CANADIAN TREE IMPROVEMENT ASSOCIATION/
ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

Tree Seed Working Group

NEWS BULLETIN

No. 32, Nov 2000

CHAIRS 'ARMCHAIR' REPORT

Holiday greetings to one and all – I wish you the best of times with family, friends and loved ones. At the CTIA meeting in Sault Ste Marie in August I was acclaimed as new chair of the Tree Seed Working Group. Firstly, I would first like to thank Howard Frame for the time he invested as chair of the group. Hopefully the future will shine brighter on tree improvement in Nova Scotia. Secondly, I would like to thank Ron Smith for his continued hard work on editing and preparing the Newsbulletin (even when his research strayed far from tree seed).

The workshop of the TSWG was a success with a range of perspectives on germ plasm storage ranging from seed banks to arboreta as living gene banks being presented. Abstracts from these talks will be part of the CTIA proceedings being prepared by Dale Simpson. The next CTIA meeting will be in Alberta in 2002. If anyone has suggestions on workshop topics or excursions, please forward your suggestion to Ron or myself.

This edition of the Newsbulletin is primarily concerned with the topic of Tree Seed Testing. Many individuals have contributed to this issue – thank you. I am hoping that by having a 'theme' or two in each issue that it will focus the contributions on similar topics, hopefully stimulate some discussion on the TREESEED Discussion Group and provide incentive for others to contribute on a topic specified in advance. I think it has worked well for this issue and hope it encourages others to contribute. I am suggesting the following topics for themes over the next few issues: Reproductive biology; Differences between wild and seed orchard produced seed; Seed in seedling production; and Gene conservation. I'm sure there are more suggestions - forward them and your articles to Ron or myself.

The Newsbulletin has been functioning for 17 years and continues to evolve with our membership. We want to hear about your successes, but also the blood, sweat and tears of your failures (so we know better). Relating to this issues theme is Ben Wang's article on testing insights in issue number 30. If anyone wants back issues of the Newsbulletin they can be obtained by contacting Ron Smith. The price is right at one Newsbulletin article ☺.

The Tree Seed Working Group began in 1983 with four objectives on promoting tree seed science and technology through:

1) Seed research from bud initiation to seed utilization;
2) Identification of seed problems relating to tree improvement and forest management;
3) Exchange of information on seed related problems
4) Advising on implementation practices

These still hold strong today, but the last concerns me. When was the last time the TSWG or the CTIA provided input on implementing practices? Is this not a current (reasonable) objective or are we too far removed from practices to be effective? The strength of the TSWG lies in its members (and their contacts) that span the gamut from pure academics, to seed producers, collectors, processors and those that use seed in producing our reforestation materials. All of your contributions are important in promoting the importance of tree seed science and technology. Be active – you'll be surprised at how quickly you will benefit.

You may notice under the contacts section that the two working parties (Cone & Seed Insects; and Tree Seed Processing and Testing) no longer exist. Peter de Groot and I have discussed this and feel strongly that the working parties are not active and there is no reason for their existence. These subjects are still very important, but there appears to be no reason to have separate working parties – it can all happen under the Tree Seed Working Group.

Summaries of two meetings are included in this edition: the XXI IUFRO World Congress in Kuala Lumpar, Malaysia and the 16th North American Forest Biology Workshop and Western Forest Association 2000 Conference. Have you attended or organized a meeting of interest to our members? All it takes is a few paragraphs to tell us about it.

I would also like to extend my congratulation to George Edwards who received a Distinguished Service Award from IUFRO for his contributions to the group as he stepped down as chair of Research Group 2.09-00 Seed Physiology and Technology. In keeping Canada present at the IUFRO table, Tannis Beardmore was elected as co-chair of this Research Group – congratulations Tannis. For those looking on the shelves for the revised "Seeds of Woody Plants in the United States" it hasn't arrived yet, but chapters completed-to-date can be viewed at http://wpsm.net. Hopefully some of the important tree seed chapters will soon be available.

I look forward to your comments and contributions. Tree seed is here to stay – let's make sure this working group is the vehicle for dealing with the challenges as we drive into the 21st century.

Dave Kolotelo
Chair TSWG

EDITORS NOTES

I would like to start my "few" words of wisdom by welcoming our new Chairman Dave Kolotelo. Dave has graciously agreed to take over from Howard Frame who has, due to significant changes in his work commitments, had to resign as Chair. Readers can note a "new" column in this issue: It called "The Chairs (Armchair) Report". In other words our new chairman is already making his mark on our Working Group!! [Editors note: Sorry Dave but I couldn't resist!!].

On a more serious note, Dave worked extremely hard soliciting contributions for this issue of the NewsBulletin - in fact 90% of this issue is attributable to his ability recruitment efforts - the contributing writers efforts not-withstanding.

I would like to personally thank Henry Kock, Dave Kolotelo, and Dale Simpson for agreeing to participate in the TSWG Workshop at the CTIA this past August. All three speakers did a great job at providing different perspectives on the subject of "The Role of Ex-situ Germ Plasm Storage in Gene Conservation". I also want to commend Dennis Joyce and crew for organizing an excellent and informative meeting and for providing great hospitality.

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Comments, suggestions and contributions for the Newsletter are welcomed by the Chairman or Editor.

Did you Hear?

Yet another "living fossil" dating back some 90 millions years was recently discovered near Sydney - Australia. The tree, which grows above 40 metres, has been christened unofficially the Nightcap Oak after its discovery in the Nightcap Range rainforest near Byron Bay, 650 km north of Sydney.

The tree is classed as belonging to the Proteaceae family;, of which native Australian banksias, waratahs, macadamias and grevilleas and South Africa's proteas are members. Cuttings from the trees have been taken and are being cultivated at Sydney's Royal Botanic Gardens.

For more info see the Dec 15 issue of the Globe and Mail

NATIONAL TREE SEED CENTRE

There was indeed a good fruit and seed crop on all species in the Maritimes this year. However, the weather/climate certainly had an impact. What started out to be an extremely early spring ended up with collections commencing later and being spread out over a longer period of time. Collections during the summer and fall occurred, for the most part, at the usual times. More effort was taken to collect seed and fruit from single trees than making bulk collections. This appears to be important for hardwood species which can exhibit a lot of tree to tree variation in germination. Also, seed is quite often requested from single trees. The winter months will be spent processing, extracting and testing.

Speaking of testing, the Centre has been encountering difficulties germinating a number of species: *Acer spicatum, A. saccharum, A. pennsylvanicum, Fagus grandifolia*. Seed is placed on moist Kimpak in germination boxes which are placed in a cooler at + 4° C. Different stratification times have been tried. Often the seed starts to germinate in stratification but after placing it in a germinator little or no more germination occurs. Ungerminated seed are fresh (contain a live embryo) when cut. The germinator environment is set for 20°C and no light for 16 hours and 30°C and light for 8 hours with a constant relative humidity of 85%. We are speculating these temperature conditions are too high for these species. Does anyone have any experiences or thoughts?

Willow Storage Experiment

Three species of willow (*Salix silicicola, S. brachycarpa* var. *psammophila* and *S. turnorii*) indigenous to northern Saskatchewan are classified by the Committee on the Status of Endangered Wildlife in Canada as 'Special Concern.' This category includes species with characteristics that make them particularly vulnerable to human activities or natural events. At some point in time it may be necessary or prudent to collect seed from populations of these species or other species for gene conservation purposes. One important question is how well can willow seed be stored. To provide an indication, three willow species (Bebbs willow (*Salix bebbiana*), pussy willow (*Salix discolor*), and red- topped willow (*Salix eriocephala*)) indigenous to New Brunswick were collected in late May - early June, 1999. The seed was extracted within 3 days of collection, the samples were cleaned and the moisture contents determined. The fresh seed was germination tested at its original moisture content. The samples were then halved and one of the sub-samples dried to a lower moisture content. The seeds were placed in cryogenic vials and stored at four different temperatures (+4°C, -20°C, -80°C, and -196°C). Seed stored at -196°C is in liquid nitrogen. The seed was tested at 3, 6 and 12 months and in the future at 2, 3, 4, and 5 years.

Although the seed have only been tested three times since being stored, some interesting observations can be made. The first and probably most important observation is that willow seed can be successfully stored at extremely low temperatures (-80°C and -196°C) without any significant detrimental effects. Seed stored at the higher moisture contents is still quite viable. Generally, germination has declined with increasing time in storage with the biggest decrease occurring for seed stored at + 4° C. The *S. eriocephala* seed has deteriorated more rapidly. The results for seed stored at its original moisture content at -20°C are shown in Figure 1.

Moisture contents were: 8.6% for *S. bebbiana*, 9.8% for *S. discolor* and 8.5% for *S. eriocephala*. Differences in germination are evident among the three species but they all follow the same trend during the 12 month period. The fall-down in germination from the time of storage to 12 months varies by species; for *S. bebbiana* it is 6%, for *S. discolor* it is 3.5% and for *S. eriocephala* it is 11%.

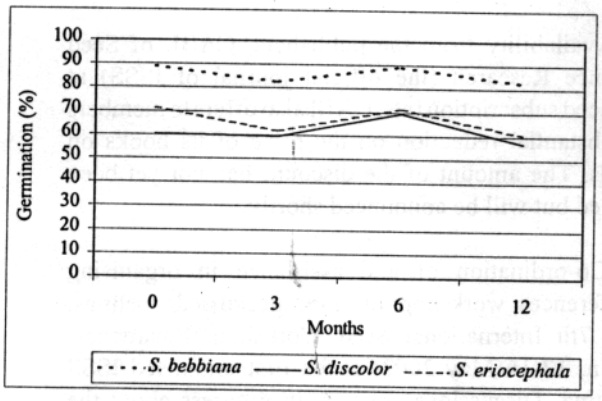

Figure 1. Changes in germination of three species of willow seed stored at -20°C.

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THE INTERNATIONAL SOCIETY FOR SEED SCIENCE (ISSS)

The International Society for Seed Science was inaugurated on January 28, 1999 at the 6th International Seed Workshop in Merida, Mexico. Its registration as a Charity with its official address in England was accepted in June 2000. Its objects are 'the advancement of education and research for the public benefit in the scientific study of seeds'.

The ISSS is a non-profit organisation with a registered office in England. Application forms for membership are available from members of the Executive Committee or from the ISSS web site at:

http://www.css.cornell.edu/ISSS/isss.htm

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Benefits to members

a) Availability from the publishers, CABI, of Seed Science Research (the official journal of ISSS) at reduced subscription rate. CABI also offers to members a substantial reduction on the price of its books on seeds. The amount of the discount has not yet been agreed but will be announced shortly.

b) Co-ordination of and assistance in organising conferences, workshops or more specialised meetings. The 7th International Seed Workshop (Salamanca, Spain, 12-16 May 2002) is the first organised ISSS meeting. Discussions are now in progress about the organisation of smaller, specialised meetings before and after Salamanca.

Note: The registration fee for attendance at ISSS meetings is reduced for members. For example, the saving on fees for registration for the 7th International Seed Workshop (Salamanca, Spain, 12-16 May 2002) is more than the membership fee for ISSS. For the 7th International Seed Workshop program and preliminary registration, see the conference web site.

[Ed. Note: See list of "Upcoming Meetings" in this issue for additional information on this conference including the web site]

c) Assistance for attendance at ISSS conferences etc (e.g., grants to student members for the Salamanca meeting have already been announced).

d) Publication of a newsletter.

e) Publication of proceedings of ISSS conferences and other meetings (sold at reduced price to ISSS members).

f) Posting of job listings.

g) Organisation of slide exchanges.

h) Sponsorship of courses in seed biology, support for educational activities, sharing of course materials, etc.

i) Involvement in political and public relations activities affecting seed research and utilisation.

j) Establishment of prizes and honours for meritorious work in seed biology.

k) Dissemination of information in various forms, including supporting e-mail groups like SEED-BIOLOGY-L, networking of members, etc.

The present members of the Executive Committee are:

Daniel Côme <come@ccr.jussieu.fr> (France), President (Trustee)
Kent Bradford <kjbradford@ucdavis.edu> (USA), Secretary (Trustee)
Hugh Pritchard <h.pritchard@rbgkew.org.uk> (UK), Treasurer (Trustee)
Michael Black (UK) (Trustee)
Ken Thompson (UK) (Trustee)
Ralph Obendorf <rlo1@cornell.edu> (USA), President-elect (Non-trustee)

Ordinary Committee Members

Henk Hilhorst <Henk.Hilhorst@algem.pf.wau.nl> (The Netherlands)
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Regional representatives

Steve Adkins <s.adkins@mailbox.uq.edu.au> (Australia) for Australia and New Zealand
Patricia Berjak <berjak@biology.und.ac.za> (South Africa) for Southern Africa
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Roberto Benech-Arnold (Argentina) and Mirian Eira (Brazil) for South America
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Jorge Vazquez-Ramos <jorman@servidor.unam.mx> (Mexico) for Mexico and Central America
Jia-Rui Fu <lssfjr@zsulink.zsu.edu.cn> (China) for China and Japan
Swati Sen-Mandi (India) for India

The Executive Committee will include one representative from the seed industry and one from affiliated Societies.

TESTING FOR SEED-BORNE PATHOGENS

The potential for conifer seed to become contaminated or infected with several fungal species makes testing for their presence a viable first step for managing seed-borne disease. Different conifer species are susceptible to contamination or infection from pathogenic fungi in varying degrees. Facts such as value of the seed, ability of any of the fungi to spread within a seedlot, ways in which the seed is collected and the fact that some tree seed is not affected by any of these fungi, result in some seed species being more susceptible than others. Also, if attacked, seed from some species represent a higher potential monetary loss. For these reasons, seed is tested for fungal pathogens in order of priority based on each species' potential to become contaminated or infected by each pathogen. Past testing has indicated the frequency with which different conifer seed species have been affected (contaminated or infected) by each species of fungi and these data, combined with known information about each fungus' life history and incidence of disease occurring on seedlings, are used in deciding priorities for testing.

In British Columbia three seed-borne fungi found on conifer seeds are of special importance to seed and seedling health. Species of fungi in the genus *Fusarium* contaminate seeds and are responsible for damping-off of seedlings and may potentially lead to *Fusarium* root rot and possibly even *Fusarium* shoot blight. Both *Caloscypha fulgens* and *Sirococcus conigenus* infect seeds. *Caloscypha* is responsible for killing seeds while *Sirococcus* infected seeds can kill the resulting germinants, and spread by spores to further infect and kill adjacent seedlings.

Seed-borne fungi are not all pathogenic or pose a threat to seeds or subsequent seedling health. It is important to remember this because although the appearance of mould or fungal hyphae growing from seeds may indicate good conditions for fungal growth in general, a reflection of improper storage, it does not necessarily indicate the seed to be inferior. A species of *Trichoderma*, a fungus known to produce antagonistic toxins, is used as a seed dressing to control damping off due to seed- and soil-borne *Fusarium* species. Many fungi are routinely found on conifer seeds, some of, which are potentially pathogenic, given the right environmental conditions, while others are relatively harmless.

***Fusarium* spp.:** Species of fungi belonging to the genus *Fusarium* are responsible for both pre- and post-emergence damping-off and can be implicated with a root rot and shoot blight of conifer seedlings. *Fusarium* is primarily spread by spores that can be borne by air, water, soil or seed. Soil-borne *Fusarium*, which can arise from overwintering of spores in soil or the introduction of spores by either air or water, is primarily a concern in bare root nurseries. However, similar mechanisms to those encountered in natural soils can occur in container settings where contaminated growing media is encountered or water- or air-borne *Fusarium* spores are introduced. In these situations *Fusarium*-contaminated growing media infects seedling roots leading in most cases to post-emergence damping-off, *Fusarium* root rot or shoot blight in this order of importance. Seed-borne *Fusarium* can lead to either of these situations but is most often responsible for pre-emergence damping-off.

Caloscypha fulgens: The common names, 'seed' or 'cold' fungus attached to this pathogen refer to its seed-borne nature as well as its ability to spread from diseased to healthy seeds during conditions of cold, such as stratification. This fungus was first reported in Ontario bareroot nurseries as pre-emergence damping-off of fall-sown pine seeds that failed to germinate and next identified as a pathogen in Britain on Sitka spruce seeds imported from North America. The fungus has subsequently been isolated from stored seeds in British Columbia, Oregon and Washington. This pathogen becomes seed-borne when cones contact forest duff or litter where C. fulgens lives.

***Sirococcus conigenus*:** *Sirococcus conigenus* causes a shoot blight of over 19 coniferous species in North America, Europe and Asia. The disease is particularly severe in British Columbia forest nurseries where it mainly affects spruces, lodgepole pine and western hemlock seedlings and to a lesser extent, Ponderosa pine. Also in British Columbia where the disease is known to be seed-borne on spruces, *S. conigenus* has recently been observed on western larch seed.

Seed testing methods

In British Columbia, screening tests called fungal assays are routinely conducted for the presence of contaminated or infected seed within seedlots stored at the BC Ministry of Forests' Tree Seed Centre. The assays are carried out following a strict set of protocols, providing confidence in the results from year-to-year as well as between testing agencies and laboratory personnel. In addition, these strict protocols allow for test repeatability.

A potential for *Fusarium* or *Caloscypha* to spread within seedlots during and following stratification exists and thus, all assays are conducted on unstratified seed. This also simplifies seed handling prior to testing.

Seed samples

Assaying seed samples from specific seedlots allows use of the results to infer rates of contamination or infection for an entire seedlot. It is important that random, representative samples of appropriate size are chosen to allow these inferences to be made, with what is considered an acceptable degree of certainty. To detect levels of *Fusarium* with a 95% degree of confidence at a relatively conservative level of 5% it is necessary to sample 500 seeds per seedlot. Detecting levels of *Caloscypha* contamination of 5% but with a greater allowance for variation around this estimate, has indicated a sample size of 250 seeds to be sufficient.

Seed-borne *Siroccocus* has the ability to spread systematically within an infected germinant and spread via spores to infect adjacent seedlings. *Sirococcus* is primarily seed-borne on spruce seed but its ability to spread to other seedlings is not limited to spruce. Spruce seed is the primary species assayed for this fungus both for its ability to kill spruce seedlings but also for the ability of infected spruce seed to become a potential inoculum source for lodgepole pine seedlings. Thus, it is desirable to detect lower infection levels of 1% within a seedlot, which requires a sample size of 1500 seeds.

Assay methods

Seed becomes contaminated by species of *Fusarium*, which includes spores or mycelium trapped in irregularities on the seed surface. Therefore, it is not necessary to surface sterilize the seed used in *Fusarium* assays as these contaminants could potentially be eliminated. The assay is performed by plating 20–25 seeds per petri dish on a *Fusarium* selective medium. The seeds are then incubated at 24/18°C and examined for *Fusarium* at 5 and 10 days after plating. Cultures are examined for characteristic fluffy hyphae with the appearance of lamb's wool. The presence of *Fusarium* is confirmed by microscopic examination until banana- or canoe-shaped, macroconidiospores can be seen. Identification of the fungus is expressed as percent contaminated seeds for the seedlot being assayed.

Detection of *Caloscypha fulgens* within seeds requires the elimination of all surface contaminants on the seed. To conduct these assays, 250 seeds are surface sterilized in a 30% solution of hydrogen peroxide (H_2O_2). After rinsing and surface drying, the seeds are plated on 2% water agar and incubated at 15°C (light unimportant). Once every 3 days thereafter the plates are examined for blue or indigo coloured, verrucose (warty) hyphae that branch at right angles, characteristic of *Caloscypha fulgens*.

Seed-borne *Sirococcus conigenus* also results in an infection and as for the cold fungus assay, it is necessary to surface sterilize the seeds which are then plated on 2% water agar and incubated at 20°C. The seed is examined for *Sirococcus conigenus* three days after plating and twice weekly for up to three weeks. Cultures are checked for fruiting bodies (pycnidia) and two-celled, spindle shaped spores. *Sirococcus conigenus* is slow to form fruiting bodies. Therefore fungi that have not been identified as *S. conigenus* are transferred to separate plates so they do not contaminate other seeds.

Assay results

Testing for the presence of seed-borne pathogens provides useful information for nursery growers. As levels of contamination or infection within a seedlot rise, the potential to negatively affect seedling germination and growth becomes significant. Knowing the percentage of infected or contaminated seeds within any seedlot provides growers the option of taking steps to minimize their impact on seedling germination and growth. While unlikely, very low levels of seed-borne contamination or infection do have a potential of spreading within a seedlot. However, potential risk to a seedlot increases as a threshold of infected or contaminated seed is approached.

Historical assay records indicate contamination or infection levels of 5% or greater within any seedlot to be significant for either *Fusarium* or *Caloscypha*. As seed-borne *Siroccocus conigenus* can become systemic in resulting germinants and spread via spores to infect adjacent seedlings, infection levels as low as 1% within seedlots become significant.

Minimizing the impact of seed-borne fungi

When seed-borne *Caloscypha fulgens* and species of *Fusarium* begin to infect or contaminate 5% or more of the seeds within any seedlot and *Sirococcus conigenus* infects 1%, steps should be taken to minimize any impact each of these organisms may have. The strategies for *Fusarium* and *Caloscypha* are aimed at putting each pathogen at a disadvantage in its ability to spread within a seedlot. These contrast methods to control the impact of *Sirococcus* infection in a seedlot, which are designed to eliminate the organism.

Fusarium contamination can intensify within a seedlot during stratification following imbibition. Spread of the fungus here results from moisture associated with seed soaking and combined with a prolonged damp, stratification. Currrent practice in British Columbia is to use running water during imbibition of all seeds, regardless of their degree of contamination. This effectively washes *Fusarium* inoculum from seed surfaces and prevents any contamination from intensifying.

Other strategies to reduce *Fusarium* levels on conifer seeds are summarized below:

- Sanitation of stratified Douglas-fir, western larch and spruce seed is recommended using a four-hour, 3% H_2O_2 soak followed by a five-minute running water rinse.
- Methods to reduce the potential for Fusarium contamination between seedlots during imbibition, include cleaning seed soaking tanks with Ivory™ dishwashing soap and hot water with particular attention given to the tank bottoms.
- Seed soaking screens should be cleaned regularly with a bleach and buffer soak solution.
- For seedlots contaminated with *Fusarium* at levels approaching or greater than 5%, strict adherence to optimal temperature and moisture conditions for each conifer species is critical for encouraging rapid germination to minimizing pre- and post-emergence damping-off.
- Succulent shoots of newly emerged germinants can be injured where they traverse the layer of coarse grit covering the cavity surface. Any subsequent lesions here become infection courts and *Fusarium* inoculum originating from the seed coat could enter stem tissues and cause rotting.
- *Fusarium* root rot usually becomes symptomatic and leads to problems in seedlings that are heat stressed.
- As well as heat, drought also predisposes seedlings to *Fusarium* root rot and growers must be diligent in avoiding these conditions for *Fusarium* infected seedlots.

The cold fungus is an apt name for *Caloscypha fulgens* as this organism has the ability to grow and spread in colder conditions than those that favour many other diseases. Thus, seedlots infected with *Caloscypha* can become rapidly overrun during a cool, moist stratification period following imbibition. Drying of seed can help overcome this, however care must be taken to ensure moisture is only removed from the seed surface. Removal of internal moisture may delay germination. The fungus kills seeds and its major effect is seen through low germination rates. With the exception of the true firs (*Abies*) and white pine, growers should sow seed from 'significantly' infected lots without stratifying. Uneven germination may be an acceptable alternative to risking disease spread. If stratification is deemed to be necessary, it is imperative that seed be sown immediately afterwards, to minimize additional cool storage. Finally, if numbers allow, seed from infected seedlots can be double or triple sown to help ensure filled cavities after germination.

Seed infected with *Sirococcus* usually manifests as *Sirococcus* blight, where killing of primary needles on germinants from the base upwards is a common symptom. The importance of this seed-borne disease lies in its ability to kill seedlings via secondary spread of inoculum from infected germinants. Cull seedlings infected with *Sirococcus* from containers and burn them to destroy any fruiting bodies on the dead foliage, which can continue to release spores. Dead seedlings should not be left to overwinter as pycnidia can form and release spores again in the spring. Finally, as spores can spread some distance via rain splash and mist, whenever possible, lodgepole pine seedlings should not be grown downwind of spruce crops known to have high *Sirococcus* infection levels.

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THE PETAWAWA GERMINATION BOX AND SEED VIGOUR CLASS

Introduction

This paper describes and discusses two important fundamental issues for laboratory tree seed germination tests. One is concerned with the micro-germination environment (i.e. germination trays, petri-dishes, germination boxes, bell-jars) while the other is the germination criteria or seedling definition. The former National Tree Seed Centre at the Petawawa National Forestry Institute had recognized these issues and initiated research to investigate them. The following is the history and results of the research developments on the two subjects.

The Petawawa Germination Box

The Petawawa germination box was developed at the former National Tree Seed Centre of the Petawawa National Forestry Institute in the early 1980s in an attempt to improve standard laboratory germination methods and procedures. In a search for a suitable germination box, many types of trays, dishes and boxes commercially available had been tested with unsatisfactory results. The germination box had to be large enough to allow large seedlings such as some of the pines and true firs to germinate and develop into the seedling stage that all essential structures can be evaluated. This size requirement was tied in with the development of laboratory germination vigour classes as will be discussed later in this paper. The need for a large germination box had arisen due to the amendment of the ISTA Rules for tree seed germination definition in 1983 when the so-called '4 times rule' (normal seedlings of tree species having epigeal germination when the primary root and hypocotyl together exceed four times the length of the seed) was changed to full seedling development with all essential structures visible. It was necessary to develop a germination box specifically designed for laboratory germination tests of tree seeds.

After two design changes, the prototype of the Petawawa germination box was consisted of two main components: one 28 cm long x 24 cm wide x 5 cm deep bottom and one 28 x 24 x 1 cm top (lid) (Figure 1). Both top and bottom are moulded out of light and unbreakable clear or opaque polycarbonate plastic with a specially designed unique universal locking mechanism to allow any combination of these components. A perforated false bottom of 26.5 x 22.5 x 0.3 cm with eight 1 cm legs was designed to fit within the box to support the germination substrate above and to provide space below as a water reservoir. The size and structure of the box was designed to achieve maximum utilization of space in currently available commercial seed germinators and to provide flexible depths of 6 to 10 cm to allow for germinant development of various species and seed sizes. The angle of the box wall was designed for nesting to save space for storage. The germination box is available in clear or opaque (black) plastic for germination in light or dark conditions.

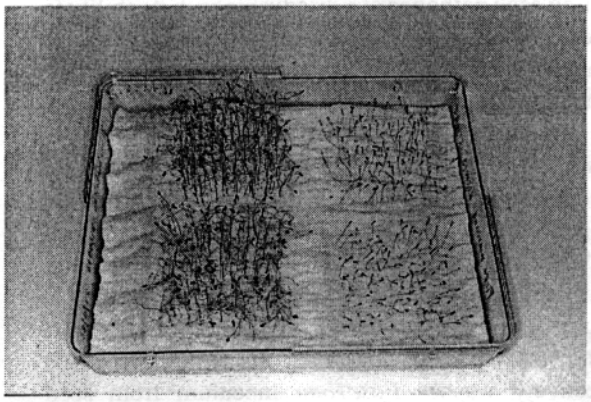

Figure 1. Photo of the base of a Petawawa germination box containing jack pine (left) and black spruce (right) germinants.

Upon completion of the final design, financial assistance was obtained from the then National Research Council's Program for Industry/Laboratory Projects (PILP) to fabricate prototype germination boxes for testing. The testing included temperature stress and light transmission by the National Research Council of Ottawa and germination tests with various species by the National Tree Seed Centre. Following the successful testing results and promising market survey, Spencer-Lemaire Industries Limited of Edmonton, Alberta introduced some minor changes in the design and was licensed to commercially manufacture the germination boxes for sale (Wang and Ackerman 1983). The germination box has been widely used for tree seed germination tests on four continents (personal communication, H.A. Spencer, Spencer-Lemaire Industries Ltd., Edmonton, AB, 2000). The Petawawa germination box has been used as part of the standard laboratory germination tests for tree seeds of various species (Wang 1999). Both the clear and opaque Petawawa germination boxes were found very useful for stage III *in vitro* propagation of apples and potatoes (Seabrook and Douglas 1987). The Pine Ridge Forest Nursery at Smoky Lake, Alberta (now Smoky Lake Forest Nursery Limited) has been successfully using the Petawawa germination box combined with the Petawawa seed vigour class as its standard germination tests.

The advantages of the Petawawa germination box are:

It is light and moulded with unbreakable polycarbonate plastic, and can make best use of the space in the commercial germinators;

Its dimension provides space and good aeration for various sizes of seeds and species, and the water reservoir maintains moist atmosphere throughout the period of moist chilling (cold stratification) and germination testing without additional watering; The unique interchangeable and the edges interlocking mechanism allows flexibility for using a bottom and a lid during early stage of germination and changing the lid to to another bottom to raise the height of the box when the germinants develop into seedlings; and

The box is durable and can stand cold and warm cycling without warping or damage and can be autoclaved at 140 kPa.

Laboratory Germination Testing by Vigour Criteria

In laboratory seed testing the primary purpose is to determine the germinability of seeds by standard methods and procedures for seed producers, users and seed control officials. Experience has shown that field emergence of many seedlots varied greatly from laboratory test results (Stein 1958) and further research is required to close the gap.

The problem appears to be related to the lack of seed vigour measurement in the standard laboratory germination tests. Although the laboratory germination is defined in ISTA Rules (1976) as " the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil", the criterion for tree seeds was the so-called four times rule which defines germination as a seed germinated and its radicle is four times the seed. In addition, many seed researchers have often used 2 mm radicle emergence as a germination criterion for evaluating treatment effects.

In an attempt to verify these germination criteria in relation to seedling development and seed efficiency, a study was carried out to compare laboratory germination test results with nursery emergence of red pine (*Pinus resinosa* Ait.) (Wang 1973). The idea was to classify laboratory germination counts by seed vigour class from ungerminated seed as vigour 8 to fully developed normal seedling as vigour 1 (Figure 2).

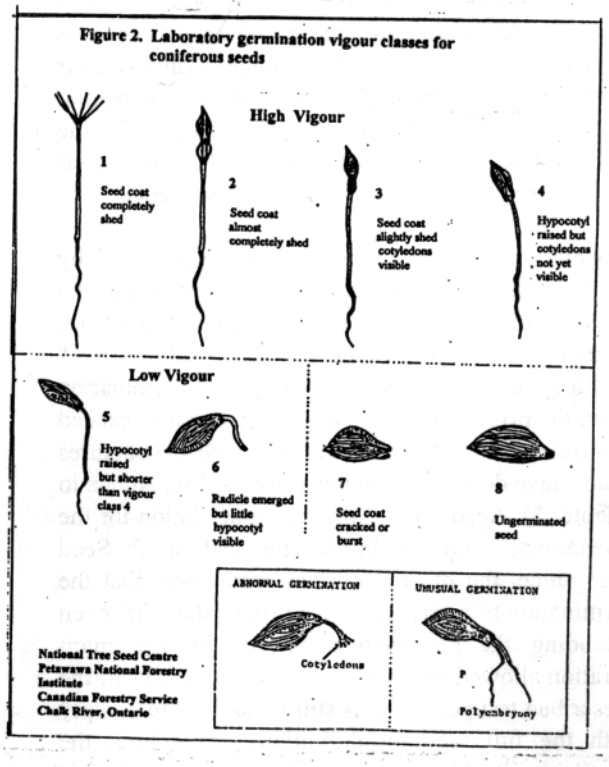

Figure 2. Illustration of the laboratory germination vigour classes for conifer seeds.

At the end of ISTA prescribed germination test period, the total laboratory germination count of vigour class 1, and combined vigour class 1-2, vigour class 1-3, vigour 1-4, vigour class 1-5, and vigour class 1-6 were compared and correlated with results of nursery germination of bareroot stock after 44 days. The hypothesis tested is that the capability of a seed to support germination and subsequent seedling growth to reach any one of these stages within a standard germination period is a better measure of field performance. The final analysis indicated that the laboratory germination of seeds with vigour class 1-3 were the most significantly correlated with the nursery emergence. In other words, in order to measure the seed vigour in laboratory germination tests, the germinants have to be able to develop at least to a vigour 3 where the germinants have fully developed strong root system and hypocotyl with partially visible cotyledons. This laboratory germination criterion was tested and verified successfully with white spruce (*Picea glauca*) seeds of 8 individual trees collected in four crop years (Wang 1976). This germination criterion was comparable to the earlier definition of normal seedling prior to 1983 when it was interpreted as the radicle to be four times the seed and close to the

criterion of full seedling development prior to amendment in 1983. This germination criterion has been adopted and applied successfully by the former Pine Ridge Forest Nursery (now Smoky Lake Forest Nursery) at Smoky Lake, Alberta. The uniqueness of evaluating laboratory germination by vigour classes is that it allows the seed analyst to distinguish high-vigour (1-4) from low-vigour (5-6) germinants of a seedlot within the ISTA prescriptions. It is also easy for the seed analyst to identify and distinguish those vigour classes from their morphological developments.

However, the full seedling definition prescribed by ISTA for tree seeds since 1983 was reversed in the 1999 ISTA Rules (ISTA 1999) to an expanded 'four times rule' which defines normal seedlings as " Seedlings of tree species having epigeal germination when the primary root and hypocotyl together exceed four times the length of the seed, provided all structures which have developed are intact" (page 156, Annex to Chapter 5). According to the recommendation for the amendment from the ISTA Tree and Shrub Seed Committee, the most important reason was that the germination of many tree species is so slow that even extending the germination test to the maximum duration allowed by ISTA (i.e. 7 days or up to half the prescribed test period), it is still impossible to comply with the full seedling definition. Therefore, the germination capacity of a significant proportion of seedlots would be underestimated. The amendment and new interpretation of the four times rule definition of normal tree seedling will tend to overestimate the true germinability of the seedlots as the new criterion can only compare with vigour class 5 at best (Wang 1973). In addition, it is difficult to evaluate those abnormal germinants and low-vigour germinants laying on the blotter or filter paper germination medium and meeting the 4 times rule criterion. At an African Training Workshop in Kenya on March 27-31, 2000 germination criteria for recalcitrant and intermediate tropical tree seeds were vigorously discussed, and concluded with a general consent that the radicle emergence (equal to the length of the seed) as well as seedling development (first set of leaves) are important and both should be recorded if possible (Thomsen and Dulloo 2000).

In the 1972 study of red pine seeds it was found that the ISTA prescribed germination temperature of alternating $20^0/30^0$ C or constant 25^0C was good for laboratory germination, but the constant 20^0 C was closer to the nursery or greenhouse temperature and had a clear-cut correlation of vigour classes 1-4 in laboratory germination with the nursery emergence. In tree seed testing the concern should be focused on how to minimize the differences between laboratory test results and nursery or greenhouse germination. Therefore, the laboratory germination test results have to reflect the germinability of the test seedlots not the viability. This is especially important when a single seed per cavity sowing is the ultimate target for efficient container seedling production. It is hoped that further research on this subject will be continued.

Acknowledgment

The author wishes to thank Steve D'Eon of Petawawa Research Forest for reviewing the manuscript.

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IMBIBITION TRIAL

A study of seed imbibition in germination dishes was carried out in three laboratories: Alberta Land and Forest Service Seed Centre (AB), BC Ministry of Forests Tree Seed Centre (BC), and the Canadian Forest Service, National Tree Seed Centre (NB) with two germination dish types (PNFI and WEST[1]). The objective of the trial was to investigate the uptake of moisture in the two germination dishes commonly used in Canada. Specific questions were 'How long does seed imbibition take in germination dishes?'; 'Is there a significant difference between dish types?'; and 'Is there a significant difference between laboratories?'. Information on the distribution of the variance across the remaining random factors (e.g. units of replication, number of seedlots etc..,) should also indicate where improvements could be made in testing efficiency.

Many facilities do not soak seed prior to stratification or germination testing and the time required for moisture uptake in dishes was not well documented. The moisture status of seed imbibed in germination dishes will also be compared to seed soaked in vials for 24 hours. This study is the first part (imbibition) comparing specific germination test methods used throughout Canada. It is anticipated that a subsequent study will focus on the differences between the two main criteria used to quantify germination in Canada (vigour classes *vs*. seed length ratios).

Materials

The study was conducted on two species: interior lodgepole pine (*Pinus contorta* Dougl. ex Loud. Var. *latifolia* Engelm.) and white spruce (*Picea glauca* (Moench) Voss) and analyses is presented separately for each species. The trial incorporated four seedlots of each species (two from BC and two from AB) to sample geographic variability in each species. The two germination dishes were prepared at all labs according to procedures used in BC (West dishes) and AB and NB for PNFI dishes (Wang and Ackerman 1983) as presented below:

[1] NOTE: West dish is the terminology used to define a square dish 4 5/16" X 4 5/16" X 1 1/8"used to hold one germination test replicate. The PNFI dish is the dish marketed by Spencer-Lemaire Ltd. and developed by Ben Wang which houses four germination replicates. These two dishes were the two most commonly used for germination testing in Canada (see Tree Seed Testing Survey article in this issue).

WEST Germination Dish Preparation:

1) place one piece of kimpack (cellulose wadding paper) in germination dish
2) add 50 ml of water
3) squish kimpack to distribute moisture and provide uniform surface
4) place filter paper on kimpack

PNFI Germination Dish Preparation:

1) pour 125 ml of water into bottom tray
2) place grid into bottom tray
3) put pre-cut kimpack on top of grid
4) pour 125 ml of water evenly over kimpack

Methods

Seed was counted into 100-seed replicates and initial fresh weight was measured and recorded. Replicates were randomly assigned to dish type (i.e. four replicates per dish type) and position at each laboratory and unimbibed seed spread onto each dish (or position within dish for PNFI dishes) so that adjacent seeds were not touching. Seed was removed from the dishes and weighed at intervals of 24, 48, 72 and 96 hours and promptly returned to the dish after weighing to avoid drying the seed. After the 96-hour measurement the seed was surface dried to provide an estimate of internal moisture content. After final measurements were recorded the seed was placed into a convection oven at 103° C for 17 hours to obtain the oven-dry weight of seed. The moisture content (MC) was calculated using the fresh weight at each time interval and the final oven-dry weight. Due to differences between seedlots in initial seed moisture content the variables to be analysed were the amount of moisture taken up after 24, 48, 72 and 96 hours (i.e. MC after 24 hours imbibition minus initial MC).

To compare seed imbibed in dishes with those imbibed in vials an additional four replicates were weighed and then imbibed in vials for 24 hours. The seed was then drained to remove excess moisture, weighed and placed into a convection oven at 103° C for 17 hours and then weighed to determine the oven-dry weight. Moisture content of the seed was then calculated.

Analysis

The trial is considered a split-split plot design as there are two restrictions on randomization: first the eight replicates are randomly assigned to the two dish types within each laboratory, then the four seedlots are randomized within each combination of laboratory, dish type and replicate. For more information on split-split plot designs refer to Hicks (1982) page 273. The trial was designed to determine the significance of laboratory, dish and the interaction of these two factors on moisture uptake and are considered fixed effects in the model. All other effects are considered random effects and the proportion of variance each source contributes to the total variance is presented. Percentage of variance for each effect is calculated as the estimated variance component divided by the sum of all variance components, multiplied by 100. The replicate factor was considered nested within laboratory as replicates are specific to laboratory. The model for the analysis is therefore:

$$MC_{@\ x\ hours} = \mu + L_i + R_{j(i)} + D_k + LD_{ik} + DR_{kj(i)} + S_l + LS_{il} + SR_{lj(i)} + DS_{kl} + LDS_{ikl} + DSR_{klj(i)}$$

where L_i is the effect of the ith Lab; $R_{j(i)}$ is the effect of the jth rep nested within the ith Lab; D_k is the effect of the kth dish; LD_{ik} is the effect of the interaction between the ith Lab and kth dish; $DR_{kj(i)}$ is the effect of the interaction between the kth dish and jth Rep within the ith Lab; S_l is the effect of the lth seedlot; LS_{il} is the effect of the interaction between the ith Lab and lth Seedlot; $SR_{lj(i)}$ is the effect of the interaction between the lth seedlot and jth rep within the ith Lab; DS_{kl} is the effect of the interaction between the kth dish and lth Seedlot; LDS_{ikl} is the interaction between the ith Lab, kth dish and lth Seedlot; $DSR_{klj(i)}$ is the effect of the interaction between the kth Dish, lth Seedlot and jth Rep within the ith Lab.

The results are presented in terms of significance of effects for fixed factors (L, D and LD) and in terms of proportion of remaining variance explained by the remaining random factors. Analysis was performed using PROC MIXED in SAS (SAS Institute Inc. 1997) Probability values are given for fixed effects, but $\propto = 0.05$ was chosen as the level to specify statistical significance of differences among the means. The help of BC Ministry of Forests Biometrician Peter Ott with the statistical analysis is graciously acknowledged.

Statistical Results

The probability values for the fixed effects are presented in Table 1 for both species and all time intervals. The Lab effect was statistically significant for all variables examined and the dish effect was not statistically significant for any of the variables. The interaction term (LD) was statistically significant for all variables except for the estimate of internal moisture following 96 hours of imbibition (MCSD). The two species had identical patterns in terms of statistical significance of the variables and effects.

The majority of the non-residual variance is explained by the Rep*Dish interaction for the variables which were not surface dried, but disappeared (estimated at zero) for the surface dried moisture content (Table 2). The total amount of variance was about one-third for the surface dried samples (2.50 vs. 7.65) on average. The error term explained a large percentage of variation for all variables ranging from 24.0% to 78.7%. The seedlot component explained a small proportion or none of the variance in the non-surface dried estimates. For the surface dried samples the variance percentage estimates for seedlots were 7.9 % for white spruce and 50% for lodgepole pine (Table 2).

Table 1. The probability values for the statistical significance of fixed effects. Results are presented for lodgepole pine and white spruce after 24 (MC24), 48, 72, and 96 hours of imbibition and following surface drying the seed after 96 hours of imbibition (MCSD). Results significant at α =0.05 are bold and shaded.

	Lodgepole Pine					White spruce				
	MC24	MC48	MC72	MC96	MCSD	MC24	MC48	MC72	MC96	MCSD
Lab	**.0165**	**.0089**	**.0209**	**.0059**	**.0004**	**.0088**	**.0028**	**.0024**	**.0063**	**.0055**
Dish	.0886	.4480	.8855	.2261	.4600	.5175	.4057	.1914	.1068	.9859
LD	**.0014**	**.0005**	**.0013**	**.0003**	.0804	**.0011**	**.0015**	**.0010**	**.0019**	.7560

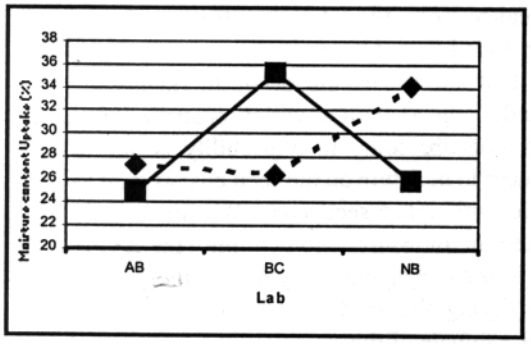
Table 2. Percentage of variance for each of the random effects. Results are presented for lodgepole pine and white spruce after 24 (MC24), 48, 72, and 96 hours of imbibition and following surface drying the seed after 96 hours of imbibition (MCSD). Sum of Variance components is bold and shaded.

	Lodgepole Pine					White spruce				
	MC24	MC48	MC72	MC96	MCSD	MC24	MC48	MC72	MC96	MCSD
Rep	0.0	0.0	0.0	0.0	0.0	18.6	0.0	9.0	0.0	2.0
RD	37.0	40.5	44.0	24.3	0.0	27.7	34.4	28.1	27.7	0.0
S	0.0	0.0	0.0	4.1	50.0	3.9	0.0	0.0	0.0	7.9
RS	5.6	4.8	10.0	2.4	3.4	0.0	0.0	2.5	0.0	0.0
DS	7.8	1.7	0.3	3.0	0.0	0.1	0.0	0.0	0.0	0.0
LDS	13.4	10.3	21.7	17.4	0.0	0.0	15.3	9.2	7.7	11.3
Error	36.2	42.7	24.0	48.8	46.6	49.7	50.3	51.1	64.6	78.7
Total	**8.94**	**7.23**	**8.03**	**7.40**	**2.94**	**11.13**	**6.54**	**5.66**	**6.28**	**2.05**

Graphical Results

To further examine the significant Lab*Dish interaction the averages were plotted by dish type over all three labs for white spruce (Figure 1A) and lodgepole pine (Figure 1B). Data on surface dry seed, after 96 hours imbibition, are not included in these averages as the interaction was not significant for this variable.

A)



B)

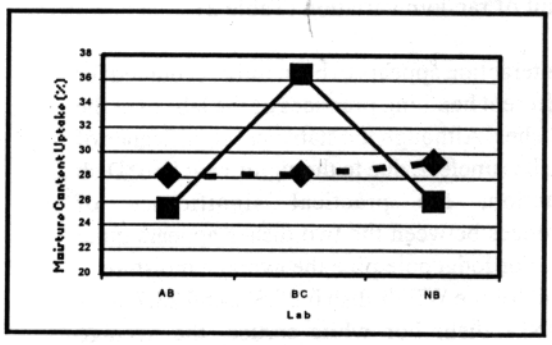
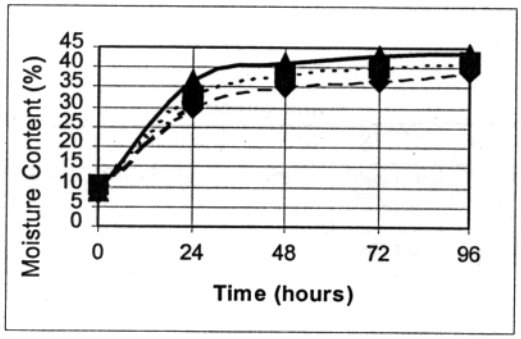


Figure 1. The graphical representation of the average Lab*Dish interaction for A) lodgepole pine and B) white spruce. ◆- - ◆ West ■ — ■ PNFI

The moisture uptake curves for lodgepole pine and white spruce, for each lab, are presented in Figures 2A and 2B, respectively. Imbibition from germination dishes was rapid reaching levels of approximately 30 to 35% after 24 hours. The corresponding moisture contents following 24-hour vial soaking were 36.8% for lodgepole pine (lab estimates ranged from 34.3 to 38.7%) and 35.7% for white spruce (ranging from 33.6 to 38.6%).

A)



B)

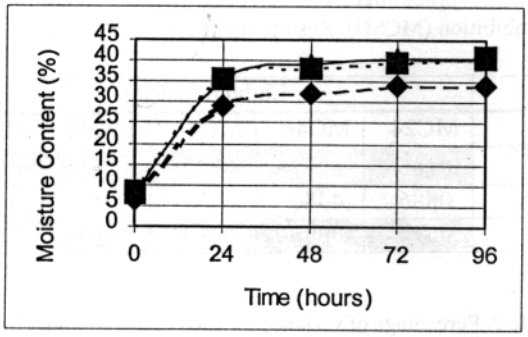


Figure 2. The moisture uptake curves for A) lodgepole pine and B) white spruce over all laboratories.

Discussion

The Lab effect was statistically significant for both species at all time intervals (including surface dried seed). The Dish effect was not statistically significant, but the interaction of the Lab*Dish was significant for all time intervals that did not incorporate surface drying making it difficult to clearly interpret the main effects. A further examination of this interaction showed that for lodgepole pine the interaction was mainly the result of high moisture contents in the BC lab for PNFI dishes and high in the NB lab for WEST dishes (Figure 1). These dishes are not the regular dishes used in the respective labs and this is the probable reason for the interaction. For white spruce the Lab*Dish interaction seemed solely caused by the high moisture content for the PNFI dish at the BC lab. For both species the surface drying of the seed following 96 hours of imbibition resulted in the interaction effect being non-significant and greatly reducing the total amount of random variation (Table 2).

The interaction appears to be mainly accounted for by different handling practices of the labs over the two dishes. Although the statistical significance of the dish is unclear, due to the significant Lab*Dish interaction, the practical significance of differences between the two dishes appears quite small. For lodgepole pine the average moisture content for the WEST dish is 29.3% and 28.7% for the PNFI dish. For white spruce, the average moisture content for the WEST dish is 28.6% and 29.3% for PNFI dishes. These differences are relatively small compared to lab differences presented in Figures 3 and 4. Laboratories can provide quite different estimates of moisture content using very similar techniques. This is partly explained by familiarity with certain techniques and the difficulty in quantifying something that is changing: the moisture content of the seed as it equilibrates to ambient conditions. Differences between labs in quantifying moisture content have also been observed to occur when more precise and sophisticated methods are used (i.e. liquid distillation methods) (Ben Wang, pers. comm. Aug. 2000).

For the non-surface dried moisture contents, the Rep*Dish interaction accounted for a large proportion of the variation. When seed was surface dried the Rep*Dish interaction was estimated to have a variance component equal to zero for both species. Surface drying the seed greatly reduced the total amount of random variation for both species. It was uncertain before the experiment began on whether we should surface dry seed for each time interval. These results certainly indicate that surface drying the seed (with reasonable guidelines) could reduce the total amount of variation found and 'clarify' the statistical significance of the fixed effects. It is recommended for future studies on moisture uptake that surface drying occur to reduce the apparently large amount of variation attributed to surface moisture content. Variability is due to differences in moisture on the seed coat as well as moisture within the seed coat. We are mainly interested in moisture imbibed into the living tissues of the megagametophyte and embryo and surface drying the seed would provide a more accurate estimate of this.

Species differences were large when the seedlot variance was examined at the surface dried condition (Table2). For lodgepole pine this term accounted for half of the variance, but in white spruce seedlot only accounted for 7.9% of the variance. Interactions (Rep*Seedlot, Dish*Seedlot

and Lab*Dish*Seedlot) also accounted for variability in moisture content, but clear trends across species or time intervals was not found. The replicate effect did explain 18.6% of the variation in white spruce after 24 hours imbibition, although it was estimated at zero for lodgepole pine. Further work looking at early imbibition may benefit by increasing the number of replicates used to increase the efficiency of the trial. The amount of residual variation was quite high and indicates a great deal of variability between cells (moisture content of a seedlot within a replicate, dish type and lab). This may be due to different individuals within a lab performing the measurements, or differential drying of cells prior to fresh weight determination.

The amount of moisture taken up directly from the media was slightly lower than vial soaking after 24 hours. The moisture content after 24 hours is adequate to initiate dormancy breaking mechanisms and the imbibitional delay is considered not important from a practical point-of-view. The BC lab currently soaks seed prior to testing and for lodgepole pine and white spruce it appears that this step can be eliminated and the seed allowed to imbibe moisture directly from the media. This would also allow for the use of the seed vacuum to count and place seeds into germination dishes. Prior to changing the methodology in the BC lab, the imbibitional delay will need to be quantified for other species routinely tested in BC, especially species with soak durations greater than 24 hours (Amabilis, grand and subalpine fir; yellow-cedar; and western white pine).

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Wang, B.S.P. and F. Ackerman. 1983. A new germination box for tree seed testing. Canadian Forestry Service, Petawawa National Forestry Institute. Information Report PI-X-27. 15 pp.

Dave Kolotelo
Donna Palamarek
Dale Simpson

Upcoming Meetings

Seventh International Workshop on Seeds
Salamanca, Spain, 12-16 May 2002

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____ Desiccation and Other Stress Tolerance and Conservation
____ Seed Ecology
____ Seed Biotechnology

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A. Stress Tolerance and Yield
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C. Seed Germination
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E. Stress and Processing Quality
F. Implications of Modified Seed Composition
G. Interactions Between Seed Research and Genomics

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Invited Speakers:

- Oxidative Stress Tolerance Mechanisms: **Reidunn Aalen** (Norway)
- Development of Desiccation Tolerance: **Dorothea Bartels** (Germany)
- Genomics and Proteomics in Seed Research: **Dominique Job** (France)
- Stress Tolerant Mutants: **Peter Jones** (UK)
- Perspectives in Seed Science: **Cees Karssen** (Netherlands)
- Genetic Variation in Seed Quality: **Maarten Koornneef** (Netherlands)
- Drought Tolerance and Yield: **Steve Quarrie** (UK)
- Temperature Stress: **Julio Salinas** (Spain)
- Seed Cdna Micro Arrays: **Lonneke Van Der Geest** (Netherlands)
- Molecular Chaperones in Plant Stress and Development: **Elizabeth Vierling** (USA)
- Seed Quality for the Milling Industry: Representative from Meneba (Netherlands)

The Organizing Committee:

Steven P.C. Groot (Plant Research International)
Henk W.M. Hilhorst (Wageningen University)

SEED TESTING INFORMATION SOURCES

The following is a list of known information on tree seed testing. It is probably incomplete and we welcome any additional references for future issues of the NewsBulletin.

ISTA - International Seed Testing Association
Secretariat, P.O. Box 308 Zuerichstrasse 50
8303 Bassersdorf, CH-Switzerland
Tel +41 1 838 60 00 Fax +41 1 838 60 01
E-mail ista.office@ista.ch

They also maintain a web page at http://www.seedtest.org

International Seed Testing Association. 1999. International Rules for Seed Testing Rules 1999. Seed Science & Technology 27, supplement

Gordon, A.G., P. Gosling and B.S.P. Wang [Eds.]. 1991. Tree and Shrub Seed Handbook. The International Seed Testing Association.

AOSA
Association of Official Seed Analysts, Inc.
201 North 8th Street, Suite 400
P.O. Box 81152
Lincoln, NE 68501-1152 USA
Tel. 1-402-476-3852 fax 1-402-476-6547
E-mail: aosa@assocoffice.net

They also maintain a web page at http://www.aosaseed.com

Association of Official Seed Analysts, 1998. Rules for Testing Seeds.

Other Sources

Edwards, D.G.W. and B.S.P. Wang. 1995. A training guide for laboratory analysis of forest tree seeds. Canadian Forest Service Pacific and Yukon Region Information Report BC-X-356. 64 pp.

Edwards, D.G. W. 1987. Methods and procedures for testing tree seeds in Canada. Canadian Forest Service Forestry Technical Report 36. 31 pp (in both English and French).

The above publications can be ordered by mail from Nina Perreault (publications), Pacific Forestry Centre, 506 West Burnside Rd., Victoria BC V8Z 1M5, by fax (250) 363-6006 or e-mail nperreault@pfc.forestry.ca.

Leadem, C.L. 1984. Quick tests for tree seed viability. BC Ministry of Forests Land Management Handbook No. 18. 45 pp.

A limited number of the above publication can be obtained by contacting Dave Kolotelo.

How do tree seeds sense temperature?

Temperature is the primary environmental factor influencing seed germination, with light and moisture conditions being of secondary importance. Germination, like most plant processes, increases with rising temperature, maximizes at an optimum temperature, then declines at supra-optimal temperatures. Temperature governs completeness and speed of germination through its effects on seed deterioration, dormancy loss, and the germination process itself. Earlier studies indicated that the temperature resulting in the most prompt and rapid germination is not always the same temperature producing the greatest number of germinants. Germination achieved under constant and alternating temperatures was found to differ also. Generally alternating temperatures have been favoured for tree seeds because they are thought to simulate field conditions during the natural germination season, and to promote the maturation of incompletely ripened seeds.

Most temperature studies have been designed to determine the minimum temperature for germination, or the optimal temperature for germination speed. They have been confined to the effects of a single temperature, or changes in temperature during a specified time period. However, the temperature dynamics of physiological processes requires that the variable *time* also be included in the analysis. Heat sum, a measure of the total energy received over time, is especially suitable for this purpose because temperature and time are incorportaed into a single relationship. Few temperature studies of forest tree seed germination have considered heat sums and, as a result, some temperature effects on germination may have been overlooked. One of the reasons is that seeds exposed to different temperatures do not receive, within an equivalent time period, the same amount of thermal energy. Thermal units do not accumulate equally at each temperature, that is, for the same length of exposure, a 2° change at 10°C is not the same as a 2° change at 20°C. A consequence of this is that greater germination may be observed at a higher temperature than at a lower temperature over a short time period, but the same germination may be achieved if the time is extended

Most plants in temperate zones are exposed to day/night fluctuations of alternating high/low temperatures, and some species appear to require such temperature variations to germinate. The question we posed was whether tree seeds respond to the number of day/night (or high/low) cycles received, or do they respond to the total energy received within a daily (24 h) period. Stated another way, we wanted to find if seeds responded to short-term temperature fluctuations (perceived as a single "day"), or if seeds integrated the total energy received (heat sums), independent of the cycles in which temperature was delivered.

Four conifer species were selected to represent different seral stages of the forest ecosystem: lodgepole pine (an opportunistic pioneer species), white spruce (a climax species), mountain hemlock (known to be germination-temperature sensitive), and western redcedar (typically non-dormant, and relatively less temperature-sensitive). For each species, two seed sources were chosen that were well separated within their natural ranges. Filled seeds were separated from empty seeds using x-ray methods. The pine and spruce seeds were stratified at 2 °C for 4 weeks, and the hemlock for 3 weeks. The western redcedar seeds were not soaked or stratified prior to testing. Seeds were then incubated for 28 days, in darkness, under eight, concurrent temperature cycles on a computer-controlled thermogradient system. The system employed thermo-electric pumps for heating and cooling so that temperatures at the germination surface remained within ±0.5°C of the programmed temperatures at all times, and changes to new target temperatures occurred within 5 minutes.

The system was programmed to deliver the eight temperature cycles with total energy varying from 400-480 degree-hours per day (Table 1). Total energy per day was expressed as degree-hours (°h), which is the mean cycle temperature (in °C) (calculated above a threshold of 0°C) multiplied by the number of hours per cycle. Thus, seeds exposed to a constant 20°C for 24h (treatment 5) would receive 20°C x 24h, or 480°h days. This energy level is equivalent to that for seeds receiving 25°C for 12h and 15°C for 12h, that is (25°C x 12 = 300°h) + (15°C x 12 = 180°h) = 480°h days (treatment 1).

Table 1. Temperature treatments programmed into the thermal gradient system

Treatment	Hours/ cycle	Cycles/ 24h	Cycle Length (h)	Cycle temperatures (°C)	Total daily energy (°h)
1	36871	1	24	25/15	480
2	6/6	2	12	25/15	480
3	3/3	4	6	25/15	480
4	1.5/1.5	8	3	25/15	480
5	24	1	24	20	480
6	24	1	24	18.3	440
7	8/16	1	24	25/15	440
8	8/16	1	24	20/15	400

For all species, earliest and most rapid germination occurred under 480°h days. The time to reach 50% germination under 440°h days was significantly longer – almost double - than under 480°h days. That is, approximately 8% less total energy (a decrease of 40°h) decreased germination speed almost 50%. Similarly, the time to reach 50% germination under 400°h days was significantly longer - (treatment 1)again almost double - than under 440°h days. At the end of the test period (28 days), germination under 480°h and 440°h days was essentially complete, with a few small differences, for all seed sources. Under 400°h days germination was incomplete in most seed sources. White spruce seeds appeared to be the least sensitive to heat sums, while the hemlock and pine, and one of the redcedar sources, were most sensitive.

The results suggest that for speed of germination, specific germination temperatures are less critical than the heat sum, or the total amount of energy, received on a daily basis. All seeds germinated more or less equally under the same heat sum, and the number of cycles had no impact. That is, a constant temperature produced the same result as an alternating temperature under 480°h days, independent of whether the temperature fluctuated once per day, or eight times per day. The same result was observed for constant or alternating temperatures under the 440°h day regime. For all species, earliest and most rapid germination occurred under the 480°h days.

For nurseries, the results suggest that some means of monitoring heat sums is desirable. Thus, if day temperatures rise above the set temperature because of excess sun exposure, night temperatures can be adjusted to provide the appropriate heat sum at the end of each 24 h monitoring period. Or night temperatures might be increased if day temperatures do not rise to the expected level. Similarly, day temperatures might be increased if night temperatures cannot be closely controlled. However, clearly there is a biological limit to this. Most north temperate conifer seeds exposed to, say, 50°C for several hours are likely to be damaged no matter what the counterbalancing low temperature might be. With more computer control being built into greenhouse design, a device monitoring heat sums at the germination surface, and capable of making adjustments to the heating/cooling system to compensate for actual temperature deviations, could potentially improve stock production efficiencies.

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XXI IUFRO WORLD CONGRESS

The International Union of Forestry Organizations (IUFRO) held its XX1 World Congress in Kuala Lumpur, Malaysia, August 7-12, 2000. Some 2400 registered delegates – slightly less than the 3000 planned for by the LOC – from 90+ countries were in attendance. When accompanying persons, guests, IUFRO staff and Local Organizing committee staff were added, the number came much closer the 3000. The Canadian contingent numbered 40 registered participants, but the dinner

held at the residence of the Canadian High Commissioner, the Hon. Jean McKloskey, brought out many more – spouses, companions and Asian students who were under Canadian sponsorship. The Congress was housed at the Putra World Trade Centre, KL, a magnificent venue that not only had an auditorium to seat 3 500, but had two other slightly smaller halls for sub plenary meetings, and was able to accommodate almost 20 concurrent sessions, all under the same roof. The only two times we had to leave the main building was to take the covered walkway across the river, Sundai Gombak, for the Welcome Dinner and the Farewell Dinner, to another building inside which had tables to seat at least 3000. Some Canadians were measuring up this building in terms of 2 or 3 hockey rinks in length! The Local Organizing Committee consisted of all the staff of FRIM (Forest Research Institute of Malaysia) which had been assigned to carrying out the multitude of tasks for such a gathering. That they succeeded in every way is an understatement. The Malaysians were excellent hosts and the Congress was a huge success.

IUFRO Research Group 2.09.00 (Seed Physiology and Technology) held a technical session on Monday August 7. Five papers, presented to a modest audience of about 35-40 people, were as follows:

Introduction of genetic resources and improved forestry seed production in Madagascar.

By Gilles Chaix, Vololoniriana Razafimaharo, Appolinaire Razafimahatratra, and Philippe Vigneron (variously from Cirad-Forêt (Forestry Department of Centre de Coopération Internationale en Recherche Agronomique pour le Développement), Programme Arbres et Plantations, B.P. 745, Antananarivo 101 Madagascar; FOFIFA (Centre de Recherche Appliquée au Développement Rural), B.P. 901 Antananarivo 101 Madagascar; and Cirad-Forêt, Programme Arbres et Plantations, TA10/C, 34035 Montpellier cedex 5 France.

Use of halogens in controlling deterioration of some tropical tree seeds.

By Alka Bhargava, Amit Sahai , A.S. Bhandari and Vishakha Kumbhare, Tropical Forest Research Institute, P.O.RFRC, Mandla Road, Jabalpur – 482 021, India

Tetrazolium test: a tool for predicting the viability of some tropical tree seeds

By Purohit, M and A. Bhargava, Tropical Forest Research Institute, P.O. RFRC, Mandla Road, Jabalpur – 482 021, India.

Planting Dipterocarp species for reforestation: is it viable?

By Marzalina M., Krishnapillay B., Nashatul Z.N.A., Ang K.C., Siti Hasanah M.S., Zaiton S., Fadzlinah Z. and Hamsinah H., Seed Technology Section, Forest Plantation Division,
Forest Research Institute Malaysia (FRIM), Kepong 52109, Kuala Lumpur, Malaysia.

Forest tree seeds at the end of the 20th. century: major accomplishments and needs.

By D.G.W Edwards, FTB Forest Tree Beginnings, 4018 Cavallin Court, Victoria, BC, Canada V8N 5P9.

The latter paper was based on a State of the Knowledge Report submitted by RG 2.09.00 earlier in the year in answer to a challenge made by IUFRO President. Dr. Burley. The SKR was a joint effort among 10 international members (representing 7 countries) of the Research Group. For the other four technical papers only abstracts will be published in the Congress Proceedings. However, the full text of the SKR is available on the RG web page, which can be accessed at:

http://iufro.boku.ac.at/iufro/iufronet/d2/ho20900.htm

The outgoing Chairman of RG 2.09.00, George Edwards (who retired from the Canadian Forest Service, Pacific Research Centre in 1996) was honored with a Distinguished Service Award. In part the citation read: Dr. D.G. Edwards is recognized for continuously serving, with the highest standards, the interests and objectives of IUFRO since 1991 when he was appointed Chair of Research Group 2.09.00 (Seed Physiology and Technology). An inaugural member of the IUFRO's seed research initiative launched in 1973, George has continued to be active at each membership level. He organized, for example, a Symposium for the seed Research Group in Victoria in 1991, which resulted in timely and often-referenced Proceedings. He also organized a technical session on seeds at the IUFRO Congress

in Finland. And he both moderated and presented a paper at the seed session in Kuala Lumpur. As a retired (1996) Research Scientist from the Canadian Forest Service, Pacific Forestry Centre, Dr. Edwards has committed time and effort to IUFRO projects and, in addition to his formal responsibilities, has also contributed substantially to the organization of this Congress in Malaysia," noted Eric Teissier du Cros (France), IUFRO Division 2 Coordinator, who presented the award at the Division 2 Business Meeting.

The next IUFRO Congress will be held in Australia in 2004.

[Ed note: George Edwards kindly provided this Congress report]

Erratum

"Upgrading seed quality of conifer seed lots: The how and glimpses of the why"
by Bruce Downey

In the last issue of the Tree Seed NewsBulletin, No. 31, I failed to mention that Bruces' article was first published in the proceedings listed below.

Gertzen., D., van Steenis, E., Kolotelo D., Summers, D., [Tech. Coord.] 1999. Proceedings of the 1999 Forest Nursery Association of British Columbia; Sept. 27-30, 1999. Vancouver, B.C. Canada. 19th Annual Proc. Publ., For. Nursery Assoc. of B.C.

My apologies to Bruce and to the coordinators of the proceedings.

Ron Smith

RECENT PUBLICATIONS

Anderson, E.A. and J.N. Owens 2000. Microsporogenesis, pollination, pollen germination and male gametophyte development in Taxus brevifolia. Ann. Bot. 86: 1033-1042.

Harsh, N.S.K. and B.M. Ojha 2000. A possible pretreatment for seeds of tropical tree species. Seed Sci. & Technol. 28: 513-514.

Hawkins, C.D.B. and K.B. Shewan 2000 Frost hardiness, height, and dormancy of 15 short-day, nursery-treated interior spruce seed lots. Can. J. For. Res. 30: 1096-1105

Karlsson, C. 2000. Seed production of *Pinus sylvestris* after release cutting. Can. J. For. Res. 30: 982-997

Oleskog, G., H. Grip, U. Bergsten, and K. Sahlén, 2000. Seedling emergence of *Pinus sylvestris* in characterized seedbed substrates under different moisture conditions. Can. J. For. Res. 30: 1766-1777.

Ruotsalainen, S., D. Lindgren, and T.J. Mullin 2000. Some formulas concerned with pollen contamination have constrained use in Lindgren and Mullin (1998). Trelatedness and status number in seed orchard crops. Can. J. For. Res. 30: 333.

Schmitz N., S. R. Abrams, and A. R. Kermode 2000. Changes in abscisic acid content and embryo sensitivity to (+)-abscisic acid during the germination of dormancy of yellow cedar seeds. J Exp Bot 51: 1159-1162

Setiawati, Y.G.B., R.T. Riding, and G.B. Sweet 1999. Determination of the stage at which failure occurred in empty control-pollinated seeds of Pinus radiata (Note) New Zeal. J. For. Sci. 29: 366-374

Trotter, D. 2000. Seed and Seedling Extension Topics. 12(1), August, 2000 [Ed. Note: Lots of good reading in this issue!!]

Varghese, B., and S.C. Naithani 2000. Dessicatioon induced loss of vigour and viability during storage in Neem (*Azadirachta indica* A. Juss.) Seeds. Seed Sci. & Technol. 28: 485-496.

Wang, B.S.P. and P. Berjak 2000. Beneficial effects of moist chilling on the seeds of black spruce (*Picea mariana* [Mill.] B.S.P.). Ann. Bot. 86: 29-36

Xia, J.-H. And A.R. Kermode 2000. Dormancy of Yellow Cedar (*Chamaecyparis nootkaensis* [D. Don] Spach) seeds is effectively terminated by treatment with 1-propanol or nitrate in combination with a warm water soak, gibberellin and moist chilling. Seed Sci. & Technol. 28: 227-240.