in 100 g	225.67	779.00	498.28	120.47	24.18	24.43	24.05	97.0	243.09	48.79
Moisture percentage	5.89	54.30	28.25	16.29	57.66	33.85	33.02	95.2	20.91	74.02
Germination percentage	3.33	92.60	44.79	27.13	60.57	38.12	37.86	98.6	33.01	73.70

The values of phenotypic variance were higher than the genotypic variance. However, for all studied traits, values for genotypic variance were very close to that of the phenotypic variance, indicating variations in these characters due to additive genes could be fixed through selection. The influence of environment was found meager in traits. Heritability indicated how much of the phenotypic variation is heritable. Heritability in broad sense was observed to be 98.6 % in germination percentage. It was however not coupled with genetic advance (33.01) of same intensity (Table 7). On the contrary, number of seeds in 100 g and 1000 seed weight recorded high heritability (97.0% & 96.0%) and were coupled with high genetic advance, 243.09 and 61.40, respectively. Genetic gain as percentage of mean was found maximum (74.02%) in moisture percentage followed by germination percentage (73.70%) and number of seeds in 100 g (48.79%). The respective low genetic gain was observed in seed width (13.43%) and followed by seed length (16.18%). Low heritability coupled with low genetic gains indicates non-additive genetic effect as explained by Srivastava (1993).

Cluster analysis

Cluster analysis of 43 seed sources is presented for different character combinations namely seed length, seed width, 1000 seed weight, seeds per 100 g, moisture percentage and germination percentage. The studies were grouped into five clusters using six different traits. In Table 8 clusters C and D were the largest consisting of 10

seed sources in each. Cluster B and E consisted of eight (8) seed sources (8) while A contained 7 seed sources. The mean values for different clusters are presented in Table 9. Mean cluster values showed significant variations among the clusters for all the parameters. Mean seed length of five cluster ranged from 1.20 (cluster E) to 1.48 (cluster C), mean seed width from 0.62 (cluster A) to 0.72 (cluster D), mean 1000 seed weight from 161.75 (cluster B) to 201.83 (cluster C), mean number of seeds in 100 g from 324.12 (cluster B) to 641.58 (cluster E), mean moisture per cent from 10.78 (cluster C) to 42.62 (cluster D) and mean germination per cent from 18.22 (cluster A) to 71.44 (cluster D) to determine the genetic relationship between the populations. Inter-cluster distance for five clusters is presented in Table 10. The maximum inter-cluster distance (3.795) was observed between D and A followed by E and C (3.589), C and B (3.279) while the least (2.227) distance was observed between C and A clusters.

Table 8. Cluster information for number of seed sources in each cluster (seed length, width, 1000 seed weight, seeds per 100 g, moisture % and germination %).

Cluster	No. of seed sources in the cluster	Seed source number and name	
A	7	17. Raipur	34. Karur
		23. Kaithal	35. Erode
		28. Daspalla	38. Kanyakumari
		33. Tiruvanamalai	
B	8	7. Lalitpur	11. Gorakhpur
		8. Jhansi	16. Lucknow
		9. Varanasi	30. Pune
		10. Bareilly	31. Chittoor
C	10	24. Barwala	39. Aruppukhattai
		29. Ludhiana	40. Chennai
		32. Coimbatore	41. Tirunelveli
		36. Namakkal	42. Krishnagiri
		37. Cuddalore	43. Pondicherry
D	10	1. Kanpur	15. Ramnagar
		2. Mirzapur	20. Pinjore
		5. Haldwani	25. Bijapur
		13. Gonda	26. Dharwad
		14. Pilibhit	27. Bhuvneshwar
E	8	8. Mathura	18. Jabalpur
		3. Etawah	19. Balaghat
		6. Mau	21. Rohtak
		7. Behraich	22. Hisar

Table 9. Cluster means for five different clusters.

No. of cluster	Seed length (cm.)	Seed width (cm.)	1000 seed weigth (g)	No. of seeds in 100 g	Moisture %	Germination %
A	1.31	0.62	166.14	568.05	14.65	18.22
B	1.33	0.63	161.75	324.12	36.39	59.47
C	1.48	0.67	201.83	485.63	10.78	19.11
D	1.43	0.72	199.43	486.77	42.62	71.44
E	1.20	0.67	165.76	641.58	35.89	52.15

Table 10. Inter -cluster distance between five clusters (Distance between cluster centriole).

Cluster groups	A	B	C	D	E
A	0.000				
B	2.881	0.000			
C	2.277	3.279	0.000		
D	3.795	2.888	3.020	0.000	
E	2.412	2.982	3.589	2.944	0.000

Discussion

Seed size may vary due to both internal (maternal) and external (environmental) conditions operating at the time of seed development (Harper et al. 1970). This differential development might have an adaptive advantage in local edaphic and climatic conditions. Winn (1991) concluded that selection of correlated traits might be responsible for individual variation in seed weight and uniform seed crops. In this wide ranging study, the results supported the position of Wright (1976) that seed weight in forest trees is generally higher in drier areas than those in wetter areas. Baker (1972) also found a positive correlation between seed weight and environmental conditions of the site in which parent plant grow. Pathak et al. (1974) showed that heavy seed weight in *Leucaena leucocephala* is generally observed in sources more towards southern latitudes. Dunlop and Barnett (1983) reported that seed size and seed weight have pronounced effect on seed germination. Generally, large seed have fast and uniform germination due to more endosperm nutrient pool (Kandya 1978). Heritability estimates have to be related to the particular population growing under particular environmental conditions. Hence, heritability estimates are useful as gross indicators of the possibility of selection for one or more traits (Namkoong et al. 1966). Johnson et al. (1955) observed that a high heritability estimates along with high genetic gain is usually more useful than the heritability values alone for predicting the resultant effect of selecting the best individual from best provenances. Therefore, high heritability estimates does not necessarily mean an increased genetic advance as reported in *Heracleum candicans* (Devagiri et al.1997) and in *Coleus forskholii* (Srivastava et al. 1986).

The seed sources together are less divergent than the ones grouped in different clusters. The clusters separated by greatest statistical distance show maximum divergence. Genetic divergence studies have been carried out to determine the genetic divergence in the provenance (Burley et al. 1971).

Conclusion

The results on variation indicate that for most of the traits, seed sources fall in separate groups. This kind of variation seems to be sporadic. Weak correlation between geographic factors and different parameters also suggests the presence of discrete population of long isolations. This study presents the benefits of having a greater understanding of provenance diversity in selecting provenances or parents for tree improvement programs. A large number of selected provenances will help in the development of seed bank or seed orchards. The cluster behavior and genetic distance would help in maintaining a genetically diverse population of high yielding provenances or population.

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Canada's National Tree Seed Centre

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Abstract

The National Tree Seed Centre was established 35 years ago to obtain, store, and provide seed of known origin and quality for research. The role has been expanded to include gene conservation of indigenous shrub and tree species. Seed is collected from natural populations or plantations of known seed origin. A typical collection consists of 5, 000 to 50, 000 seed. The Seed Centre is a well-known supplier of seed for research and requests are received from around the world. Seed is primarily requested from Canadian species and about 80% of the seed provided is from these native species. Storage temperature and seed moisture content are critical in order to ensure long-term viability. Seed of recalcitrant species is stored at 4 C with some air exchange and collections are made frequently to maintain a viable supply. Seed of orthodox species is dried to a moisture content of 5-8% and stored in hermetically sealed glass jars at –20°C. Under these conditions the viability of seed of many species can be maintained above 75% for at least 50 years. Access to cryogenic storage is also available and is currently being used to develop storage protocols of recalcitrant species. Seed quality is monitored by conducting germination tests on a regular basis. Testing criteria prescribed by International Seed Testing Association and Association of Official Seed Analysts are used. Developmental research is an important component of the Seed Centre's activities. Some key areas include testing and developing better storage methods, refining seed processing techniques, and developing germination test protocols.

Acceleration of Emergence of English Oak Seedlings Grown in Containers

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Abstract

Rentability of the production of seedlings of the English oak in containers is seriously reduced as a consequence of prolonged germination of seeds and seedling emergence. These processes can be significantly accelerated by sowing into containers acorns deprived of about 1/3 of their original length by cutting off the apical ends of their cotyledons. The mean germination time is shortened from about 8 to 4-5 weeks. The increase in the number of seedlings of the investigated seed lots caused by this operation surpassed in some cases by 20%. Cutting and exposing the cross-cut surfaces of the cotyledons makes individual inspection and selection of the acorns possible and so additional increase of the emerged seedlings.
The height of seedlings, obtained from some lots of cut acorns, was even more than those from intact seeds. Also the number and the dimensions of the seedlings from the shortened acorns have not been reduced after careful 2-hour drying as well as by a 2-hour imbibition in water. Sowing acorns with the radicle end down has extended somewhat the duration of the mean germination time in contrast to the acorns sown horizontally.

Keywords: acorns, seed, germination, seedling emergence, growth, containers

The Effect of Desiccation on the Seed Germination of *Laurus nobilis* L.

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Abstract

In this project we examined bay laurel (*Laurus nobilis* L.) seed viability in relation to seed moisture content. Fresh seeds, with their original moisture content (37.0% f. wt), when submitted to a pregermination treatment (soaking in water for 10 days to remove the external fleshy coat and subsequent stratification at 4±1°C for 2 months), displayed a germination percentage of 55.1%. When the seed moisture content was reduced by 2.0% (from 37.0% to 35.0%) in an oven at a constant temperature of 30°C, the germination percentage rose to 81.0%. When the seed moisture content was reduced to 30.0%, 25.0%, 20.0%, 15.0% and 10.0% by using the same method, the germination percentages decreased to 44.0%, 33.2%, 24.0%, 1.1%, 1.0%, respectively. Reducing the seed moisture content to 28,7% and 23,5% by drying the seeds in alternating room conditions (25°C day/15°C night) resulted in an increase of seed germinability to 84.3% and 90.9%, respectively. The drying of the seeds for a longer time reduced the seed moisture content to 21.0%, 19.6% and 17.0% and seed germination to 66.8%, 49.4% and 48.0%, respectively. Reducing seed moisture content below 15.0% resulted in practically nullifying seed germinability. The fact that bay laurel seeds cannot retain their germinability at lower moisture contents demonstrates that it is a species with recalcitrant seeds.

Keywords: desiccation, germination, *Laurus nobilis*, seed, recalcitrant

Introduction

Bay laurel (*Laurus nobilis* L.) is an evergreen aromatic shrub or small tree that is an indigenous species of the Mediterranean (Kavvadas 1959). It is a dioecious plant, whose fruit is a drupe with a single seed of 1-1.5 cm diameter, oval shaped and black when ripe. The plant has been known since ancient times and has been associated with several myths and is considered a symbol of victory, glory and honor (Kavvadas 1959). The pharmaceutical properties of its leaves and fruit have also been known since Dioscorides (Skroubis 1990). Its utilization in cooking and perfumery is still important today and it is widely cultivated as an ornamental plant in gardens and parks (Arabatzis 1998). Moreover, it is used symbolically in religious celebrations (Baumann 1993).

Investigations of the bay laurel seed germination behaviour have shown that the external fleshy pericarp causes dormancy (Takos 2001) while embryo dormancy is also referred (Sheryshov 1975, Mkervali 1977a, Mkervali 1977b, Vadochkoriya and Loladze 1986, Takos 2001). Seed dormancy is interrupted by soaking seeds in water at room temperature, for approximately 10 days, in order to remove the pericarp and then stratifying the seeds at 4±1°C for 1.5 to 2 months (Takos 2001).

The seeds of most plant species have an orthodox storage behavior (Dirr and Heuser 1987, Thanos and Georghiou 1988, Bonner 1990, Thanos et al. 1992). This means that their seeds attain moisture contents of 10.0% or less without losing viability. If viability is lost long before seed moisture reaches this level, then one concludes that the seeds are "recalcitrant" (Bonner 1996) and are usually short-lived seeds (Roberts 1973). Species with recalcitrant seeds include several tropical plants (Arentz 1980, Bilia et al. 1999) as well as plants of the temperate zone, such as *Castanea, Quercus, Aesculus* and certain species of the genus *Acer* (Bonner 1990, McCreary and Koukoura 1990, Dickie et al. 1991, Merou and Takos 1995, Bonner 1996). The classification, as far as storage behavior is concerned, of some lesser known species, such as bay laurel, (Takos 2001) has not yet been done although there are indications of recalcitrance. The drying of bay laurel seeds causes a reduction of seed germination (Takos 2001); nevertheless, the critical moisture contents that produce a loss of seed viability have not been determined. The logical way to investigate this is to desiccate the seeds uniformly and periodically sample for moisture content and germination. The objective of the present research was to examine the viability of the species *Laurus nobilis* L. during desiccation at different temperatures and moisture levels.

Materials and Methods

Ripe seeds were collected from a row of trees in the city of Drama, Greece, which is at an elevation of 90 m above sea level, in early December 2000.

Determination of the (initial) moisture content of fresh seeds

After collection, the seeds were taken to the laboratory and after they were stirred carefully, their moisture content was determined according to the ISTA (1999) procedure. Rather than two seed specimens, as specified by ISTA (1999), five seed specimens were used, each weighing 4-5 g; they were cut into pieces with a scalpel and after being weighed, they were placed in an oven at 105 ± 1^{o}C for 17 ± 1 hours. Then they were weighed again and the seed moisture content was determined for each specimen separately (Edwards 1987, Hartmann et al. 1997, ISTA 1999). The mean of the five measurements, whose difference did not exceed 0.3-2.5%, constituted the seed moisture content, which was rounded off to the nearest 0.1% (ISTA 1999). Seed moisture content was expressed as a percentage of the fresh weight (%, f. wt).

Seed moisture reduction using a fast process (in an oven at 30^{o}C) - Calculation of seed moisture

The seeds were divided into lots containing 400 seeds each (400=4X100 for the germination test) and then, they were weighed and placed in a recycled air oven at a temperature of 30^{o}C. To establish the desired seed moisture content (35.0%, 30.0%, 25.0%, 20.0%, 15.0% and 10.0%, f. wt), the seeds were weighed constantly until they reached the predetermined weight that corresponded to specific seed moisture content. The specimens were weighed every hour on the first day of the experiment and three times a day on subsequent days (in the morning, in the afternoon and in the evening). After the seeds reached the desired weight, they were removed from the oven and were subjected first to pregermination treatments (see below) and then to a germination test.

Seed moisture reduction using a slow process (under normal conditions, 25^{o}C day/15^{o}C night)- Calculation of seed moisture

Regarding desiccation under normal conditions, the seeds were divided into lots of 400 seeds each and placed on Petri dishes on the laboratory bench at room temperature (25^{o}C day/15^{o}C night). The seeds were weighed with the initial moisture content and then every fifteen days a 400-seed lot (4×100) was removed and its moisture content was calculated on the basis of weight loss incurred and was expressed as a proportion of fresh weight. Then, the lot was subjected to pregermination treatments and then to a germination test (see below).

Pregermination treatment

The pregermination treatments that done on both fresh seeds and seeds with reduced moisture were: (a) soaking in tap water for ten days, changing the water every two days (to remove the external coat by manually rubbing the seeds); and (b) cold stratification (4 ± 1^{o}C) in moist river sand for 60 days (Takos 2001).

Germination test

In the germination tests, the seeds of each lot (4 replicates of 100 seeds each) were put in Petri dishes (20cm diameter) half covered by moist river sand; then the Petri dishes were put in a germination chamber. The temperature in the germination chamber was set at 25^{o}C for 8 hours with 1000 lux light coming from cold light bulbs and at 20^{o}C for 16 hours in the dark. The first count was made on the 7th day and then a count was taken every four days till the tenth week. The appearance of a 2-mm-long radicle was the criterion for germination. The total germination percentage was counted as the average of the four replicates. The germination value (GV) was calculated using the equation GV=PV x MDG (Czabator 1962), where GV is the germination value, PV is the peak value, which is calculated as the quotient of the highest value of the cumulative germination percentage, divided by the number of days from the beginning of the test, and MDG is the average daily germination.

Statistical analysis

The experimental design for the determination of the germination percentage and rate was a randomized complete block with four replicates. The statistical analysis (ANOVA) was done with the help of the computer software package SPSS (Norousis 1997). The means were separated according to Duncan's test at the 0.05 level of probability.

Results

Desiccation method –seed moisture content reduction

Tables 1 and 2 show moisture loss in ripe bay laurel fruit (seeds) under two different desiccation methods: (a) in

an oven and at a constant temperature of 30° C and (b) in alternating room temperature (25°C in the day /15°C at night). The desiccation rate in the oven was fast in the beginning and the moisture was reduced from 37.0% (initial moisture) to 35.0% in approximately 7 hours, to 30.0% in one day and 25.0% in three days. Then the desiccation rate slowed down, and the moisture content fell to 20.0% in 13 days and 15.0% in 34 days. The desired moisture content (10.0%) was reached in 43 days (Table 1).

Table 1. Bay laurel seed moisture content reduction through desiccation in an oven (30°C).

Desiccation days	Seed moisture content (%, f.wt.)
0	37.0
<1 (7 hours)	35.0
1	30.0
3	25.0
13	20.0
34	15.0
43	10.0

Table 2. Bay laurel seed moisture content reduction through desiccation under alternating room conditions (25°C day/15°C night).

Desiccation days	Seed moisture content (%, f.wt.)
0	37.0
15	28.7
30	23.5
45	21.0
60	19.6
75	17.0
90	15.2
105	15.2
120	15.3
135	13.7
150	12.8
165	12.8
180	12.8

As shown in Table 2, seed moisture content reduction through seed desiccation at alternating room temperature was performed at a slower rate compared to desiccation in an oven. After fifteen days of desiccation, the moisture content was reduced to 28.7%, after 30 days to 23.5 % and after 45 days to 21.0%. Then the rate of moisture reduction was slowed down even further. Thus, after 60 days the moisture content was 19.6%, after 75 days it was 17.0% and after 90 days it was 15.2%. After the 90th day, seed moisture content reduction progressed at an extremely slow rate. After 105, 120 and 135 desiccation days, the moisture content became 15.2%, 15.3% and 13.7%, respectively. Finally, moisture content stabilized at 12.8% after the 150th day.

Seed germinability

The effect of seed moisture content reduction through seed desiccation in an oven and under normal conditions had on seed germination is shown in Table 3. At first, moisture reduction had a positive impact because the seed germination percentage increased from 55.1% for seeds with the initial moisture content to 81.0% after a brief period of desiccation in an oven and 84.3% to 90.9% after partial desiccation under alternating room conditions; the difference in the values of these increased percentages was not statistically significant. Further reduction of seed moisture content resulted in a gradual reduction of germination, which differed statistically from the above-mentioned high germination values. Specifically, for seeds desiccated in the oven, when the moisture content was 30.0%, 25.0%, and 20.0%, germination fell to 44.0%, 33.2% and 24.0%, respectively. Germination dropped in a similar way when moisture content reduction was achieved under alternating room conditions: for moisture contents of 19.6%, 17.0%, 15.2%, 15.2% and 15.3% the germination percentages were 49.4%, 48.0%, 11.0%, 6.3% and 2.1%, respectively. The reduction of the seed moisture content to a value ≤15.0% using both desiccation methods led to very low seed germination percentages that were not statistically different from one another (Table 3). Therefore, it seems that when the moisture content drops below 15.0% seed germinability becomes practically null.

Table 3. The impact of seed moisture content reduction on germination.

Seed desiccation method % seed moisture content	Germination %*	Germination Value (GV) *
Initial moisture, before desiccation =37.0%	55.1c	1.03b
Seed desiccation in a kiln (30°C)		
35.0%	81.0a	9.55a
30.0%	44.0cd	1.36b
25.0 %	33.2de	1.35b
20.0 %	24.0e	0.18c
15.0 %	1.1f	0.00d
10.0 %	1.0f	0.01d
Seed desiccation at alternating room temperature (25°C day/15°C night)		
28.7 %	84.3a	4.30e
23.5 %	90.9a	7.17f
21.0 %	66.8b	0.96b
19.6 %	49.4c	0.85b
17.0 %	48.0c	0.32g
15.2 %	11.0f	0.10c
15.2 %	6.3f	0.03d
15.3 %	2.1f	0.00d
13.7 %	0.8f	0.00d
12.8 %	0.0f	0.00d

*The values that are followed by the same letter do not have a statistically significant difference p= 0.05, Duncan's test.

Germination value (GV)

Germination values are shown in Table 3. Partial and brief desiccation produced an impressive increase of the germination value. The highest germination value (9.55) was attained in seeds with seed moisture content of 35.0%, following seed desiccation in an oven at 30°C. Germination value was also high after desiccation under alternating room conditions for 15 and 30 days reaching values of 4.30 and 7.17 and seed moisture contents of 28.7% and 23.5% respectively. Germination values were very small for seeds with lower moisture contents and seeds with the highest (initial) moisture content.

Discussion

As demonstrated by this research, a small reduction of the initial seed moisture content produced an increase in germinability. This increase in germinability may be due to the continuation of the embryo after-ripening process, which takes place during seed desiccation. The increase in germination seen as a result of a slight loss in seed moisture content after a brief period of desiccation has also been observed in both recalcitrant and orthodox seeds. For example, Sycamore Maple (*Acer pseudoplatanus*) seeds, which are recalcitrant, display a slight increase in germinability after the partial desiccation of its seeds (Hong and Ellis 1990). The same phenomenon was observed with another recalcitrant seed, Horse chestnut (*Aesculus hippocastanum*), where a brief period of desiccation increased germinability (Tompsett and Pritchard 1998). However, the overall behavior of bay laurel seeds during the progressive reduction of their seed moisture content showed that their germinability is reduced when moisture is lost and that their germinability remains high only within a narrow range of high seed moisture contents. The gradual reduction of the seed moisture content with both desiccation methods resulted in the accelerated reduction of the germination capacity to the point of nullification. The results of the experiments as well as of previous research, which demonstrated that the desiccation of seeds caused a reduction of seed germination (Takos 2001), prove that the bay laurel seeds are recalcitrant, because they cannot be dried to a moisture content below 10.0% without losing viability (Roberts 1973, Bonner 1996). By desiccating bay laurel seeds, this research revealed the typical behavior of recalcitrant seeds. The "critical" minimum bay laurel seed moisture content that is necessary for the preservation of even a low level of viability is approximately 15.0%, depending also on the desiccation method. The corresponding "critical" moisture content for the recalcitrant seeds of other species is 25.0-30.0% for *Quercus* species (Bonner 1990), 35.0% for *Coffea arabica* (Bonner 1990), 20.0-22.0% for *Inga uruguensis* (Bilia et al. 1999) and 10.0% for *Acer pseudoplatanus* (Dickie et al. 1991).

Conclusion

The results of this research demonstrate that bay laurel seeds are recalcitrant. A low reduction of the original seed moisture content resulted in an increase of germinability, while further progressive seed moisture content reduction produced a significant reduction of germinability. The critical level of seed moisture content, the point at which seed viability becomes practically null, was determined to be equal to approximately 15.0%.

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Pine Seed Architecture

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In a study of the evolutionary trends associated with seeds, measurements and observations were carried out on 76 different seedlots from 68 taxa or 58 pine species, a fair representation of more than half of the species in the genus *Pinus* (and both subgenera *Pinus* and *Strobus*). The emphasis was placed on the cost of seed coat construction (on a biomass basis), as expressed in units of embryo plus megagametophyte (i.e. packing vs content). In every seedlot tested, a negative linear curve was obtained by regressing seed coat (as a ratio of seed coat mass over e+mg mass) on e+mg. However, when the average pair values of e+mg and seed coat ratio from all seedlots were plotted together, a positive linear regression was obtained, implying an evolutionary trend of a relatively increase in 'packing investment' for increasing 'contents' (e+mg) (Figure 1). It is concluded that an evolutionary trend of a relatively increased seed coat 'protection' with increasing embryo and megagametophyte biomass (seed content) does prevail, in contrast to the 'geometry rule' (as explained above).
Further measurements of seed dimensions resulted in assigning an index of sphericity for each taxon; these values were negatively regressed with either seed size or biomass. Finally, a number of additional traits and characteristics for each taxon (obtained from the literature) were correlated with seed architecture, size, volume and sphericity.

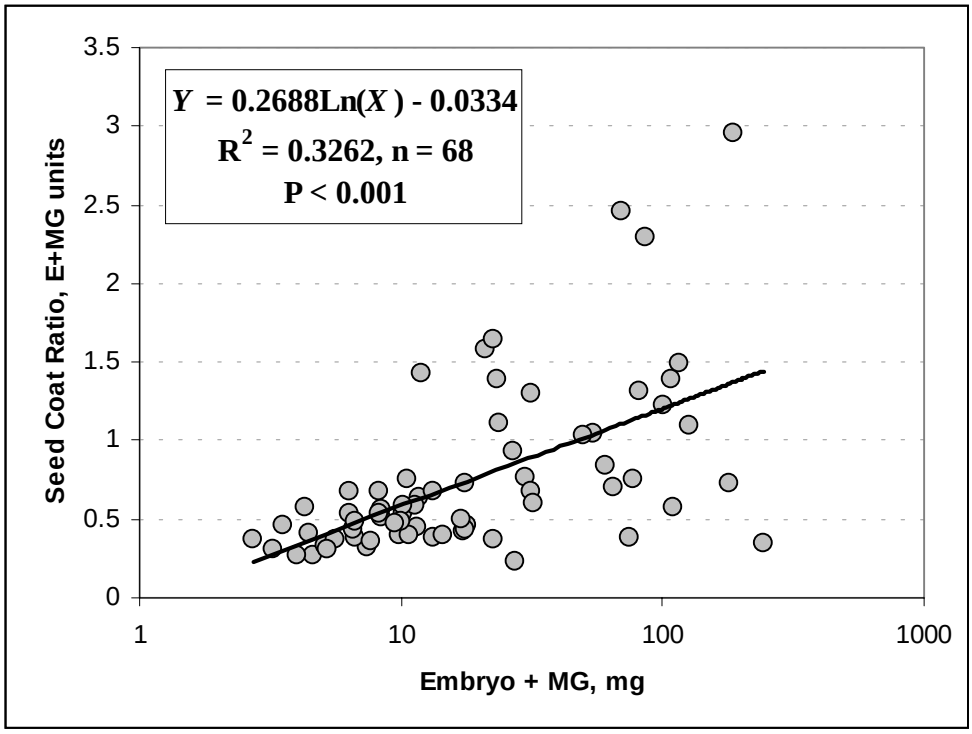

Figure 1. Regression of seed coat ratio values (expressed on a mass basis, in units of the respective embryo plus megagametophyte) over embryo plus megagametophyte mass. Each circle in the diagram corresponds to a particular, separate taxon (species or subspecies) of *Pinus*, total of pairs (n) is 68.

Do Trees and Shrubs Have Persistent Soil Seed Banks?

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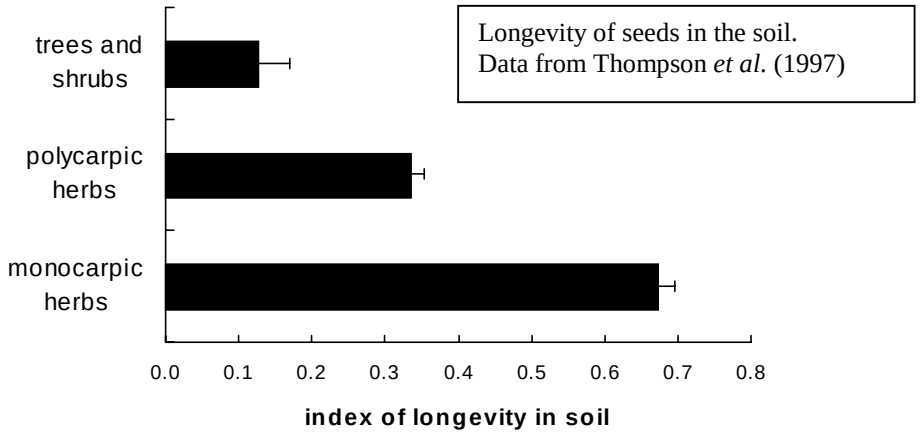
It is widely assumed that the great majority of woody plants do not accumulate persistent soil seed banks, and most of the evidence appears to support this conclusion.
However, there are two difficulties with the published evidence. First, most is derived from studies that identified the contents of the seed bank by germinating them. Since tree and shrub seeds are often dormant, many may have remained undetected. Second, since tree and shrub seeds are relatively large, we would expect them to be present at low densities. Most seed bank studies sample the soil inadequately, making this problem worse.



Here I examine the germination biology of tree and shrub seeds for clues to the probability of accumulation of a seed bank. Many tree and shrub seeds are dormant, often deeply so, but this is little help – dormancy and persistence in the soil are essentially unrelated. Much more important are germination requirements *after* dormancy is broken, in particular whether seed have a light requirement for germination. Unfortunately there appears to be an almost complete lack of evidence on this (Baskin and Baskin 1998).
There is evidence, some convincing, some less so, that some trees and shrubs can accumulate persistent seed banks (Marks 1974; Nakagoshi 1985). On the other hand, it seems likely that the high levels of predation suffered by large seeds prevent most species from accumulating really long-term seed banks. There is room for much more work on this under-researched topic.

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Near Infrared Spectroscopy as a Potential Technique to Upgrade Seed Lot Quality of Conifers

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Abstract

The potential of near infrared spectroscopy as a rapid technique to separate insect-infested and filled seeds of *Larix decidua* Mill., as well as empty and filled seeds of *Pseudotsuga menziesii* (Mirb.) Franco is demonstrated. Near infrared transmittance spectra, expressed in the form of log (1/T), were recorded from single seeds with 1225 Infratec analyzer from 850 – 1048 nm at 2 nm interval. Discriminant models were developed with partial least square regression (PLS) using the digital spectra as a regressor and a y-vector of artificial values (1 for filled and –1 for insect-infested/empty seeds) as a regressand. The result showed a 100% separation of empty and insect-infested seeds from filled seeds of *P. menziesii* and *L. decidua* respectively. Difference spectra and PLS weights indicated that the origin of spectral differences between insect-infested and filled seeds of *L. decidua* was attributed to differences in the amount of major reserve compounds that were low in infested seeds. Empty and filled seeds of *P. menziesii* were also discriminated based on differences in storage reserves that were completely absent in empty seeds. The results shed light on the prospect of developing near infrared spectroscopy-based sorting equipment for a rapid seed cleaning process in the future.

Keywords: *Pseudotsuga menziesii*, *Larix decidua*, empty seeds, insect, NIR

Introduction

Establishment of new forest plantations has increased globally over the last decade despite continued losses of the world's natural forests. The current global forest resources assessment report indicates that new forest plantation areas are being successfully established at the rate of 3.1 million hectares per year (FAO 2001). The success of sustainable reforestation/afforestation endeavors demands a continuous supply of high quality seeds for the production of seedlings in nurseries or for direct sowing out in the field. In addition, the cost of containerized seedling production and the success of seedling establishment by direct seeding punctuates the need for "precision sowing", which in turn depends on access to high quality seed lots of the desired species.

Many conifers produce a substantial quantity of empty seeds due to pollination failure or post-zygotic degeneration (Owens *et al*. 1990, El-Kassaby *et al*. 1993). Although floatation techniques for removing empty seeds from a seed lot are available, the efficacy varies between species, and complete separation is far from being achieved (e.g. Bergsten and Sundberg 1990). Insect infestation is another important factor that reduces the viability and vigor of a seed lot. Many insect species have been reported feeding on cones and seeds of conifers (e.g. Dajoz 2000). Insect predation reduces the germinability of a seed lot by damaging the embryo or exhausting the reserve food. Early infestation often causes abortion of the attacked ovule or the whole fruit, while attacks occurring later during fruit/seed development result in empty and damaged seeds (Janzen 1972, Hedlin *et al*. 1981). Furthermore, certain insect species continue to feed on seeds during storage and cause a great amount of loss in seed crops. Currently, there is no method to sort out internally infested seeds from a seed bulk, although chemical and other treatments are being used to kill hidden insects. It is, therefore, readily apparent that an efficient technique is needed to remove such unproductive seeds from a seed lot in order to enhance the lot's germination performance and subsequent seedling production.

Here, we demonstrate the potential of near infrared spectroscopy (NIRS) as a rapid technique to distinguish filled, empty and insect-infested seeds of two valuable conifer species, *Pseudotsuga menziesii* and *Larix decidua*. NIRS is a technique that can detect chemical composition and moisture content in biological materials based on the absorption of near infrared (NIR) radiation by bonds between light atoms, mainly C – H, O – H and N – H. These bonds generally have high vibrational frequencies and result in overtones and combination bands that are detectable in 750-2500 nm wavelength region of the near infrared spectrum (Osborne *et al*. 1993). NIRS has been found effective in detecting internal insect infestation in *Cordia africana* seeds (Tigabu and Odén 2002a), insect-damaged seeds of *Albizia schimperiana* (Tigabu and Odén 2002b), empty seeds of *Pinus patula* (Tigabu and Odén 2002c) and in separating viable and dead-filled seeds of *Pinus sylvestris* (Lestander and Odén 2002).

Materials and Methods

Preparation of seed samples

Samples of *Pseudotsuga menziesii* and *Larix decidua* seeds were obtained from a seed archive of the Forest Seed Science Centre, Department of Silviculture, Swedish University of Agricultural Sciences Umeå. They were stored in a cold room at -5 °C in glass bottles. Seeds were sorted into filled, empty and internally infested by X-radiography (ISTA 1999). Seeds were considered as filled if the embryo cavities together with megagametophte were visible and as empty when both the megagametophte and embryo were absent. Insect-infested seeds were those with visible larvae hidden in the seed. A total of 54 insect-infested and 60 filled seeds of *L. decidua*, and 80 empty and 80 filled seeds of *P. menziesii* were collected separately for scanning by NIR spectroscopy.

Acquisition of NIR Spectra

NIR transmittance spectra, expressed in the form of log (1/T), were collected from single seeds of each species with 1225 Infratec analyzer (FOSS Tecator, Sweden) from 850 to 1048 nm at 2 nm interval. Individual seeds were placed on a single seed adapter at 20 fixed positions and the average of 32 monochromatic scans was taken from each seed. Prior to scanning of every sample set (20 individual seeds at a time), a reference measurement was taken on the standard built-in reference of the instrument.

Model development and validation

A separate Principal Component Analysis (PCA) was performed first on data sets of each species as a basis for outlier detection and to get an overview of the data. Consequently, 1.67 % of filled seeds and 3.7 % of infested seeds of *L. decidua*, as well as 1.25 % of filled seeds of *P. menziesii* were found to be outliers. The deviation of these samples from the rest of the observations could be attributed to stray light effect, which in turn was due to failure to fully cover the slit of the single seed adapter as a result of smaller seed size. They were discarded from the final data sets. In addition, PCA score plots did not show a clear grouping patter of the two seed fractions, especially for *L. decidua* samples, which indicated systematic noise, such as light scattering, path length difference *etc*., in the data.

The data sets were divided into calibration sets for developing the discriminant models and test sets for evaluating the prediction ability of computed models as shown in Table 1. In order to resolve spectral features, the calibration sets were pretreated with orthogonal signal correction (OSC) procedure. OSC is a mathematical filter based on partial least square regression under the constraint that the weights in OSC are calculated to minimize the covariance between the spectral data, **X** and the response, **y**. Then components orthogonal to the response that contain unwanted systematic variation are subtracted from the original spectral data to produce a filtered descriptor matrix containing the variation of interest (Wold *et al*. 1998). Since no true response variable, **y,** existed in our data sets, artificial values of 1 for filled and –1 for empty and infested seeds were assigned.

Table 1. Number of seed samples used in the final calibration sets to develop the discriminant models and as test sets to evaluate the prediction ability of computed models.

Species	Seed fraction	Calibration sets	Test sets	Total
P. menziesii	Filled seeds	40	39	79
	Empty seeds	40	40	80
	Total	80	79	159
L. decidua	Filled seeds	40	19	59
	Infested seeds	40	12	52
	Total	80	31	111

Discriminant models were derived with Partial Least Squares (PLS) regression using the digitized spectra as a regressor and a y-vector of artificial values, 1 for filled and -1 for empty and infested seeds, as regressand (Martens and Næs 1989). For each species, discriminant models were developed on full spectral range (850-1048 nm) as well as on selected NIR absorption bands (880-940 and 1000-1048 nm). NIR absorption bands were selected based on the observed absorption peaks in the difference spectra and in the weight plots of the full spectrum model. The number of significant PLS factors to build the model was determined by cross validation. To do this, one seventh of the samples were taken out from the data set at a time, a calibration was developed with the remaining samples, and the removed samples were predicted. This was repeated for all samples, and a calibration was selected using the number of significant factors recommended by the software. A factor was considered significant if the ratio of the prediction error sum of squares (PRESS) to the residual sum of squares of the previous dimension (SS) was statistically smaller than 1.0, or if the predictive power (Q^2 = 1.0 – PRESS/SS) is larger than a significant limit. The optimal number of significant factors to build the model was, however, determined by examining the explained variation for y (R^2Y) and the prediction power (Q^2) for each PLS factor.

Finally, the computed PLS models were used to discriminate unknown samples in test sets. Prior to prediction, the same OSC filter used to transform the raw spectra in the calibration data sets automatically transformed the raw spectra in test sets into OSC-filtered spectra. The unknown samples in tests sets were assigned into different seed fractions based on a rejection threshold, which was set at 0.0. Seeds were considered filled if predicted values were greater than the rejection threshold, and all others were considered as either empty or insect-infested seeds. Recognition rate, defined as the proportion of number of filled, empty and insect-infested seeds predicted correctly to the total number of each fraction in test sets, was computed for each model. All model computations were performed with Simca-P, version 8, software (Anonymous 2000, Copyright: Umetrics AB, Sweden) and on mean-centered data sets.

Spectral regions sensitive to differences between filled and empty seeds of *P. menziesii*, as well as between filled and insect-infested seeds of *L. deciduas*, were determined from difference spectra and PLS weight plots. Difference spectra, computed by subtracting the average spectrum of filled seeds from empty seeds of *P. menziesii* and from infested seeds of *L. decidua*, indicate wavelength regions of interest. PLS weights also indicate which **X**- variables (wavelengths) contributed for discriminating the seed fractions. Thus, weight plots can be compared to NIR absorption of specific functional groups to get the unique chemical information distinguishing filled seeds from empty and infested seeds.

Results and Discussion

Table 2. Description and statistical summary of PLS models calculated based on different wavelength regions. Where A = the number of significant PLS factors to build the model, R^2X = the explained spectral variation, R^2Y = the variation between seed fractions explained by the model, Q^2_{cv} = the predictive power of a model according to cross validation and Q^2_{test} = the predictive power of a model for external test sets.

Species	Wavelength range (nm)	A	R^2X	R^2Y	Q^2_{cv}	Q^2_{test}
P. menziesii	850-1048	1	0.981	0.945	0.942	0.919
	880-940	1	0.998	0.945	0.942	0.933
	1000-1048	2	1.000	0.958	0.955	0.962
L. decidua	850-1048	2	0.986	0.830	0.824	0.984
	880-940	2	1.000	0.819	0.814	0.986
	1000-1048	4	1.000	0.921	0.906	0.999

NIR spectroscopic data were modeled with PLS regression to discriminate empty and filled seeds of *P. menziesii*, as well as filled and insect-infested seeds of *L. decidua*. The statistical summary for the computed PLS models is shown in Table 2. For *P. menziesii*, more than 97 % of the spectral variation (R^2X) was explained by 1 or 2 significant PLS factors (A) according to cross validation. This spectral variation, in turn, substantially

described the variation between empty and filled seed (R^2Y) with an excellent overall predictive power for the calibration (Q^2_{cv}) and test sets (Q^2_{test}). Similarly, PLS models explained a larger proportion of the spectral variations and described very well the variation between filled and insect-infested seeds of *L. decidua* with few significant factors. For both species, the model dimensions for 1000-1048 nm region were slight higher than the other models, which could be due to non-linearity problem as witnessed from the residual plots. In such cases, additional PLS factors, compared to the number of interferences, are needed to account for non-linear effects between composition, interferences and individual spectral variables (Martens and Næs 1989). In general, the model statistics indicated that NIR spectroscopy data contained much information, and the computed PLS models were more efficient, dimensionally less complex, and easier to interpret.

Discriminant plots for the first two PLS factors displayed clean and symmetrical clusters of empty and filled seeds of *P. menziesii*, as well as insect-infested and filled seeds of *L. decidua* in the calibration sets from full spectrum models, 850-1048 nm (Fig. 1). The first PLS factor, thus, summarized the relevant spectral information for distinguishing the two seed fractions in both species. The second PLS factor, however, described unexpected phenomena, such as non-linearity, which in turn could be attributed to heterogeneity in seed size and the size of wings remaining attached on the seed. The non-linearity problem was more pronounced in seed samples of *L. deciduas.* The second PLS factor was not significant for *P. menziesii*.

The recognition rate of empty and filled seeds of *P. menziesii* in the test set is shown in Table 3. Both full spectrum model and models derived from selected NIR absorption bands resulted in complete separation of empty and filled seeds. Likewise, insect-infested and filled seeds of *L. decidua* in the test set were perfectly recognized by all models (Table 4). In general, the results showed that NIR spectroscopy data contained much information that can efficiently be modeled to distinguish empty and insect-infested seeds from filled seeds of *P. menziesii* and *L. decidua* respectively. The success of discriminating the two seed fractions in both species with selected NIR absorption bands sheds light on the prospect of developing an automatic filter-type-sorting instrument in the future that is less expensive than the monochromatic instrument.

Table 3. Recognition rate of empty and filled seeds of *Pseudotsuga menziesii* in the test set by PLS models derived from different wavelength ranges.

Wavelength	Filled seeds		Empty seeds	
range (nm)	% member	% non-member	% member	% non-member
850-1048	100	0.0	100	0.0
880-940	100	0.0	100	0.0
1000-1048	100	0.0	100	0.0

Table 4. Recognition rate of insect infested and filled seeds of *Larix decidua* in the test set by PLS models derived from selected wavelength ranges.

Wavelength	Filled seeds		Infested seeds	
range (nm)	% member	% non-member	% member	% non-member
850-1048	100	0.0	100	0.0
880-940	100	0.0	100	0.0
1000-1048	100	0.0	100	0.0

Analysis of PLS weights and difference spectra of filled and empty seeds of *P. menziesii* indicated similar absorption regions (Fig. 2). In the 900-950 nm region, a small bump appeared around 912 nm, and a weak peak was observed around 1020 nm in the longer part of the NIR spectra. The 900-950 nm region corresponds to C – H stretch third overtones, and molecules responsible for absorption in this region are CH_3, CH_2, which are the common chemical moieties of fats, and oil (Osborne *et al*. 1993). The observed peak in the vicinity of 1020 nm

is characteristic of N – H stretch second overtone due to absorption by proteins (Murray and Williams 1987, Osborne *et al*. 1993). Apparently, the origin of spectral differences between filled and empty seeds is attributed to the variation in the amount of lipids and proteins. This variation arises from the fact that empty seeds are totally devoid of the major storage organ, the megagametophyte, where the reserve compounds are often stored. It also appeared on the weight spectrum that the absorption region characteristic of lipids influenced the model more than the longer region of the spectrum where protein moieties showed typical absorption. Lipids and proteins are the major seed storage reserves in *P. menziesii*, with the megagametophyte serving as the principal storage organ (Owens *et al*. 1993). In a study made on oil content and fatty acid composition of conifer seeds, Wolff *et al*. (1996) reported that the oil content of *P. menziesii* seeds amounts to 30%; and among the fatty acids, linoleic acid is the most abundant, accounting for 49.53 % of the total fatty acid compositions followed by Δ5 olefinic acids (23.54 %), pinolenic acid (18.37 %) and oleic acid (17.75 %). This finding accords with our previous study on *Pinus patula* seeds where absorption bands typical of fats and oils were found to be the major source of variation to classify empty and viable seeds with NIR spectroscopy (Tigabu and Odén 2002c).

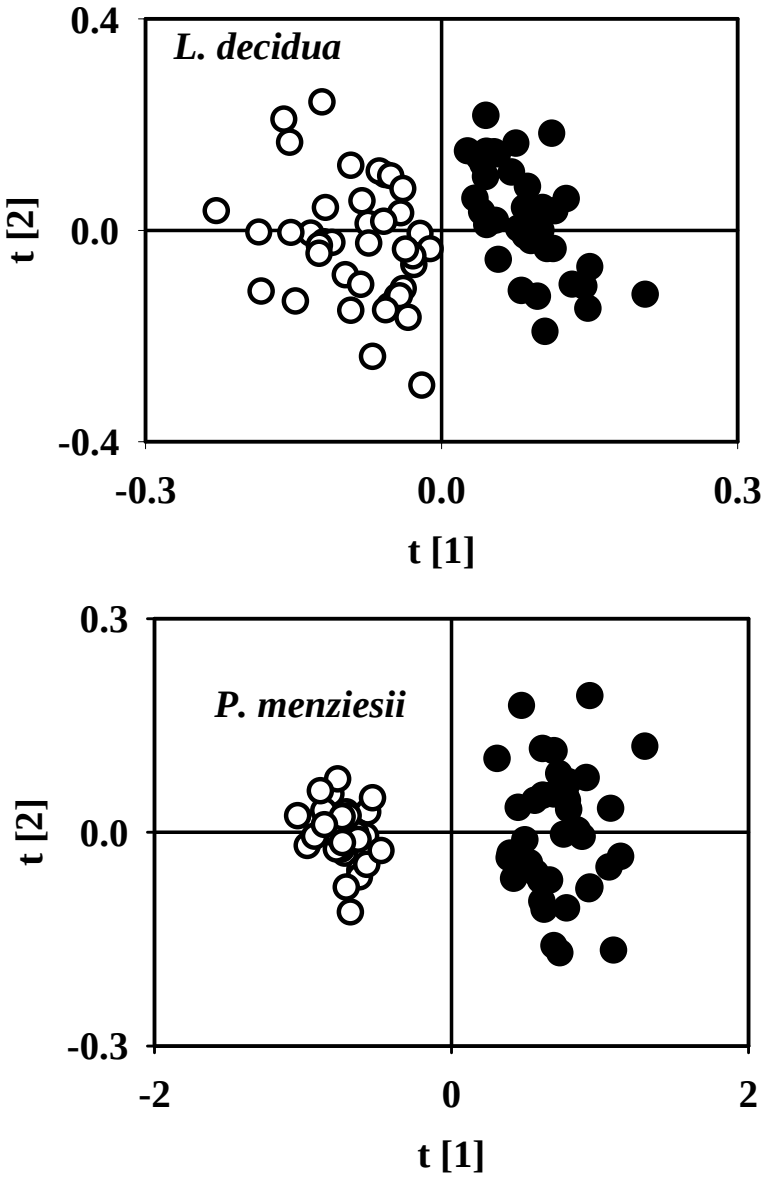

Figure 1. Score plots for the first two PLS factors (t_1 versus t_2) based on full spectrum models, showing clustering of filled and empty seeds of *P. menziesii*, and filled and insect-infested seeds of *L. decidua* in the calibration sets. Note that the second factor was not significant for *P. menziesii*, just to make scatter plot possible.

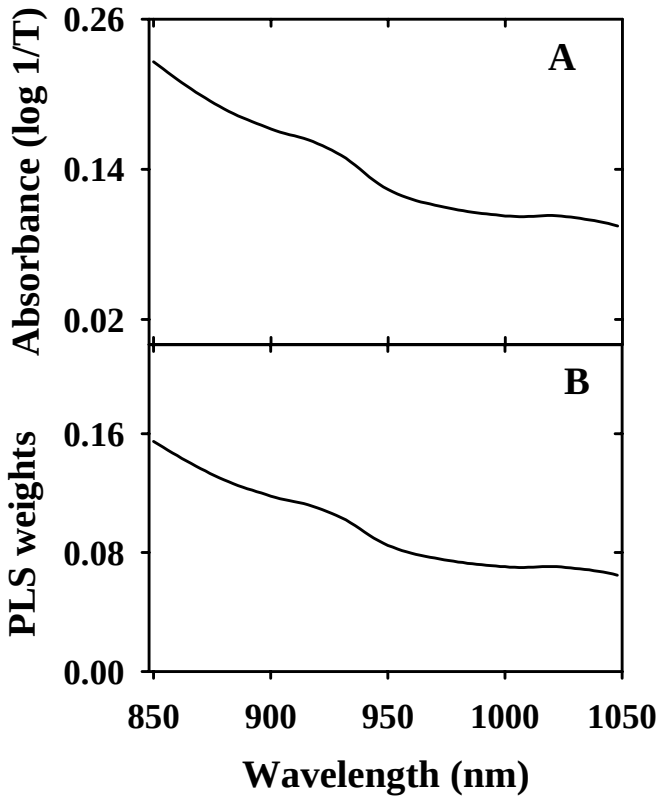

Figure 2. Difference spectrum (panel A) and PLS weights for the first factor (panel B) from the full spectrum model. Difference spectrum was obtained by subtracting the average spectrum of filled seeds from that of empty seeds of *P. menziesii*.

Difference and weight spectra of *L. decidua* revealed two absorption peaks in the 880-940 nm region, the center being at 894 and 916 nm, and one broader peak at 1030 nm in the 1000-1048 nm region (Fig. 3). The 880-940 nm region is characteristic of second overtone C –H stretching vibration of fatty acid moieties, CH_3, CH_2, as well as oil (Osborne *et al*. 1993). The latter region, 1000-1048 nm, corresponds to N –H stretch second overtone (Murray and Williams 1987), and the observed absorption peak at 1030 nm is specifically assigned to the amide functional group, RNH_2 (Osborne *et al*. 1993). Thus, NIR spectroscopy detected differences in the amount of lipids and proteins between filled and insect-infested seeds of *L. decidua*. It was apparent from the difference spectrum that filled seeds absorbed more of the incident radiation than insect-infested seeds. Closer examination of insect-infested seeds by a cutting test revealed that both the embryo cavity and the megagametophyte were absent, and only the larva was found enclosed within the seed coat. This suggests that either the feeding larvae have completely depleted the reserve compounds or the attack might have occurred early during seed development, and hence resulted in empty seeds. It has been shown that early infestation often causes abortion of the attacked ovule or the whole fruit and attacks occurring later during fruit development result in empty and damaged seeds (Janzen 1972, Hedlin *et al*. 1981). The weight spectrum also depicted that the absorbance region characteristic of lipids was more influential than the protein band. This is because *L. decidua* seeds have high lipid content with varying proportions of the major fatty acids: linoleic (43.10 %), Δ5 olefinic (30.36 %), pinolenic (27.39 %) and oleic (18.76 %) acids (Wolff *et al*. 1996).

Our result conforms to previous studies made on insect infestation in wheat kernels by Ridgway and Chambers (1996, 1999). They showed that starch content decreases with infestation, and hence accounted for the discrimination of healthy and infested kernels. We also found a decrease in lipid and protein contents with infestation as a basis for the discrimination of infested and sound seeds of *Picea abies* (Tigabu and Odén, manuscript in preparation). Contrary to our finding, however, some earlier studies reported typical absorption bands from insect cuticular lipids and chitin as an important origin of spectral difference between infested and healthy kernels (Dowell *et al*. 1998, Baker *et al*. 1999). Perhaps, this could be related to the size and developmental stage of hidden larvae in *Larix decidua* seeds. Effect of size and developmental stage of larvae on the detection sensitivity by NIR spectroscopy has been reported by Dowell *et al*. (1998). According to this study, NIR spectroscopy can detect 3rd and 4th instar rice weevils as small as 2 mm^2 with 95 % confidence, although the minimum detectable size can go as low as 1.1 mm^2 for lesser grain borers.

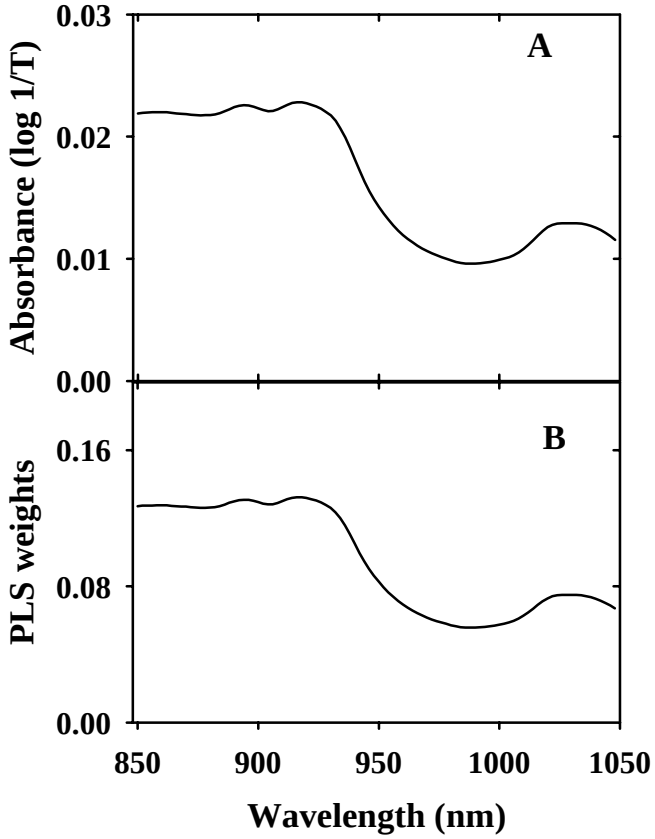

Figure 3. Difference spectrum (panel A) and PLS weights for the first factor (panel B) from the full spectrum model. Difference spectrum was obtained by subtracting the average spectrum of filled seeds from that of insect-infested seeds of *L. decidua*.

Conclusion

Our results showed that empty and insect-infested seeds are successfully discriminated from filled seeds of *P. menziesii* and *L. decidua* respectively with NIR spectroscopy. Thus, it can be concluded that NIR spectroscopy has a great potential to remove unproductive seeds from seed lots, thereby upgrading tree seed lot quality. Unlike density-based separation techniques currently in practice, NIR spectroscopy offers several advantages. It reduces the time and energy needed for sample preparation, rapidly scans seed samples at a rate of *ca* 12 sec/seed, and hence benefits seed companies by cleaning a large quantity of seeds in a very short time. Consequently, future research should be geared towards developing automated sorting equipment. Since selected NIR absorption bands resulted in complete separation of empty and insect-infested seeds, it is economically feasible to consider filters than monochromatic gratings during designing of sorting equipment.

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Damages Caused by Fungi in Norway Spruce, *Picea abie*s (L.) Karst. Seeds

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Introduction

Cone pathogens of *Picea* spp. cause malformation of cones and lower seed crop. Crop losses have been reduced in some cases by spraying trees with fungicides, even if the timing of treatment is vague. Fungi were revealed inside the seeds during microscopic studies of seed samples for other purposes. The aim of this study was, therefore, to present novel information about fungal damages found in spruce seeds.

Material and Methods

The structures of seeds and pathogens were examined using scanning electron microscope (FESEM) and light microscopy (LM).

Results

Two main types of spores were found either inside the seed coat or in the outermost layer, the sarcotesta. The seed coat opened faster and wider than usual in mature, moistened spruce seeds. Detached cells containing aeciospores of *Chrysomyxa pirolata* extruded from the opened seed coat. Aeciospores appeared particularly abundant in the nucellar layers. The sarcotesta was frequently infected by *Thysanophora penicillioides*, which is a common endophyte in Norway spruce needles. Wax cover, typical of mature conifer seeds, was in this case largely missing.

Discussion and Conclusions

Fungal damages appeared in the structures sheltering the embryo and the megagametophyte from desiccation and oxidation. Destruction of these tissues together with rapid opening of the seed coat impairs storage viability of seeds and causes economic losses. The occurrence of fungi in the nucellar tissue proves that female flowers are infected during pollination, when the integument is open at the micropyle. The results confirm convenience of this period for treatments against rust fungi in spruce seeds. Disease control may have economical importance particularly in spruce seed orchards.

Keywords: conifer seeds, pathogens, morphology, SEM, LM

Seed and Germination Characteristics of *Swietenia mahogani* (L.) Jacq.

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Abstract

Seed weight and germination characteristics of *Swietenia mahogani* (L.) Jacq. seeds collected from different strata of the tree and positions in the fruit were examined. Capsules were collected from the lower (L), middle (M) and upper (U) part of the tree. Furthermore, the capsules were divided into basal (near peduncle, B), central (C) and apical (A) positions.

The results showed that the heaviest seeds were those collected from the central and basal part of the fruit (354.16 to 405.76 mg) irrespective of position in the tree. The seeds from the apical part of the fruit were the lightest (216.13 to 242.30 mg). Similarly, the percent germination of the heavier seeds was significantly higher than the lighter seeds (80.5% to 98.5% vs. 47.5% to 54.25%). Germinative energy appeared to have similar patterns as % germination although the fruits from the MC, UB, and LB exhibited the significantly highest values (62.75% to 72.25%). As expected the lightest seeds have the lowest % GE (19.25% to 29.25%). The lightest seeds (LA MA & UA) have the longest pre-germination period ranging from 13.75 to 17.5 days. The pre-germination period of the heavier seeds ranged from 10.25 to 13.75 days. Weight correlates significantly with % germination, % germinative energy and pre-germination period.

The study suggested that capsules of *Swietenia mahogani* could be collected from any part of the tree. Seed processing should separate the lighter seeds from heavier seeds to ensure higher germination performance of the seed lot and subsequently better nursery stocks.

Keywords: *Swietenia mahogani* (L) Jacq., small leaf mahogany, seed collection, seed weight

Introduction

Several factors are recognized to affect seed production and the resulting seed quality, namely: age and size of trees, crown exposure, tree vigor, heredity and climate (Kramer and Kozlowski, 1960). Trees growing in open areas, dominant and codominant trees are usually the most prolific seeders. Better photosynthetic activities in these trees are presumed to increase available carbohydrates resulting in higher seed yields (Kramer and Kozlowski, 1960). Fowells and Schubert (1956 cited by Kramer and Kozlowski, 1960) observed that practically all the cones produced by ponderosa and sugar pines in California were all from dominant trees. Dominant trees of white fir produced about 88% of the fir cones, while codominant trees accounted for only about 12%. The work of Verne (1953 cited by Kramer and Kozlowski, 1960) on oak tree revealed that outer exposed parts of tree produced more acorns than those on partially shaded portions. He further noted that south facing crowns produced more acorns than those facing east or north.

Literatures describing the effect of position of seed in multi-seeded fruits to seed quality are very limited. Studies indirectly supporting this hypothesis are available which refer mainly to the effect of size, weight or density to seed and seedling quality. Seed weight and density are invariably varied. In the words of Kramer and Kozlowski (1960): "*The size of seed is extremely variable, differing from year to year with weather conditions, from tree to tree, and even with location in the cone or fruit. For example, the largest seeds usually are larger than those at the ends."* The authors attributed the differences to variations in efficiency of food translocation to various fruits and parts of fruits.

A listing of various studies describing how size, weight or density has contributed to seed performance is presented. In 1925, Hoffman observed that large and small seeds of sweet corn germinate at the same rate, but plants from large seeds reached canning maturity five days before those from small seeds. Culpepper and Moon (1941) also noted the size of seedlings from small, immature seed was much less than that of seedlings from mature seeds. Kneebone and Cremer (1955), in a study with five grass species reported that large seeds were superior in days to 50% emergence, in seedling height and fresh weight. In a similar study of ten grass species, Kittock and Patterson (1962), found that within a species, the correlation between field emergence at three weeks and seed weight was 0.98. Black (1959) reviewed seed size effects in small seeded species like those of the genera *Trifolium, Melilotus and Medicago*. Within a species and seed lot, large seed gave superior field

emergence especially when the seeds were sown deeply. The study showed that field emergence increased with seed weight. Heydecker (1972) listed other studies proving the improved performance of seedlings coming from large seeds. This includes: peas (Nobbe,1876); red clover (Schacl, 1970); lettuce (Scaife and Jones 1970); a variety of vegetables (Hewston 1964); and rice (Oelke, *et al.* 1969) (all 5 previous references cited by Heydecker, 1972).

An acceptable explanation on the better performance by larger and heavier seeds was appropriately described by McDaniels (1969) and Copeland (1976). In their own words, "*Aside from greater quality of storage materials and food products available in larger seeds, seedling fresh weight, seedling mitochondrial protein, and mitochondrial biochemical activity are reported to be positively correlated with seed weight. The increased quantity of mitochondrial protein of seedlings produced from heavy seeds is indicative of a higher respiratory rate and a greater amount of energy (ATP) production, giving heavier seeds a greater potential than lighter seeds.*"

Some researchers reported contrasting results. Moore (1943) found that the emergence of the largest seeds of a sample of *Trifolium incarnatum* sorted into 5 grade sizes was less than that of the smaller seeds sown at different depths of sowing. It was attributed to some genetic abnormality or to mechanical damage during harvesting which may have affected the large seeds in a sample than the small seeds, as with *Phaseolus* (Faris and Smith 1964). Austin (1972) also found that the largest seeds in commercial lots of radish (*Raphanus raphanistrum*) seed had a lower percentage germination and field emergence than seeds of an intermediate size. This may have been the result of mechanical damage for the siliquae of the radish when being threshed which require high drum velocities and close concave settings for efficient seed removal, and damage to the seed when the pods and seeds are very dry.

In the light of these interesting results, this study was designed to determine the effects of collecting seeds from different heights of the tree and position in the fruit on the seed characteristics and germination performance of small leaf mahogany (*Swietenia mahogani* (L.) Jacq.). Specifically, differences in weight and germination characteristics of seeds from various position in the fruit and tree were identified.

Materials and Methods

Seeds used in this study were collected from a small leaf mahogany (*Swietenia mahogani* (L) Jacq.) tree west of the Administration building of the University of the Philippines Los Baños-College of Forestry and Natural Resources during its maturity season. The tree has a height of 15 m and a diameter of 110 cm.

The position in the tree was determined by arbitrarily dividing it into three sections, the upper (U), thirteen (13) m, the middle (M), ten (10) m, and the lower (L) seven (7) m from the ground. For each stratum, intact capsules were collected. From the collected capsules, ten (10) representative fruits were randomly selected and studied. Three positions in the fruit were identified, (1) basal (B), which is nearest the peduncle, (2) apical (A), which is the opposite end of the basal, and (3) central (C) which is between the basal and apical. To insure consistency, the first three rows of seed from the apex were assigned as the apical, the next three rows, the central and the remaining rows as the basal seeds. Seeds from each respective treatment combination were mixed and randomly selected for weight determination and germination test. Treatment combinations were designated using two letters, the first letter representing the position in the tree and the second letter the portion in the fruit it was obtained, e.g. UC, means seeds from fruits collected from the upper part of the tree and the central portion of the fruit.

Whole seeds (including the wings) were weighed to the nearest 0.1 of a milligram. Germination test was conducted by randomly selecting four (4) 30-seed samples for each treatment combination. Seeds were germinated in germination trays lined with filter paper inside a Seedburo germinator set at a 12-hour cool (27°C) and warm (32°C) cycle with the light condition coinciding with the warm cycle. A germinated seed was defined as one with a well-developed radicle and plumule.

The following germination parameters were used to compare differences between the treatments: (1) percent germination, which is the percentage of normal seedlings germinating at the end of the test; (2) percent germinative energy, the cumulative percentage of normal seedlings up to the peak day and; (3) pre-germination period, the number of days before germination commences. Germination was terminated when no germination was observed to occur for three (3) consecutive days.

Analysis of variance, mean comparison by Tukey's HSD Test and correlation analysis between seed weight and the different germination parameters were performed using SAS/STAT (version 6.03 for PCs).

Results and Discussion

Seed Weight

Weights ranged from a high of 416.1 mg to a low of 114.6 mg or a difference of more than 300 mg per seed. The mean seed weight is 330.7 mg with a standard deviation of 79 mg. The significantly heaviest seeds came from the central and basal sections of the fruit irrespective of position in the tree (Fig. 1). On the average, the UC seeds are the heaviest (405.76 mg), followed by the LC, MB, MC, UB and MB. The weight values, however, are not significantly different. Seeds from MA, LA, UA are all significantly lower than the rest of the seeds. It will also be noticeable that standard error of the mean of the lighter seeds is relatively higher than the heavier seeds. Based on the morphological features of the lighter seeds, variation in sizes is obvious and very common. The seeds from the basal and central portions of the fruit are more uniform in size and based on the results obtained, even their weights are relatively homogenous. Based on the previous cited study by Kramer and Kozlowski (1960), the more efficient translocation of photosynthates could account for the heavier weights of the seeds. More food reserves could have been translocated to the seeds proximate to the peduncle resulting in better seed development as manifested by the heavier weights.

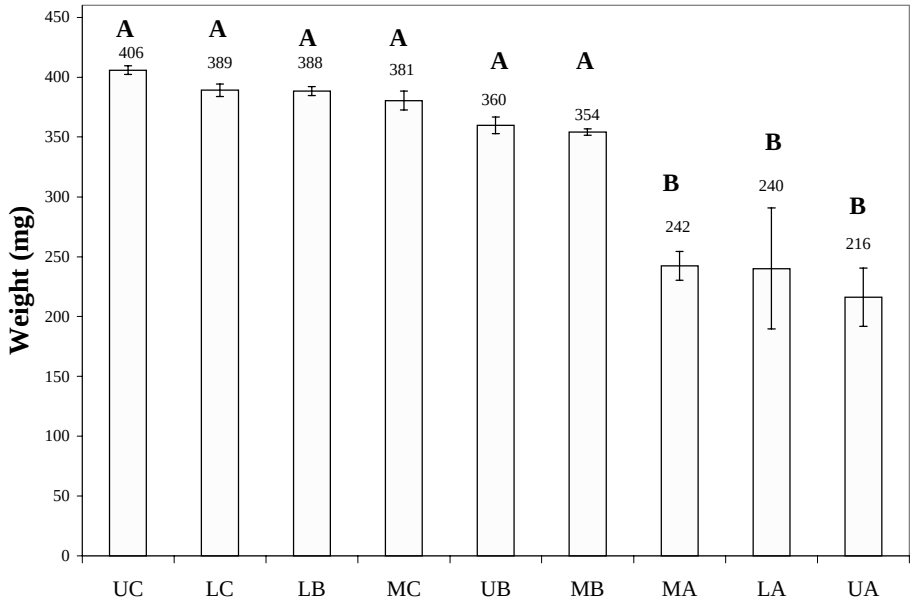

Figure 1. Average weight of *Swietenia mahogani* (L.) Jacq. seeds collected from different parts of the tree and part of the fruit (Legend: UA – seeds from upper part of the tree, apical part of the fruit; UC - seeds from upper of the tree, central part of the fruit; UB - seeds from upper part of the tree, basal part of the fruit; MA – seeds from middle part of the tree, apical part of the fruit; MC - seeds from middle of the tree, central part of the fruit; MB - seeds from middle part of the tree, basal part of the fruit; LA – seeds from lower part of the tree, apical part of the fruit; LC - seeds from lower of the tree, central part of the fruit; LB - seeds from lower part of the tree, basal part of the fruit). Means with the same letter(s) are not significantly different.

Germination Performance

A. Pre-germination Period (PGP)

The seeds from the MC, MB and UC showed the shortest pre-germination period (PGP) ranging from 10 to 11 days (Fig. 2). LB, UB and MC seeds come in second with 12 days. However, difference between the 10 to 12 day-PGP is not statistically significant. This is also true for UA with a PGP of 14 days. However, MA and LA seeds with a PGP ranging from 16 to 18 days were significantly longer in PGP than the previous seed sources. However, LB, UB, LC, UA, and MA (12-16 days) were statistically the same. UA, MA, and LA (14-18 days) were likewise not statistically different. It appears that differences in pre-germination period (PGP) are not as evident as the weight differences. Nevertheless, it is still clear that the seeds from the apical portion are in the tail end of germinating seeds, meaning that delays in germination of these seeds are expected. In fact, comparing the

extreme values, there is a difference of more than one week (8 days), which under nursery conditions is very important.

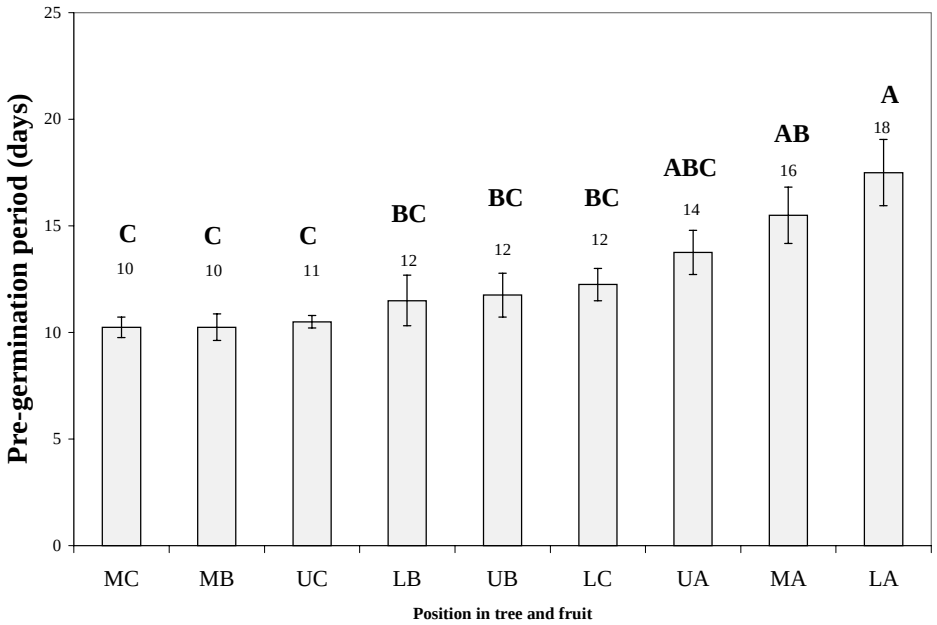

Figure 2. Pre-germination period of *Swietenia mahogani* (L.) Jacq. seeds collected from different parts of the tree and part of the fruit (Legend similar to Fig. 1). Means with the same letter(s) are not significantly different.

B. Percent Germination (% G)

The viability percentage of seeds in the apical position is significantly lower irrespective of position in the tree (MA, LA, UA). Values ranged only from 48 to 54% (Fig. 3). The rest of the seeds whether it came from the lower, middle and upper part of the tree but obtained from the central and basal portion of the fruit are significantly higher. Values ranged from 81 to 99%. The trend is very similar to the weight of the seeds. The results could indicate that the well-developed seeds (heavier) have better viability properties than the lighter seeds. Morphologically, these seeds would have bigger embryos and cotyledons. Physiologically, as cited earlier, it may contain more mitochondrial protein resulting in higher mitochondrial activity that would enhance the biochemical activities required in the production of ATP needed by the germinating embryo (Copeland, 1976).

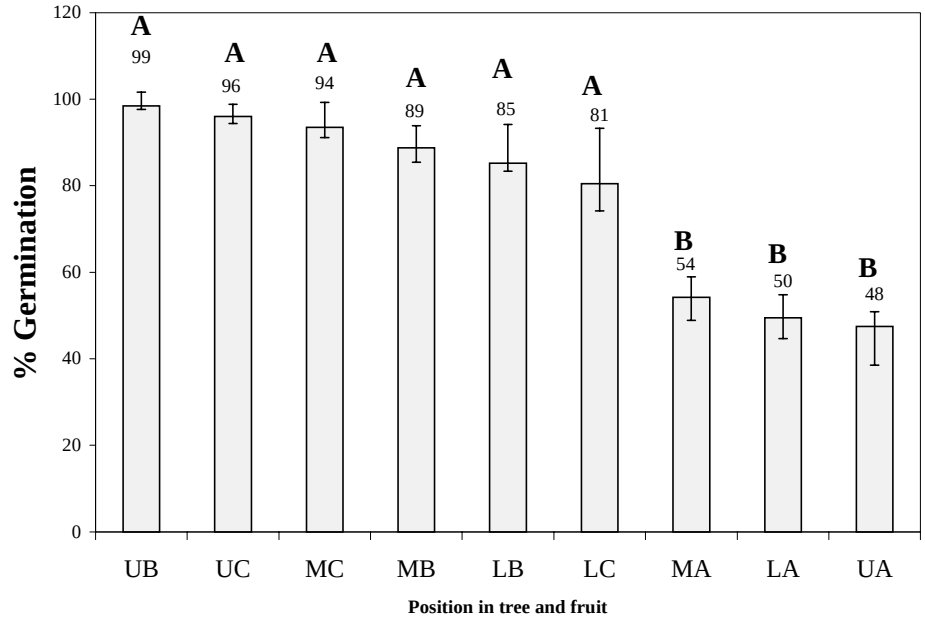

Figure 3. Percent germination of *Swietenia mahogani* (L.) Jacq. seeds collected from different parts of the tree and part of the fruit (Legend similar to Fig 1). Means with the same letter(s) are not significantly different.

C. Germinative Energy (GE)

Germinative energy (GE), which is used to measure vigor in this study, has no clear-cut statistical differences (Fig. 4). Values from 49 to 72% were considered not statistically different. These are for MC, UB, LB, UC, MB, and LC. The rest MA, UA and LA were significantly lower with values ranging from 19 to 29%. However, the lowest value (49%) among the seeds with higher %GE (LC) is not significantly different from the seeds of lower vigor. In like manner, MA (54%) and LC (49%) were not significantly different from the least vigorous seeds MA and UA. Though not as complicated as the PGP results, still the results have no evident differences unlike the % G and weight data. Nevertheless, it is still provides consistent proof that seeds from the apical position of the fruit are not as vigorous as those from the basal and middle sections of the fruit.

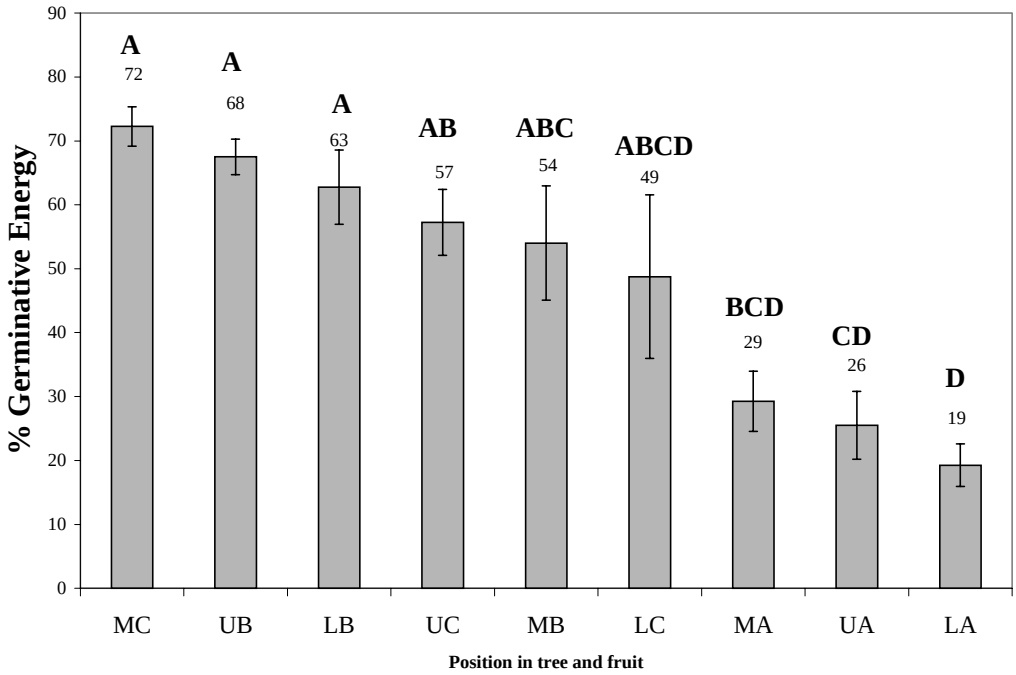

Figure 4. Percent germinative energy of *Swietenia mahogani* (L.) Jacq. seeds collected from different parts of the tree and part of the fruit (Legend similar to Fig. 1). Means with the same letter(s) are not significantly different.

All the germination parameters correlate significantly with seed weight. Among the germination parameters, % G has the highest correlation coefficient (r=0.80, Table 1). % GE has the second highest correlation coefficient with 0.64, while PGP has a correlation coefficient of -0.57. This clearly demonstrates that germination characteristics of seed particularly viability is highly affected by the weight of the seeds, i.e. heavier seeds have better germination and vigor levels coupled with shorter pre-germination periods.

Table 1. Correlation analysis between seed weight and the germination parameters of small leaf mahogany.

Germination Parameter	Pearson correlation coefficients/(Probabilities)*
% Germination	0.80801 (0.0001)*
Germinative energy	0.64687 (0.0001)*
Pre-germination period	-0.57558 (0.0002)*

Conclusions

The germination characteristics of the *Swietenia mahogani* (L.) Jacq. seeds are clearly influenced by the source of the seed in the fruit segment but not position in the tree. Seeds from the basal and the central portions of the

fruit are heavier, more viable and vigorous and has shorter pre-germination period. The hypothesis that efficient translocation to developing seeds appear to control weight and germination characteristics of the seeds. The foregoing results become important during seed collection and processing of this species. When mechanical seed extraction of the species is utilized which may involve drying of the mature dehiscent capsules, seeds may have to be upgraded through machines that adequately distinguishes seeds by weight, e.g. gravity table separators, air screen cleaners to separate the light from the heavy seeds. In developing countries where manual seed processing is the common practice, workers have to be instructed to separate the seeds from the apical end of the fruit to avoid including them in the lot. If the capsules are opened through sun drying, an additional step of upgrading to remove light seeds is necessary to insure better quality seeds for the nursery stock production.

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Thermal Conditions for Dormancy Release of *Cornus sanguinea* L. Seeds

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Abstract

Seeds (in stones) of *Cornus sanguinea* L. extracted in autumn from fully ripe fruits are deeply dormant. In the seedbed, in natural conditions, they do not start germination erlier than before the second or the third spring. After sowing pretreatment at warm-followed-by cold stratification, they germinate fast (3-5 weeks) and at a high level (about 90%) when cyclically alternating temperature 3~15^{o}C or 3~20^{o}C (16+8 hours/day) is applied. The optimal temperature for seed pretreatment is when the warm phase of stratification runs for 14 weeks at 15~20^{o}C changing diurnaly (24+24 hours/two days) and after that for 24 weeks at the constant temperature of 3^{o}C.

Keywords: stratification, germination

Biochemical Basis of Desiccation–Sensitivity in Tropical Forest Tree Seeds

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Abstract

Mechanism of recalcitrant storage physiology in seeds is poorly understood. The critical water content of desiccation tolerance varies greatly among different desiccation-sensitive seed species. In an attempt to understand the mechanism of desiccation tolerance/sensitivity two economically important tropical forest tree species; sal [*Shorea robusta*] and neem [*Azadirachta indica*], with different critical moisture contents were selected for study. The critical moisture content [CMC] for sal and neem was 36.5 and 14.8%. We have monitored changes in cellular leakage, lipid peroxidation product, superoxide anion and free radical processing enzymes in drying mature sal and neem seeds during storage at ambient condition [25 ± 2^0C]. The decline in viability below critical moisture contents in these seeds was associated with sharp increase in electrolyte leakage, a marker for membrane perturbations. Cellular leakage was positively correlated with accumulation of lipid peroxidized products [LPP] and superoxide anions in deteriorating seeds. Manifold rise in the levels of LPP and superoxide was discernible in seeds dried below CMC. The pattern of antioxidant enzyme viz., ascorbate peroxidase [AP], glutathione reductase [GR] and catalase [CAT] was similar in sal and neem seeds in response to dehydration. Low levels of enzyme activities were recorded in undried seeds. Dehydration of these undried seeds induced gradual increase in enzyme activities. Enzyme activities reached peak levels in seeds dehydrated to CMC and then declined sharply in the deteriorating seeds. However, differential pattern of superoxide dismutase [SOD] was discernible in desiccating sal and neem seeds. The 100% viable undried seeds dried up to 37.6% moisture content exhibited higher levels of SOD but reduced abruptly on further drying with the loss of viability, like AP, GR and CAT. Where as, in neem seeds, relatively very low activity of SOD was maintained in the 100% viable undried and seeds dried to 14.8% moisture content [CMC]. Desiccation of neem seeds below CMC enhanced SOD activity manifold. Absolute failure of antioxidant enzyme system perhaps leads to recalcitrant storage physiology in sal seeds whereas partial impairment e.g., loss of AP, GR and CAT only with high levels of SOD in seeds desiccated below CMC leads to intermediate storage behaviour in neem seeds. The intermediate behaviour of neem seed is further substantiated by recording the induction of SOD activity below CMC in response to dehydration induced superoxide signal. Thus, free radical processing enzymes, and not the superoxide and LPP *per se*, play a detrimental role in offering ultimate protection against desiccation-induced loss of viability.

Changes in Heterogeneity of Pine Seeds During Ageing in Low-Oxygen Atmosphere

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Abstract

Pine (*Pinus sylvestris* L.) seeds were stored for 30 days at 12, 22, or 37°C in hermetically sealed containers in a low-oxygen atmosphere. A decrease in germination percentage was accompanied by an enhancement of both room temperature phosphorescence and electrolyte leakage from the seeds. The initial unimodal distribution of seeds by room temperature phosphorescence was transformed into a multimodal one, with three major components corresponding to vigorous, nonvigorous, and dead seeds. It is assumed that ageing of individual seed is a stepwise, rather than gradual, transition from the viable to the nonviable state through an intermediate metastable state. The dynamics of the structural transitions during seed ageing can be monitored by measuring the intensity of the room temperature phosphorescence of air-dry seeds.

Keywords: accelerated ageing, germination percentage, low-oxygen storage, *Pinus sylvestris*, room temperature phosphorescence

Introduction

During the storage of seed lots the number of viable seeds declines. Prior to their death, seeds gradually lose their vigour (reviewed by Smith and Berjak 1995). In our previous studies (Veselova et al. 1996; 1999; Veselova 2001) on electrolyte leakage, length of axial organs, and room temperature phosphorescence (RTP) of air dried pea seeds, we noted that an increase in seed heterogeneity during storage and accelerated ageing was caused by the appearance of new discreet fractions in seed lot. If the individual seed gradually lost its quality, it would be impossible to distinguish any fraction in seed lot during ageing. In other words, the existence of several fractions at once suggested that ageing-dependent changes in properties of individual seed occur in a step-wise manner, rather than gradually.

We wanted to analyse changes in seed lot heterogeneity under different storage conditions with another seed species. Pine (*Pinus sylvestris* L.) seeds were chosen with various moisture contents, stored at different temperatures under low-oxygen conditions.

It is well known, that seed deterioration is delayed and thus seeds survive for longer durations when they are stored under low-temperature, low-oxygen, and low-moisture conditions (Roberts and Abdalla 1968; Ellis et al. 1982; Bewley and Black 1994). The seeds of legume and cereal grasses as well as sunflower seeds stored for several years in hermetically sealed containers in a low-oxygen atmosphere retained their sowing qualities to a greater extent than in air (Kartashova et al. 1984). Hypoxic conditions suppressed pest and microflora infection and changed the activities of some seed enzymes. For example, dehydrogenase activity in the seeds was reduced and the commonly used assay of seed quality based on the topographic staining of seeds with tetrazolium salts seems to be unsuitable for seeds stored in hypoxic conditions (Kartashova et al. 1985).

To determine the changes in seed lot heterogeneity, room temperature phosphorescence (RTP) was measured. The RTP-method allows for the determination of difference in individual seed moisture content (MC) (Veselova et al. 1999).

Materials and Methods

Pine *(Pinus sylvestris* L.) seeds had an initial germination of 98% and moisture contents (MC) of 8, 10, or 13%. These seeds were subjected to accelerated ageing for 30 days at 12, 22, or 37^0C in hermetically sealed containers. Twice a day, all hermetically sealed containers with seeds were blown for three to five minutes with nitrogen gas containing 1 to 2% of oxygen. The flow rate was 0.05 – 0.1 l/min per kg of seeds. Difference in MC of the seeds at the beginning and at the end of the experiment did not exceed 1% fresh weight.

The pine seeds (four replicates with 100 seeds in each replicate) germinated at 20-22^0C between filter paper moistened with tap water. The rate and percentage of seed germination was determined on the 7 and 15 days

after seed was placed on moistened filter paper. Root length was measured in 400 seedlings 5 days seed was placed on filter paper.

Because RTP level decreases with increasing MC, before RTP measurement, the treated pine seeds were equilibrated with air at 60% RH at 22-24°C for two weeks to a constant weight.

Individual seed RTP was measured with a Becquerel-type phosphoroscope. The double-disk system provided intermittent illumination of a seed with 6 ms light pulses (light intensity at seed level was 300 W/m^2); RTP was measured during dark periods, within 3 - 18 ms after switching off the excitation light. The measurement of phosphorescence of one seed needs 2-3 s.

The seed coat has a high optical density; therefore the exciting light does not penetrate into the seed. Room temperature phosphorescence is emitted mainly from the seed surface. In addition, the dark coloured pigments of the seed coat lower the luminescence emitted from the inner part of a seed. In experiments with fungal conidia, it was shown that the RTP level decreases with increasing melanin content of the cells (Shumaev et al. 1992). Therefore, it was necessary to decoat seeds before RTP measurements were taken. Electrolyte leakage was also examined after incubating 1 g of seeds in 10 ml of distilled water for 20 h. Water conductivity was measured with a conductometer (Model 571, Poland); the assays were repeated five times. The results given in the Table and Fig. 1 are the means +/- their standard error.

Results

Germination rate and germination percentage of pine seeds with various MC declined from 97 to 0% after storage at various temperatures (Table 1). The higher seed MC and storage temperatures resulted in an increased decline in % germination. Changes in germination rate were more pronounced in comparison with changes in germination percentage.

Table 1. Germination rate (numerator) and germination percentage (denominator) of seeds with various MC stored for 30 days at various temperatures in a low-oxygen atmosphere, 1-2%.

Initial moisture content of seeds, % fresh wt	Storage temperature, ^0C		
	12	22	37
8	$\frac{95 \pm 2.03}{97 \pm 1.35}$	$\frac{91 \pm 2.5}{93 \pm 0.6}$	$\frac{20 \pm 2.1}{57 \pm 4.14}$
10	$\frac{93 \pm 1.06}{95 \pm 1.06}$	$\frac{76 \pm 2.08}{85 \pm 2.9}$	$\frac{4 \pm 0.71}{13 \pm 2.25}$
13	$\frac{80 \pm 1.24}{85 \pm 0.75}$	$\frac{15 \pm 2.9}{16 \pm 2.84}$	$\frac{0}{0}$

Average RTP level and electrolyte leakage increased with decreasing germination percentage in 8, 10, or 13% moisture content seeds, which were kept in a low-oxygen atmosphere for 30 days at 12, 22 or 37°C (Fig. 1). Correlation coefficients were 0.98 for both dependencies, namely, average RTP and electrolyte leakage versus germination percentage. The relationship between them deviated from linearity only when less than 15% of the seeds were able to germinate.

The rate of electrolyte leakage characterizes the extent of cell membrane disturbances (Simon and Harum 1972), whereas the RTP intensity characterizes the state of cell surface biopolymers and their MC (Veselovsky and Veselova 1990). The parallel changes in these two independent characteristics indicate that the alterations in ageing seeds are integral and that RTP measuring can be used for testing the viability of pine seeds.

The method allowed us to detect RTP of individual seed and thereby to reconstruct the curve of seed distribution by RTP. As shown by earlier work, the individual seed distribution by RTP reflects the seed distribution by MC because the RTP level is inversely proportional to seed MC (Veselova et al. 1985).

In the initial seed and in the seed batch containing seeds with 95% germinability, there was only one seed fraction; the RTP distribution of this seed fraction was close to a normal distribution (Fig. 2a). The batch containing seeds with 85% germinability displayed a bimodal RTP distribution (the distribution had two

maxima). The second peak was formed by seeds, whose RTP was two times higher than the initial seed. In the seed batch containing seeds with 57% germinability, a third peak appeared, which was induced by the seeds with RTP level three-fold higher than RTP of the initial seed (Fig. 2b). In the seed batch containing seeds with 13 - 16% germinability, there were several fractions differing in RTP. Non-germinated seeds displayed a unimodal RTP distribution, with a single peak significantly shifted to higher RTP level.

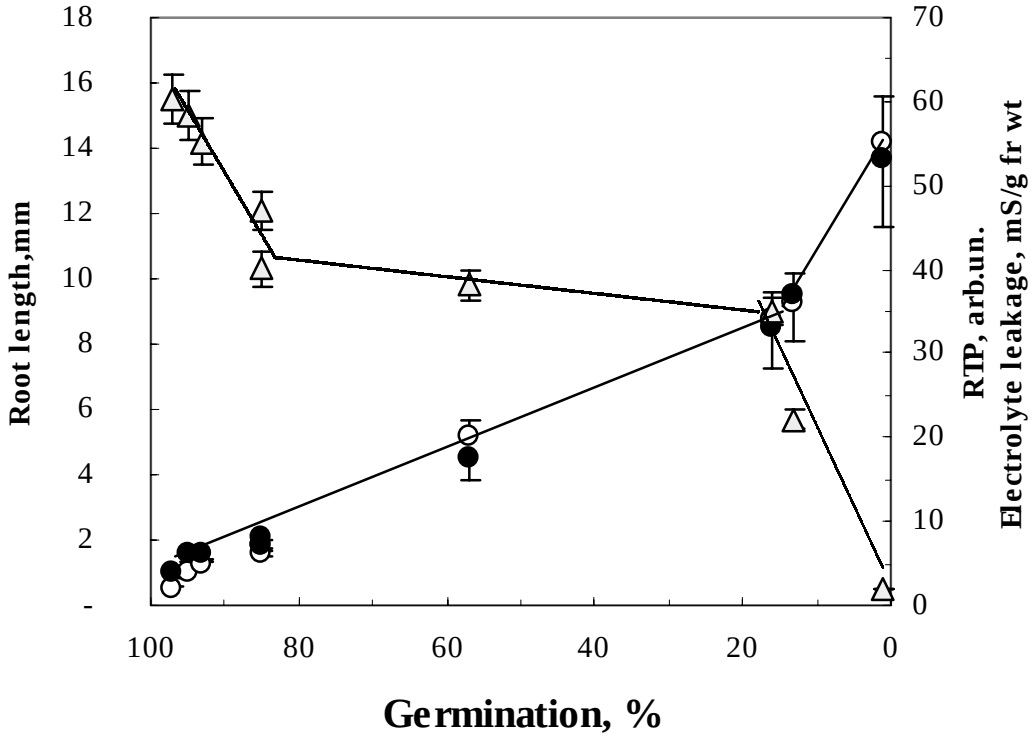

Figure 1. RTP of air-dry seeds (open circles), electrolyte leakage from soaked seeds (closed circles), and root length (triangles) of 5-day-old seedlings as a function of germination percentage of seeds with various moisture content that have been stored for 30 days in a low-oxygen atmosphere at various temperatures.

Bearing in mind the three-fold enhancement of RTP in the dead seeds as compared to viable air-dry seeds (Veselova et al. 1985), we can conclude that the percentage of the dead seeds is lower than the value obtained by counting the number of non-germinated seeds. Judging from the RTP intensity, the seed lot, which contained 85% of the germinated seeds, did not contain any dead seeds. The seed lot, which contained 57% of the germinated seeds, appeared to contain no more than 25% - 30% of dead seeds, and lot with 15% germinating seeds should contain at least double the percentage of viable seeds.

Therefore, the tetrazolium method underestimated the percentage of viable germinating seeds, because in the seeds stored in a low-oxygen atmosphere, the embryos are only faintly colored with tetrazolium salts, but the RTP measurements show that not all viable seeds can germinate.

It is believed that cell membrane damage during seed imbibition causes loss of seed germinability and that accelerated ageing enhances the appearance of such disturbances (Tilden and West 1985; Smith and Berjak 1995; Vertucci, 1989). For example, we observed that not all actively respiring seeds from a batch of artificially aged barley seeds were able to germinate (Ugol'nokov et al. 1992). By measuring RTP in soybean seeds, we have shown the appearance of a fraction of non-vigorous seeds. These viable non-vigorous seeds did not germinate because the embryos were unable to disrupt the seed coat. After the seeds were scarified, they were able to germinate (Veselova et al. 1988). Hence, the difference between the percentage of germinated and viable (according to RTP measurements) pine seeds can be related to the loss of seed vigor during ageing; this conclusion follows from a delayed germination (a disparity in the percentage and rate of germination can be seen from Table 1) and shorter length of seedlings developed from the aged seeds (Fig. 1).

It is interesting that the seedlings with short roots appeared from seed lots with germination percentages below 85%. Here the distribution of air-dry seeds by their RTP became bimodal, displaying a new fraction of seeds

with a doubled RTP level. The short-root seedlings appeared as long as this fraction was present in the seed distribution by RTP. When this fraction disappeared, the seeds fail to germinate.

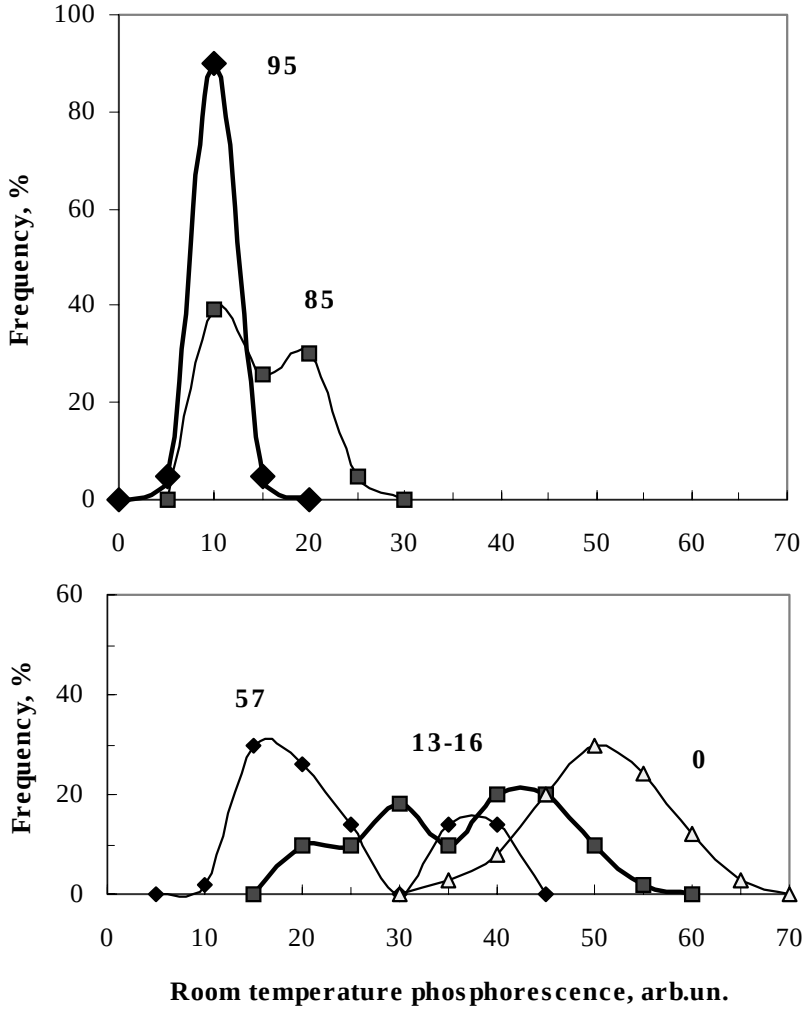

Figure 2. The distributions of air-dry pine seeds by RTP after accelerated ageing. The numbers near curves indicate average germination percentage of a seed batch. Each curve was drawn using the data for 40 seeds.
(a) RTP distribution of the starting seed material coincides with that of seeds with 95% germinability (first peak). Note that RTP distribution of seeds with 85% germinability displays the first and second peaks,
(b) Seed distribution by RTP in a seed batch, in which dead seeds were present. Note that the first peak disappeared and only second and third peaks are present in the seed distribution by RTP in the seed lot with 57% germination percentage.

Our data show that pine seed deterioration is a stepwise, rather than gradual process, which results in the appearance of discrete fractions in the seed distribution by RTP. The average size of the short-root seedlings remained almost unchanged with germination percentage decreasing from 85 to 15%, and such seedlings did not emerge from the seeds during further deterioration (Fig. 1). The structural transitions underlying these phenomena seem to occur as early as in air-dry seeds and lead to an abrupt transition of some seeds from one fraction to another. These sharp alterations in the seed state can escape the investigator, if seed ageing is monitored using characteristics averaged over the whole seed lot.

Discussion

The time during which the seeds maintain their viability (average lifetime) depends on their genotype and storage conditions (temperature, humidity, oxygen content in air, etc.) (Bewley and Black 1994; Roberts 1986). To monitor seed condition and estimate the optimum storage period, seed viability is routinely tested by

measuring seed germinability and vigor. Then the average lifetime of seeds in the seed lot and its dispersion are determined assuming that the lifetime distribution of seeds is normal (Maguire, 1977). However, each seed can germinate only once. Therefore, this method prohibits any ways of discovering when an individual non-germinated seed would die, and how long an individual seed would remain alive if it was not permitted to germinate. Investigation of the morphological, physiological, and biochemical processes responsible for seed deterioration allows one to predict the lifetime of the seeds (Maguire 1977). However, the application of these methods is associated either with seed hydration and dormancy breaking or with the destruction of seeds. Thus, none of the methods can monitor the state of each individual seed during storage.

We believe that the method of RTP measuring of air-dry seeds, combined with other methods, can help in the study of dynamics of seed deterioration during storage. This conclusion is based on the relationship between RTP and germination percentage of air-dry seeds (Veselova et al. 1996), which was confirmed here (Fig. 1), and on the possibility of fast and repeated non-destructive measurements of RTP in individual seeds.

The suitability of the RTP method for testing seed quality is confirmed by the fact that the RTP level is closely correlated with electrolyte leakage from the pine seeds (Fig. 1). The practical advantage of RTP measurement over the estimation of electrolyte solution conductivity after seed soaking is its brevity: the former takes only seconds, whereas the latter takes hours. Therefore, the performance of these two methods differs by three orders of magnitude.

Quantitative estimation of RTP in the seed lots and individual seeds allowed us to investigate the specific features of viability loss by pine seeds during storage.The proportionality between germination percentage, RTP, and electrolyte leakage from seeds, was observed in that range of experimental conditions, in which the empiric rule of Harrington (Harrington, 1963) is obeyed. This rule dictates that an increase in seed moisture content by 1 % reduces their lifetime (and thus germination percentage) to the same degree as a temperature rise by 5°C. For example, when pine seeds differing in moisture content (10 - 13%) were kept at 22°C for 30 days, their germination percentage decreased from 85 to 16% (Table 1) and RTP rose from 18 to 38 arb.un. (Fig. 1). A similar effect on the germinability and RTP of pine seeds having 10% moisture content was produced by increasing storage temperature by 15°C (from 22 to 37°C) (Fig. 1 and Table 1).

However, at seed germination percentages below 20% (Fig. l and Table 1), the dependence of RTP and conductivity on seed germinability deviated from linearity. As follows from the seed distribution by RTP (see Results), not all viable seeds that germinated in the seed lots had a moderate germination percentage. These seeds had a lower level of RTP than the dead seeds. Because the presence of the former the total RTP was lowered as compared to that expected from the germination percentage of the seeds.

By measuring RTP, we have revealed that, irrespective of storage conditions, an intermediate fraction of seeds appeared in the homogeneous starting material, along with nonviable seeds. In our opinion, this fraction does not pre-exist in the initial seed population, but rather appears during the transition of individual seeds to a new state. Pine seeds from this fraction had a lower germination rate, and the root growth of their seedlings lagged during the first two weeks after germination. The average root length was almost the same in the seeds, whose germination percentage decreased from 85 to 15% (coefficient of variation was low 10%). Similar data has been obtained by other authors. For example, short-term accelerated ageing of soybean seeds was found to inhibit seedling growth, but further ageing did not impair the growth of young seedlings, despite a dramatic decline in germination percentage from 60 to 30% (Sun and Leopold 1993). The loss of seed vigour during ageing seems to be a stepwise, rather than a gradual process. It is significant that such deterioration occurs in air-dry seeds.

These phase transitions in seed condition can be detected because of the close dependence of RTP on the amount of water absorbed by the biopolymers. This method provides a means for detecting a change in seed moisture content of 0.1 - 0.2% fresh wt (Veselovsky and Veselova 1990). The loss of seed viability is accompanied by a decrease in their water content by 2%, i.e., from 8 to 6%. Taking into account that the water content of seeds is determined by the microstructure and conformation of biopolymers, it seems likely that seed ageing is accompanied by phase (cooperative) transitions in the biopolymer microstructure.

Conclusion

Using RTP measuring we have shown that the increase in seed heterogeneity upon ageing of pine seeds in low-oxygen atmosphere, as well as upon ageing of pea seeds in air conditions (Veselova et al. 1999) was caused by the appearance of a new fraction of low vigour seeds in an initially uniform seed lot.

Acknowledgments

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Symbiosis – What Can you Offer to Strengthen our RG?

Jack A. Vozzo
Chair, RG 2.09.00
USDA Forest Service, Southern Research Station, Starkville, Mississippi - USA

I would ask each of us to pause a minute and just look around the room.

The diversity of our Research Group is our strength. While each of us is an individual with personal and family responsibilities, we all share in common the professional goal to understand seed research and technology. Some of us are nurserymen, others are seed collectors, some teach, a few are in commerce, and we all do some level of research. I say all do some research because not one single person yet understands the complete picture of how, why, when do seeds mature, and store, and germinate to grow. But, collectively, we represent a dedicated group that will initiate and contribute to seed science.

Today we add to our objectives by meeting and discussing the fundamentals and the innovations that interest us. May I suggest that this week we reach out and find new collaborators and new ideas as we offer our own applications? Let us all go out of our way to meet new people. Ask them for new ideas and thoughts as much as we tell them of our own. We have such an opportunity this week related to our common goal in the RG 2.09.00 Seed Physiology and Technology.

As Chair, I want to know your ideas of what is interesting, what needs to be done, what are your talents? Specifically, can we channel all our collective talents to define a few RG projects that will, as a group, contribute to our goals? To accomplish any objective, we will need all members to participate ….. the nurserymen, the seed collectors, the teachers, the businessmen, the researchers…

Think about how we can accomplish some collective contribution … pause a minute and just look around the room.

Food Preferences of Mice and Voles May Be a Critical Determinant of Forest Succession in White Pine Ecosystems of Canada

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Abstract

Eastern white pine (*Pinus strobus* L.) does not regenerate well in the absence of fire, or without mechanical exposure of mineral soil, while balsam fir (*Abies balsamea* L. Mill.) is a common understory species on sites occupied by white pine. Seed predation has been shown to have a critical impact on white pine regeneration (Duchesne *et al.* 2000). The objective of this investigation was to determine whether micromammals such as mice and voles play a role in seed predation in white pine ecosystems. For this, we compared seed predation from exclusion boxes that limited seed access to micromammals to seed predation from boxes that did not limit access of seed predators. Predation of white pine seeds was four times greater than balsam fir seed predation even when seeds of white pine and balsam fir were left as a mixture on the forest floor. There was no difference between seed predation from exclusion and opened boxes, suggesting that mice and voles are important seed predators, which influence forest succession.

Keywords: *Pinus strobus*, *Abies balsamea*, succession, seed predation, mice, vole

Introduction

White pine (*Pinus strobus* L.) is an economically important species in eastern Canada and Northeastern United States. Contemporary management of white pine is conducted through an intensive two-cut shelterwood system, a technique that yields adequate white pine regeneration in seventy percent of stands (Corbett 1994). Ecosystems that fail to regenerate under shelterwood management are often replaced by early successional deciduous ecosystems dominated by trembling aspen (*Populus tremuloides* Michx), paper birch (*Betula papyrifera* Marsh) and balsam fir (*Abies balsamea* L. Mill.). Pre-European settlement ecosystems were fire dependant (Heinselman 1981) and it is presumed that the maintenance of white pine ecosystems is closely linked to fire (Mc Rae *et al.* 1994) for the creation of a proper seedbed. However, under the current fire exclusion policies, the understory of white pine ecosystems tend to be invaded by balsam fir and/or red maple (*Acer rubrum* L.) whereas white pine regeneration is often lacking (Corbett 1994; Day and Carter 1990; Methven and Murray 1974).

The interaction between light conditions, soil organic matter and soil moisture has been blamed for the regeneration failure of conifers in northern ecosystems (reviewed by Herr *et al.* 1999). White pine is moderately tolerant of shade (reviewed by Wetzel and Burgess 1994) whereas balsam fir is very tolerant of shade (Baker 1949). Thomas and Wein (1985a; 1985b) showed that balsam fir seeds are more sensitive to moisture stress than those of white pine in greenhouse conditions, while Herr *et al.* (1999) showed that organic material at the surface of the soil reduces white pine germination when soil moisture is a critical factor. Recently, Cornett *et al.* (1998) determined that balsam fir germination and early seedling establishment is more sensitive to soil organic matter than that of white pine in conifer-deciduous mixed woods of Northern Minnesota. For white pine, the negative impact of soil organic matter on seed germination can be negated with shading if there is sufficient soil moisture to support germination (Herr *et al.* 1999).

Seed predation has been implicated in the failure of artificial and natural seeding programs for many years (see for example Smith and Aldous 1947; Spencer 1954; Abbott 1961; Pank 1974) including direct seeding of white pine (Horton and Bedell 1960). Although seeds can be removed by birds, insects and a few other species of small mammals, mice and voles are generally associated with the failure of seeding programs (Abbott and Hart 1960; Martell 1979; Pank 1974; Vander Wall 1994). Duchesne et al. (2000) demonstrated that white pine seeds are predated at a greater rate than balsam fir seeds while there were no differences in the response of early germinants of these two species to physiological extremes of soil moisture and shading. Although we speculated that mice and voles might have been responsible for white pine and balsam fir seed predation, our experimental system did not allow us to discriminate between predation conducted by other groups of seed predators and predation by mice and voles (Duchesne *et al.* 2000).

The objective of this investigation was to ascertain the impact of mice and voles on seed predation as compared to other groups of seed predators.

Materials and Methods

Study site

This experiment was conducted in a white pine ecosystem (Table 1) with abundant balsam fir regeneration (4000 seedlings and saplings/ha) and poor white pine regeneration (< 100 seedling and saplings/ha). This site is located near Echo Bay, Ontario, Canada, (46°25 'N, 84 ° 02 ' W), approximately 28 kilometres southeast of Sault Ste. Marie, Ontario within the Algoma Section (L.10) of the Great Lakes-St. Lawrence Forest Region (Rowe 1972). White pine was extensively harvested from the site in the 1920s (J. Krohn, pers. communication) and there is no evidence of fire scars for the past 70 years. Understorey vegetation consisted mostly of *Aralia nudicaulis* L., *Aster macrophyllus* L., *Pteridium aquilinum* (L.) Kuhn. and *Corylus cornuta* Marsh. The soil is a Gleyed Gray Luvisol over a thick (>10 m) clay-loam deposit (Agriculture Canada 1983).

Table 1. Characteristics of dominant vegetation at the Echo Bay site. Algoma district, Ontario.

Species	Stems/ha	Mean age (years)	Mean DBH (cm)
Pinus strobus L.	20	115	42.3
Picea glauca (Moench) Voss	23	112	36.1
Populus tremuloides Mchx	120	55	31.2
Abies balsamea (L.) Mill.	520	21	11.3

Seed predation

Unstratified seeds of white pine and balsam fir [National Forest Genetics Resource Centre Seed Bank of PNFI, White pine: seed lot No 8930585, 97% viability *in vitro*; Balsam fir: seed lot No 9130077.0, 61% viability] were placed in: 1) exclusion boxes that limited seed predation to mice and voles, or 2) unclosed boxes that did not limit access to the seeds by all potential predators.

The exclusion boxes consisted of 30 cm x 30cm x10 cm wooden structures with a horizontal 2 cm x 30 cm opening permitting access to mice and voles but limiting access to other species, larger species that do not normally search the forest floor for single seeds. The bottom of the exclusion boxes consisted of nylon mesh (1 mm) taped to the bottom of the rings to prevent the seeds from mixing with forest floor material while allowing rainwater to drain. Three treatments were used for this experiment: 1) boxes with one hundred seeds of balsam fir; 2) boxes with one hundred seeds of white pine; and, 3) boxes with one hundred seeds of white pine and one hundred seeds of balsam fir. Ten exclusion boxes for each treatment were used for a total of 30 boxes which were randomly distributed throughout the forest stand.

This experiment was also replicated using unclosed boxes consisting of lidless exclusion boxes to determine overall seed predation rates. For this, ten exclusion boxes were used for each treatment for a total of 30 boxes which were randomly distributed throughout the forest stand.

After two weeks, the content of the boxes was transferred to plastic bags and the number of whole seeds subsequently counted in the laboratory. This experiment was repeated three times from October 4, 1999 to October 18, 1999, November 3 to November 17, 1999, November 21 to December 5, 1999.

Statistical analyses

Effects of treatments on seed predation were compared using analysis of variance (ANOVA) after the data was arcsine-transformed to ensure homoscedasticity. When ANOVA results indicated a significant treatment effect, Tukey's HSD test was performed on each reduced data set to determine which levels of the treatments differed (Sokal and Rohlf 1981).

Results

White pine seed predation (61.18%) was significantly different from balsam fir seed predation (14.19%) (Table 2, P =

0.005). The rate of predation of each seed species was not significantly affected by mixing species together. As well, there was no difference between the predation rate of seeds in exclusion boxes and the predation rate of seeds in open boxes.

Table 2. Average predation of white pine and balsam fir seeds in seed trays in a non-regenerating white pine stand. Trays with single species contained 100 seeds of either balsam fir or white pine whereas trays with mixed species were contained 100 seeds of both species. (N=10). Within lines, values followed by a different letter are significantly different at P<0.02. There were no significant differences within columns at P<0.05.

	White pine	Balsam Fir	White pine/balsam ratio
Rings:			
Single species	57.23 a	19.73 b	2.9
Mixed species	69.74 a	15.56 b	4.5
Exclusion boxes:			
Single species	55.62 a	13.45 b	4.1
Mixed species	62.14 a	9.23 b	6.7

Discussion

In this investigation we demonstrated seed predation by mice and voles to be a contributing factor in the regeneration of white pine ecosystems. Although seed predation has been implicated in the failure of artificial seeding programs of white pine, this investigation provides evidence that seed predators actively select white pine seeds over balsam fir seeds and that they reject balsam fir seeds. This conclusion is based on our observation that the predation rates of balsam fir seeds was not affected when both species were mixed together. Our results are similar to those of Abbott and Hart (1960) who demonstrated that deer mice (*Peromyscus maniculatus* Wagner) and red backed voles (*Clethrionomys gapperi* Vigors) prefer red spruce (*Picea rubens* Sarg.) seeds over balsam fir seeds. Moreover, mice and voles consumed and/or cached a quantity of red spruce seeds greater than the natural seed crop, thus seed predation was speculated to be a limiting factor determining the low ratio of red spruce to balsam fir regeneration in the Northeast (Abbott and Hart 1960).

Conifer seeds are predated by several animal species (Smith and Balda 1979, West 1992). The use of exclusion boxes in this investigation allowed us to determine that micromammals, which are mice and voles, consumed the bulk of seeds left in our study site. Our results are consistent with the findings that small rodents are the most important seed predators in North American forests (Smith and Aldous 1947, Smith and Balda 1979). In particular, mice of the genus *Peromyscus* tend to prefer eating seeds (Banfield 1975, Brown 1964, Bruce Wagg 1963, Drickamer 1970, Everett *et al.* 1978, Hamilton 1941, Jameson 1952, Kirkland 1990, Manson *et al.* 1998, Nowak 1991, Ostfeld *et al.* 1997, Williams 1959, Wolff and Dueser 1986). In practice, population density and female fertility of the deer mouse (*Peromyscus maniculatus*) in the boreal forest can largely be affected by seed supplies (Gashwiler 1965, Gashwiler 1979, Hooven 1976, Halvorson 1982). Deer mouse is the principal post-dispersal seed predator within its habitat range (Abbott 1961, Alhgren 1966, Gashwiler 1970, Sullivan and Sullivan 1982; Von Treba *et al.* 1998). Although we did not investigate the abundance of mice and voles in our research site, population densities of 7 animals/ha found in central Ontario (I.D. Thompson, unpublished results) are comparable to the those found by Abbott (1961).

Differential seed predation may have long lasting impacts on ecosystem succession. For example, Cornett *et al.* (1998) demonstrated that regeneration success of white pine seeds after two years was increased 3-fold by controlling seed predation using caging experiments whereas the regeneration of balsam fir was only marginally improved by controlling seed predation. One management implication of our findings is that controlling seed predation following artificial or natural seeding should improve white pine succession.

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Semina Palmarum: the Seed Conservation Biology of Palms

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Abstract

Palms are one of the most threatened groups of flowering plants globally, making an assessment of their conservation prospects urgent. So far there is only information on the seed storage characteristics of about 100 species, or < 5 % of the family. Semina Palmarum aims to double this baseline knowledge on palm seed conservation potential by working on 200 species in the next 5 years. Species are being selected on the basis of their conservation status, taxonomic and habitat coverage. The study operates at two levels: 1) for all species, a basic assessment of physical characteristics, germination rate at a single temperature, desiccation tolerance and germination morphology; 2) for selected species, the identification of optimal storage and germination conditions. In a first screen of about 30 species from 20 genera, there was prior knowledge of seed storage response for only six of the species. For the other species, at least nine of them (the others are still under testing) have been shown for the first time to have desiccation tolerant seeds. The project has links with Botanic Gardens and other institutes around the world.

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