CANADIAN TREE IMPROVEMENT ASSOCIATION/
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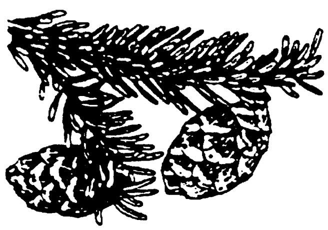

Tree Seed Working Group

NEWS BULLETIN

No. 40 December 2004

PROBLEMS AND INFORMATION NEEDS FOR TREE SEED

THIS ISSUE AT A GLANCE

CHAIR'S 'ARMCHAIR' REPORT

Hello! It is my pleasure to welcome you to the 40th Edition of the CTIA Tree Seed Working Group (TSWG) News Bulletin. Before reminiscing about our heritage I'd like to thank everyone who attended and participated in the CTIA meeting this past summer. I have received a great deal of positive feedback concerning the meeting and workshops. A synopsis of the Tree Seed workshop is presented in an enclosed article, but I would like to thank, up front, all of the speakers who made presentations on 'Quality Assurance in the Seed Handling System" as well as everyone involved in our lively discussions.

The Tree Seed Working Group began in 1983 after successful seed workshops at the 18th (Duncan) and 19th (Toronto) CTIA meetings. The group has consistently produced two News Bulletins a year for the past 20 years and has hosted tree seed workshops at most CTIA meetings over this time span. The group has maintained the following four objectives over its 21-year history: **To promote 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) the exchange of information on seed related problems, and by
4) advising on implementation practices

The objectives still seem relevant and help to define the scope of our Working Group. A large number of people have contributed to the TSWG in terms of articles, workshop presentations, and their dedication to tree seed science and technology. Firstly, I would like to identify and thank our past TSWG chairs:

Ben Wang	Chair 1983 to 1985
Yves Lamontagne	Chair 1985 to 1987
Graham Powell	Chair 1987 to 1991
Guy Caron	Chair 1991 to 1997
Howard Frame	Chair 1997 to 2000

The chairs may provide the face of the organization, but the group's success is on the backbone of the TSWG News Bulletin Editor. We have had some excellent editors over the years and their contributions have been incredible. Thank you.

George Edwards	Editor 1983 to 1985
Hugh Schooley	Editor 1985 to 1995
Ron Smith	Editor 1995 to 2002
Dale Simpson	Editor 2002 to present

There are many more individuals who have dedicated energies towards the TSWG. I cannot identify all, but I would like to acknowledge a few other individuals:

Peter De Groot has been involved in the TSWG from News Bulletin #2 (at least) and co-ordinated the Cone and Seed Insects Working Party (CSIWP) for many years. The TSWG also had a Tree Seed Processing and Testing Working Party that was headed by Dave Bewick and then myself. These working parties were dissolved in 2000 not because these topics are unimportant, but because the working parties were not active and these subjects can be handled effectively under the TSWG umbrella.

You may wonder who is the Tree Seed Working Group? The answer is basically anyone with an interest in tree seed science and technology who participates in our News Bulletin or our workshops. Currently the News Bulletin is distributed to about 250 individuals and about 40 libraries. The audience is primarily those working in Canada, but about 7% of the News Bulletins go to workers in the United States and an additional 9% goes to a variety of other Countries throughout the world.

Before presenting my two-cents on this edition's theme I'd like to announce that the theme for News Bulletin #41 is "**Cone and Seed Pests**". This is certainly an important topic that is having a huge impact in BC seed orchards. It is currently considered our greatest impediment to meeting seed demands over the next decade. The BC Pest Management group with the Tree Improvement Branch has agreed to help write and solicit articles. Please contribute to keeping the News Bulletin active, current, and National in scope. Deadline for articles is May 20, 2005. Thank you.

The theme of this News Bulletin is **"What are your biggest problems or information needs with respect to tree seed?"** It allows for some reflection on what we need to do to move forward in keeping tree seed science current in a rapidly changing world. I will elaborate on a few ideas starting from a big picture view to some specifics that I believe are critical. My biggest concern is in the number of people who are actually working in the area of tree seed science and technology. This is not new and most of us can remember Ron Smith's 1998 commentary in News Bulletin #29 "Tree Seed Research: Is anyone doing any?". We have experienced a large drain in Canada with the retirements of George Edwards, Robert Farmer, Carole Leadem, John Owens, Ben Wang, and Joe Webber who were leaders in our field for many years. The area does not attract many new people and this is not because we have answered all the pertinent questions, especially with the rapidly changing genetic base of our seed. The field currently does not present a very promising future for students and they cannot compete for research dollars with state-of-the art molecular techniques.

I do not want it to appear like I am against molecular biology, but I strongly think there is a need for a multi-pronged approach to solving tree seed problems. With more science being on the cutting edge of technology the gap between research and operational implementation is widening. The results of scientific research cannot always be easily implemented into operations and technology transfer does not receive strong support today. I strongly believe that we are loosing our prong of how to deal with questions related to seed anatomy and morphology. These topics are rarely taught in Universities as there does not 'appear' to be practical applications and over time our expertise in this area will disappear. There is some great work happening in conjunction with Simon Fraser University and the Plant Biotechnology Institute in Saskatoon using MRI technology as a method of visualizing water and oil distribution in seeds, so there is hope. Look forward to more information on this valuable technology in the next issue.

In addition to not enough basic scientific researchers, technology transfer personnel, and operational researchers I don't think we are very efficient in accessing research that has already been performed. I keep coming back to a quote from Dr. Jeffery Burley in an article entitled **The restoration of research,** in which he states "*Research information and practical experience of the management of forests and trees have accumulated for over a century; however, much research has been repetitive in ignorance of earlier work, much has been ignored by current policy-makers or resource managers, and much has not addressed current issues*". I think this hits several key points, but the one I'd like to emphasize is the need to better appreciate and have more awareness of research that has already been performed, considering its benefits as well as its limitations in tree seed science today. The electronic age and reliance on electronic literature searches that primarily focus on the last decade (or two) ignore a large foundation of tree seed science and technology. I'm hoping to take a bite out of this general issue over the next year – stay tuned.

Moving to more specific needs I have a few topics to put forth as problems or needs:

1) We need a good method of quantifying seed dormancy. Fortunately a paper is *in press* that addresses this topic and should provide a standard method for quantifying seed dormancy. For those at the 2002 Tree Seed Workshop in Edmonton you obtained a sneak preview on the topic.

2) Many jurisdictions are moving towards greater use of seed orchard produced seed. The transition is happening quickly and it raises a variety of questions (or should). We should continue to be diligent in addressing questions of after-effects, family variability, and better quantifying what exactly we produce in seed lots from imperfectly random mating.

3) The last item I would like to highlight is pests and a better understanding of fungi and insects that reduce the efficiency of our operations. Specifically more information on basic biology of coneworms (*Dioryctria* spp.) and the route and properties of *Fusarium* spp. contamination are priorities.

Thank you to everyone who has contributed to our 40th anniversary edition. Have a Happy and Safe holiday season.

Dave Kolotelo
Chairperson

EDITOR'S NOTES

Welcome to the 40th issue of the TSWG's News Bulletin! I had the good fortune to be invited to give a paper at the Tree Seed Workshop held prior to the 19th CTIA in Toronto in 1983 and participated in the meeting that followed which formalized the formation of the Tree Seed Working Group. The first issue of the News Bulletin was published December 1983. Now 20+ years and 40 News Bulletins later the Group continues to thrive and be active. It is an honor to be involved.

I am sure you will find something of interest in this issue. There are several shorter submissions dealing with the theme as well as a couple of longer articles. You will also find a number of general interest and newsy articles. It is this diversity that I think continues to make the News Bulletin interesting and successful.

At this time I want to wish you all a Joyous Holiday Season and the Best for the New Year.

Dale Simpson
Editor

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Comments, suggestions, and contributions for the News Bulletin are welcomed by the Chairperson or Editor.

THE BIGGEST PROBLEMS OR INFORMATION NEEDS WITH RESPECT TO TREE SEED FOR THE PROVINCE OF QUÉBEC

The Ministère des Ressources naturelles, de la Faune et des Parcs from Québec provides all the tree seeds used for seedling production. Québec's objective for 2005 is to produce 80% of all the seedlings from improved sources. About 135 million seedlings are planted each year. Seventy percent of the coniferous seedlings are produced in 20 private nurseries with the balance produced in 6 government-owned nurseries. All coniferous and hardwood seeds are processed and stored at the provincial Tree Seed Center in Berthier.

The major coniferous species used for seedling production are, in decreasing order: black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), white spruce (*P. glauca*), Norway spruce (*P. abies*), eastern white pine (*P. strobus*), red pine (*P. resinosa*), balsam fir (*Abies balsamea*), and eastern larch (*Larix laricina*). Those species are produced from seeds, mainly in containers. Black spruce, white spruce, Norway spruce, and hybrid larch are also produced from cuttings.

Over 1 million hardwood seedlings are also produced from 15 species. The principle hardwood species grown are: yellow birch (*Betula alleghaniensis*), red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), and white ash (*Fraxinus americana*). About 1.5 million hybrid poplar cuttings are produced per year.

Seed research in Québec is conducted both by the Tree Seed Center and the Direction de la recherche forestière. Our interests are wide but mainly our goals are to: 1) Improve seed orchard production by using electrostatic pollination. Preliminary results for black spruce show that the number of seeds produced by cones pollinated with the electrostatic pistol can be doubled. 2) Facilitate cone harvest with top pruning. Tests have started in second-generation black spruce and white spruce seed orchards. 3) Improve extraction methods at the Tree Seed Center to increase seed lot quality. For example, since 2001, mean germination rate of white spruce seed lots increased from 85 to 93%. 4) Improve seed stratification methods for some hardwood species (white ash, black ash (*F. nigra*), black maple (*A. nigrum*), and American basswood (*Tilia americana*)). 5) Determine sowing factors to optimize the utilization of seeds for seedling production. These factors will allow adequate nursery sowing and will avoid seed waste. 6) Determine a germination test frequency for each coniferous species in order to reduce the number of seed germination tests done at the Tree Seed Center without reducing the quality of the results.

For general information, see the ministry web site: http://www.mrnfp.gouv.qc.ca/english/forest/quebec/quebec-system-management-plants.jsp (in English) http://www.mrnfp.gouv.qc.ca/forets/entreprises/entreprises-semences.jsp (in French, more exhaustive)

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AN EXAMPLE OF THE NEED TO MAINTAIN GENETIC DIVERSITY

From evolutionary, population adaptation, and climate change adaptation perspectives, I think one of the main issues for seed collection activities is the maintenance of genetic diversity with a special focus on the type of diversity being maintained. Seed orchards are an excellent idea for maintaining genetic diversity and as a basis for allowing natural selection to determine the genetic and adaptive constitution of the next generation. However, for the past 10 years, I've been focussing on the conservation of genetic diversity in marginal natural populations along the northern margins of their geographic range under the assumption that these populations may be the best genetic sources for northward migration in the event of anticipated climate warming. One problem in these smaller, isolated, marginal populations is inbreeding and its often deleterious effects on reproductive capacity. However, such populations may also be very active areas of evolution of the species because of the activity and consequences of evolutionary forces such as inbreeding, drift, and natural selection. I think the loss of these populations through harvesting (before we can take advantage of the generations of natural selection, mild inbreeding, and genetic drift that have often produced the best-adapted genotypes for continuing northward migration) should be seen as a serious issue by the tree improvement community. I would like to see the tree improvement community voice their collective concern and support for the maintenance of a representative sample of these older, natural populations at their northern range margins in recognition of their potential value as important genetic resources and seed sources for adaptation under anticipated climate change.

With many in the forestry community now talking about how forests will adapt to climate change, I think it's a great time to be working with seed issues because these seed issues are a fundamental aspect of how well the forest sector and forests adapt to climate change.

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WHAT ARE THE BIGGEST PROBLEMS OR INFORMATION NEEDS WITH RESPECT TO TREE SEED IN SASKATCHEWAN?

Unless a person has been working within the realm of tree improvement or had experience with seed orchards, there lacks a general understanding of wild tree seed development. Many times silviculturists are forced to collect seed without proper information. For example, taking a helicopter to nearby areas where there are sufficient cones in order to hopefully fill the quota of seed needs for the up-coming year.

More often than not, a clear plan of objectives does not exist when it comes to maintaining or ensuring that there is a supply of quality seed. This can be effected by one or more of the reasons below:
1) Future Forest Planning – knowing the volume of seed by species that is needed for a future period of time.
2) Collecting from "quality" stands - seed collection stands should be sought out in advance and monitored for seed yields. Perhaps a protection zone can be put around these areas. Often stands are selected for their closeness to roads and not enough emphasis is put on tree quality (is it growing straight, is it prone to disease?).
3) Proper timing of cone collection. Improper understanding of embryo development. Collecting cones at the wrong time can result in poor quality seed.
4) Collection methods. These days there are numerous ways to collect cones and often cost dictates which method is used. Hiring of untrained crews or improper training of the crews also affects the success of the collection.
5) Quality Control Measures of the entire process (from finding stands to storage of seed). If there are no guidelines then there are no standards to measure against.

Therefore, what is needed to advance tree seed science?
1) Current education material/information for inexperienced to learn from. Perhaps short courses for company personnel. A regional perspective would need to be maintained.
2) Companies/Government need to hire qualified individuals with silviculture experience.
3) Develop regional quality control measures utilizing information obtained from people with experience.

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POTENTIAL AREAS FOR FUTURE TREE SEED RESEARCH

Summary

There is considerable scope for tree seed research to improve the reliability and success rates of all three methods of woodland establishment: 1) nursery sowing plus transplanting of seedlings, 2) direct sowing, and 3) natural regeneration.

Nursery and direct sowing would both significantly benefit from higher quality seed. Higher germination percentages, quicker and more uniform emergence, and seed better able to germinate over a wider range of conditions are three potential improvements. Methods to prolong the storage life of recalcitrant seeds and better removal of dead and dying seeds are two others.

Improved, faster and more versatile seed pretreatments for overcoming seed dormancy would not only provide major benefits in the areas of nursery and direct sowing but also in the quality assurance realm of seed testing. An ultimate aim for tree seed research should be the development of techniques to enable tree seeds to be pretreated, dried back to normal storage moisture contents, stored for any period of time and yet still be in a non-dormant state. Then they could be supplied from dry-storage and be ready for immediate sowing.

Many woodland managers would prefer to use more natural processes in the creation and maintenance of uneven-aged or continuous-cover forests. Identifying the fates of naturally dispersed and direct sown tree seeds, predicting seed longevity in soil seed banks, plus a better understanding about what makes tree seeds germinate or not, (particularly the phenomenon of seed dormancy) would significantly improve success rates for both natural regeneration and direct sowing.

Introduction

There are generally three methods used to establish trees from seeds: 1) nursery sowing followed by transplanting seedlings to their final planting site, 2) direct sowing where seeds are sown onto the final site of the proposed crop, and 3) natural regeneration (or more accurately, 'human-assisted natural regeneration') where 'natural' colonisation/establishment from seed banks is promoted by a number of artificial, pre-planned operations such as leaving seed trees, applying carefully timed ground disturbance, minimising seed predation, and protecting seedlings from weed competition and browsing.

Throughout the world, there are significant problems in the establishment of forest trees via all of these methods. Even weed seed characteristics are much better understood than those of trees because greater research efforts have been directed for longer periods of time towards controlling weed problems in food crops than towards improving afforestation and reforestation techniques. Consequently, the quality and performance of most tree seeds lags about 50 years behind agricultural, horticultural, vegetable, and flower seeds. Some of the more significant tree seed problems, which are potentially amenable to research solutions, are considered below in the chronological sequence in which they occur.

Flowering/Fruiting

Tree seed production is highly erratic. In some years trees produce lots of seeds, in others virtually none. But there are no cost-effective methods for stimulating consistent seed production in anything other than seed orchard systems. Seed supply is therefore a problem especially for species with seeds that cannot be stored.

Methods for assessing flowering and fruiting in natural stands are not only extremely subjective, but they are also still in their infancy and only available for a relatively few species. The development of more objective flowering/fruiting assessments would enable advance prediction of 'good' versus 'bad' seed years, lead to fewer uneconomical seed collections, and increase the chances of successfully managing subsequent phases of 'human-assisted, natural regeneration'.

Processing

Trees habitually produce large proportions of empty and dead seeds, and insect and fungal infections are not uncommon. With the exception of removing empty seeds, processing techniques for separating live, healthy seeds from unproductive ones are relatively inefficient and only applicable to a few species. As a consequence, few countries even attempt to remove dead seeds from any of their seed lots. Hence germination percentages for tree seed lots can be quite low, and unlike commercially available agricultural, vegetable, and flower seed lots there are no minimum germination standards. Better removal of dead and dying seeds would lead to higher germination percentages, more efficient nursery production, and increased opportunities for direct seeding. Improved detection and control of seed borne insects and fungi would reduce seed/seedling losses in nurseries.

Seed Storage and Longevity

The seeds of many tropical rainforest and some temperate tree species such as oaks (*Quercus*), chestnuts (*Aesculus* and *Castanea*), and sycamore (*Acer pseudoplatanus*) are extremely perishable. They are shed at a high moisture content and are killed by very little drying. Such seeds cannot be stored without significant deterioration over very short time-scales (e.g., weeks to months) and are often referred to as recalcitrant. The seeds of these species usually tend to be large and as a consequence of their size and perishability they are by far the most expensive tree seeds (on a per live seed basis). Methods are urgently sought to prolong the storage life of such rapidly deteriorating seeds, both for conservation and commercial reasons.

In contrast to seeds with recalcitrant properties, the majority of tree seeds can be dried and stored relatively successfully and are said to be orthodox. However, whereas the seed longevity of many agricultural and horticultural crops can be predicted at different moisture contents and under different temperature regimes from mathematical formulae, most tree seed storage is done empirically. Research is required on these tree species to not only enable the maximum potential longevity of seeds to be predicted in commercial seed stores and *ex-situ* conservation facilities, but also to monitor/predict seed deterioration in natural seed banks to improve the chances of successfully managing natural regeneration.

A small minority of tree seeds appear to fall into an intermediate storage category between orthodox and recalcitrant. Preliminary evidence suggests that these seeds are less tolerant of drying and shorter-lived than the majority of seeds with orthodox storage characteristics. But they are not as desiccation sensitive or highly perishable as recalcitrant seeds. Early indications are that *Acer platanoides, Calocedrus decurrens, Fagus sylvatica*, some true-firs (*Abies* spp.) and some cedars (*Cedrus* spp.) may fall into this category.

Seed Dormancy, Pretreatment, Testing and Propagation

Agricultural, horticultural, vegetable, and flower seeds are nearly all supplied from dry storage ready for immediate sowing. Hence most farmers, horticulturists, and gardeners are used to buying seeds, planting them, (perhaps watering them) and watching them grow into plants. Unfortunately, virtually all tree seeds exhibit dormancy. Dormant seeds are live, fully mature, and perfectly healthy. But even when they are provided with apparently ideal conditions for germination (ample water, good aeration, and a suitable temperature) they obstinately refuse to germinate. To overcome this dormancy, most tree seeds require some form of artificial, pre-sowing treatment. Unfortunately, effective pretreatments are not known for many species; in other instances, pretreatments are only effective on some seed lots or a small proportion of seeds in any particular seed lot. Even for seeds where pretreatments are considered to be relatively good, they are nearly always awkward to carry out, take months or even years to complete, and require frequent monitoring.

Better, faster, and more versatile seed pretreatments for overcoming seed dormancy would provide several major benefits: 1) improved measurement of seed quality and forecasting accuracy of nursery emergence from seed test results, 2) increased opportunities for adopting precision sowing and container production methods, 3) more consistent production of higher quality nursery stock at lower cost, and 4) greater chances of success for direct sown tree seeds. The increased understanding of tree seed dormancy, pretreatment techniques, and germination characteristics which would accompany the above research would also significantly improve the predictability and hence management of natural regeneration.

An ultimate aim of tree seed research should be the development of techniques to enable tree seeds to be pretreated, dried back to normal storage moisture contents, stored for any period of time, and yet still be in a non-dormant state and ready for immediate sowing. This is no more than is taken for granted with the seeds of virtually every other crop.

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CONE INDUCTION AND SEED PRODUCTION NEEDS

Seed orchard effectiveness has been greatly improved thanks to research in flower stimulation, pollen management and artificial pollination. However, deployment of orchard seed lots requires further information on many issues. In my opinion, the most important are induction treatment frequency, flower induction/differentiation process, ovule and seed development, and seed extraction.

In the last 20 years, progress was made on the refinement of flower stimulation techniques (regarding timing, technique of application, dose of active ingredient). As these techniques have generally additive effects the best results are obtained with combined treatments. The success rate and the gains achieved can be considered satisfactory though they still depend on site conditions, genotype, and physiological age. For example, combining root-pruning, girdling, Gibberellin$_{4/7}$ application, and nitrogen fertilizers proved to be the most successful treatment in French Douglas-fir (*Pseudotsuga menziesii*) meadow orchards. However, repeated treatments and frequent cone production weaken the trees. Trees treated several times often show needle damage, reduced vigor, and they do not respond as well as untreated individuals to subsequent treatments. Our data suggest that biennial application may not be compatible with sustainable seed production. Is it preferable to use highly effective treatments every third or fourth year or a less stressful treatment every second year? No answer can be found in the literature because the experiments cover too short a period of time. New experiments should be established for this specific purpose.

The last great advance made in the understanding of the mechanisms involved in flower initiation is related to the discovery of the effectiveness of Gibberellin$_{4/7}$ in the Pinaceae. It was nearly 30 years ago. Basic research should continue in that field.

Initiating flower primordia is one thing, producing large quantities of seeds is another one. Nonnegligible loss of flowers, ovules, and seeds has been reported in many papers. Efforts should be devoted to research aiming at increasing seed efficiency (actual number of full seeds per cone / potential number of seeds). That implies a better understanding of the pollination mechanism and embryo development. As insects seem to play a leading role in flower, ovule, and seed abortion, at least in pines, cooperation with entomologists should be reinforced. Moreover, simple treatments such as irrigation, fertilization, and girdling that increase carbohydrate availability in the crown portion where the cones develop proved to improve seed traits. More information is needed on the effect of nutrition on seed development.

Taking into account the numerous efforts devoted to seed production enhancement, every possible means must be used to reduce seed loss in the final stage, that is seed extraction. We know that a lot of viable seeds remain in the cones of recalcitrant species like European larch (*Larix decidua*). Moreover, the extraction rate varies from one clone to another and increases the natural deviation from panmixia. For these reasons, it is desirable to improve the effectiveness of the seed extraction machinery used in seed plants (or at least to assess the % of potential seeds actually extracted).

Ideally, seed orchards have a panmictic mating system. That means that all the genotypes produce the same number of gametes, that these gametes mate at random, and that there is no selfing and no pollen contamination. In fact, there are always deviations to panmixia and their extent depends on flowering conditions. Some countries have adopted a protocol for rating seed orchard seed lots. The genetic quality (genetic gain, effective population size) of each seed lot is assessed according to the parental gamete contributions. The development of such protocols should be encouraged.

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PRIORITY AREAS IN SEED BIOLOGY RESEARCH

Introduction

Not enough is known about the biological processes involved in seed germination. Despite the work done on seed provenance testing and tree breeding, there has been relatively little work on seed biology. When it comes to seeds, there are many 'information gaps'. However, identifying the 'biggest problems and information needs' presents more of a challenge.

A failure to consider seed biology ignores the potential contribution of seeds to research. For instance, the evolution of conifers represents over 200 million years of successful defense against disease, insects, and environmental stress. Although the genetic code is fully present in conifer seeds very little of the genetic 'memory' is utilized. Once seed defenses are unraveled, we could have a powerful tool that will enable us to fully utilize the natural defenses of trees.

The production of seed by trees is irregular; hence there is the necessity to store seeds for regeneration purposes. The loss of seed viability over time adds to the cost of storage and hinders regeneration efforts. Of course, users still prefer young, fresh seed. With age, stored seed may germinate and grow more slowly, compared to young, fresh seed. Beyond a certain age, seeds lose viability and have to be tested to ensure they reach minimum standards.

Oxidative Stress, Antioxidants and Seed Senescence

Living, respiring, organisms require oxygen to stay alive and germinating seeds consume large amounts of it. Oxygen is not a stable element and forms toxic free radicals when organisms are under stress. Oxidative stress and free radical formation may diminish seed longevity and its ability to germinate but it has been found, in aged white pine seed, that the use of antioxidants can partly reverse seed deterioration (Blake 2002a).

Even small variations in the most commonly used seed treatments may inhibit germination and cause slow growth. For example, repeated seed wetting and drying are variously reported to increase, decrease or have no effect on germination, depending on the study. Although pre-sowing treatments, e.g., pre-germination and 'fluid drilling', have been advocated, their benefits are not always apparent. Although there are reports of early root emergence and faster seedling establishment following repeated wetting and drying cycles, other workers have failed to find any real benefit from dehydration treatments. Also, drought-hardening seed treatments (e.g., immersion in an osmoticum such as mannitol or PEG) were reported to increase seedling hardiness, but the dramatic slowing of growth reduced any competitive advantage.

The seed stage is the most desiccation-tolerant part of the life cycle of a tree, however, seed viability and desiccation tolerance decline greatly under warm moist storage. Desiccation tolerance is maximal in the seed but declines during germination, along with a reduction in lipid-soluble antioxidants. Seed viability requires active biosynthesis of phospholipids. High viability, in young, fresh, mature seeds, coincides with maximum phospholipid synthesis. As seeds age, viability declines and there is a loss of membrane integrity that carries-over into the seedling stage. Membrane leakage occurs in aging seeds when free radicals degrade membrane lipids. In the desiccation-intolerant seeds of silver maple (*Acer saccharinum*), membrane leakage was found to increase during seed dehydration (Pucacka 1989).

Use of Antioxidant Seed Treatments

The synthetic antioxidant Ambiol enhances the low germination rate observed in artificially-aged seeds, including those exposed to radiation (Vichnevetskaia and Roy 1999). Ambiol also increased germination rate and early seedling growth in black spruce (*Picea mariana*) (Borsos-Matovina and Blake 2001). A single seed pre-treatment with Ambiol (0.1, 1 or 10 mg/L) accelerated seed germination (by 25%), hastened emergence, and also protected seedlings against drought and extreme temperatures. Treated canola seed was twice as likely to have emerged 10 days after treatment (Vichnevetskaia and Roy 1999). Not only were anti-oxidant treated seedlings more stress tolerant, but they also grew faster. Ambiol pretreatment of seeds was found to 'switch-on' gene synthesis (Vichnevetskaia and Roy 1999) and genes could be 'switched-off' by a subsequent treatment with either a general protein synthesis inhibitor (cyclohexamide) or a specific inhibitor of RNA-synthesis (actinomycin-D) (Kritenko and Blake, unpublished data).

The Role of Seed Phenols

The role of phenols in stratification and germination requires more study. Phenols are often thought to be merely by-products of metabolism, but they may also stimulate or inhibit plant growth and development. Washing of stone pine (*Pinus cembra*) seeds resulted in more rapid germination which was attributed to leaching of inhibitors from the seed coat (Martinez-Honduvilla and Santos-Ruiz 1978). Germination was stimulated as much by removal of the seed coat as by stratification. However, the beneficial effects of seed coat removal may result from an increase in oxygen and water uptake by seeds; so a role for phenols has still to be confirmed.

Stratification improves the speed and uniformity of germination in some species, but many questions remain. Not all greenhouse operators stratify conifer seeds of boreal conifers, as a matter of course.

Stress-signaling compounds are often phenolic compounds, (e.g., salicylic acid). Phenols are major constituents of pine seeds and levels of phenolic acids decline significantly in the seed coat during stratification (Murphy and Noland 1981a).

Although they play a role in stratification and plant defence reactions, the role of seed phenols remains unclear. The activity of the PAL (phenylalanine ammonia-lyase) enzyme (which initiates phenol production), is highly correlated with germination, growth, and development. In contrast to treatments, such as soaking and storing seeds at room temperature, which lowered PAL activity, stratification caused PAL activity to increase by 50% after a two-week lag period (Murphy and Noland 1980).

Levels of phenolic acids, including cinnamic acid, p-coumarin acid, and ferulic acid, were relatively low in dormant sugar pine seeds, but increased substantially in the embryos during stratification at 5°C. The active synthesis of phenols in sugar pine embryos did not support an inhibitory role for phenols in germination (Murphy and Noland 1981b).

Reaction to Mild Doses of UV-B Reveals Plant Defence Reactions

Not only are phenols active in plant defence, but they also play a role in growth and development. We used UV-B and Ambiol (a 5-hydroxybenzimidazole derivative), as model systems to study the role of phenols in plant defence reactions. Ambiol was added as a 24-hour seed soak, in a range of concentrations (0.01 to 10 mg/L).

Low (ambient) doses of UV-B are known to stimulate the production of the enzyme PAL, which is the first step in activation of the phenol-synthesis pathway. We used a PAL-inhibitor (AIP – 2-aminoindan-2-phosphonic acid) to block the earliest stage in phenol formation in plants. Hardiness induction was compared in UV-B-treated and untreated plants, with and without AIP. UV-B increased hardiness but the stimulation was reversed by AIP (i.e., plants treated with UV-B + AIP were unable to tolerate drought or extreme temperatures). In the absence of AIP, UV-B treatment caused phenols to accumulate, which increased plant hardiness to extreme temperatures.

Since the PAL-inhibitor (AIP) also prevented phenol synthesis, this suggests that phenol formation (quercitin, kaempferol and anthocyanin) is a requirement for plant hardiness. The UV-B-filtering effects of the glass and plastics used in commercial greenhouses could explain why transplants are often unthrifty (Teklemariam and Blake 2004).

UV-B and antioxidants both 'switch-on' plant defence genes. We used UV-B, as a model system, to determine whether there is a common basis in plants that enables them to withstand a variety of different types of stress (such a mechanism, which is called 'Systemic Acquired Resistance', or SAR, has been suggested to underlie plant resistance to stress). PAL was found to activate phenol synthesis and certain phenols were identified as 'signaling' compounds, that appear to initiate plant defence reactions to stress. The SAR concept would explain why plants exposed to one stress (UV-B) acquire resistance to other types of stress (Teklemariam and Blake 2003; Teklemariam and Blake 2004).

Membrane Protection

Stress acts at the level of the cell membrane. Membrane protection, once activated, would increase the tolerance of high and low temperatures. An increase in stress resistance (and reduction in membrane leakage), was observed in UV-B-pretreated seeds. This resulted from PAL-induced phenol production. The PAL-inhibitor, AIP reversed the beneficial effects of mild UV-B treatment on plant hardiness. This confirmed the importance of PAL synthesis in membrane hardiness and stress tolerance (Teklemariam and Blake 2004).

Seed Pretreatments Enhance Growth and Stress Tolerance

Experimental verification of the benefits of some types of seed pre-treatment (e.g., fluid drilling, seed wetting and drying) is often absent. However, many studies show that a single (24-hour) seed-soak, with either Ambiol or natural plant extract (BioProtect) increases and stimulates germination, growth and stress tolerance (Blake 2002a). Antioxidants were shown to spare membranes, protect photosynthesis and enhance growth under drought (Rajasekaran and Blake 1999), which provides an explanation for the improved environmental resistance that was observed following seed pretreatment with the antioxidant (Rajasekaran and Blake 2002).

Operational Use of a Natural Antioxidant (BioProtect) in Canada: Field Results

Since 2002, over 4 million seedlings have been treated with the natural plant extract, BioProtect. This product has been used in reforestation projects from New Brunswick to British Columbia. We have not attempted to track the performance of all these seedlings, but feedback from forest company and nursery personnel has been positive. Of course field performance is the only real indicator of the value of any product or procedure that is used in the nursery. Therefore we have produced a short review of several trials that were established to demonstrate the effectiveness of the natural antioxidant, BioProtect.

In 2001, trials were established using black spruce, white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*) in Thunder Bay and Dryden, Ontario. Early results (fall 2001) indicated that BioProtect had resulted in improvements in field performance.

In the fall of 2004, one of these trials at Thunder Bay was re-measured. For white spruce, treated seedlings were slightly taller and 28% larger in stem volume. Survival for BioProtect treated seedlings was 85% compared to 76% for untreated seedlings. For black spruce, BioProtect seedlings were 20% taller and 60% larger in stem volume after 4 growing seasons. In addition, survival was 94% compared to 68% for untreated seedlings. For additional information on seed treatments and their effectiveness, please refer to our web site: www.ambiolinc.com.

Conclusions

The effects of seed deterioration are carried over into the seedling stage. The use of poor seed provenances or inappropriate seed treatments slows the growth of transplanted seedlings. Once growth slows and membranes start to leak, seedlings become uncompetitive. In stressed seedlings, oxidation reactions alter protein and RNA synthesis and these changes cause lipid peroxidation and membrane leakage (using the malondialdehye (MDA) assay) (Teklemariam and Blake 2004).

The use of antioxidants is a form of seed 'chemotherapy' which benefits aged seed (Blake 2002b). Such a treatment is cheaper and more effective than seed testing. The antioxidant Ambiol 'switches-on' dormant genes and the resulting synthesis of PAL accelerates germination. Early seed germination and faster seedling growth were observed following antioxidant treatment and the growth advantage carries over into the field. Treated seedlings were larger, more tolerant of drought, and of high and low temperatures. Antioxidant treatment also protected seedlings against some greenhouse diseases, including damping-off. The advantages of a single, antioxidant seed treatment were still apparent four years after transplanting to the field. A growth advantage that lasts through the greenhouse stage and through the first four years of growth in the field could last over the life cycle of a tree.

These results, when taken together, suggest that trees can acquire 'systemic acquired resistance' (SAR). The membrane sparing ability of antioxidants and a mild dose of UV-B suggest a mechanism that could be used to harden seedlings to most, if not all, types of stress (Genaud and Metraux, 1999). Although many of the effects of antioxidants resemble those of ambient UV-B, more study is required to fully understand the nature of systemic resistance (SAR). Research into the process by which the seed develops its extreme environmental resistance and passes its stress resistance onto seedlings and trees, is obviously a high priority.

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TREE SEED WORKSHOP OVERVIEW

At the 29th meeting of the CTIA, the Tree Seed Working Group presented a workshop on "Quality Assurance in the Seed Handling System". Two additional speakers (George Edwards and Conor O'Reilly) also presented volunteer papers. I'll present an overview of the talks and try and recall the issues raised. If I've missed something, please elaborate on it in the next News Bulletin.

I introduced the workshop topic and defined

Quality Assurance [QA] quite broadly as " the evaluation, monitoring, and management of information and practices related to activities within the seed handling system". QA evolved from manufacturing with the goal of reducing variability. In forestry, variability plays an important role in ensuring trees survive to rotation age. Emphasis should be on reducing process variability and measurement subjectivity (= standardization). The variation we are faced with in cones and seeds is a price we pay for maintaining genetic variability. This differentiates us from the agricultural and bedding plant seed industries where genetic variability is minimized and classical QA is more appropriate. This needs to be appreciated as we borrow technologies from these industries. I have done some reading on the topic and would like to suggest seven QA 'truisms' to help initiate or improve your system:

1) Focus on customer satisfaction ,
2) Concentrate on reducing process variability,
3) Decisions should be based on data,
4) Root causes of problems should be sought out by disciplined investigation,
5) Data recording must be performed consistently,
6) Barriers between departments need to be broken down, and
7) QA needs to provide significant added value and it needs to demonstrate it effectively to management.

Here is an overview of the speakers and topics covered during the workshop. I planned to have a half-day workshop, but we ended up finishing at about 3:30. I'm not sure if that's an organizational failure or success, but I was pleased with the presentations and lively discussions we had on a variety of topics. I'll try and cover some of the main points, but here are the presenters – Thank you very much:

Chris Walsh — Crop Maintenance and Collection in Seed Orchards
Al Foley — Quality Assurance in Cone and Seed Processing
Dale Simpson — Quality Assurance in Seed Testing
Donna Palamarek — Quality Assurance in Seed Storage
Dave Kolotelo — Quality Assurance in Seed Preparation
Susan Thorpe and Fernando Rey — Quality Assurance in the Nursery

George Edwards — Thermo-kinetics of water absorption, with special reference to Noble fir seeds
Conor O'Reilly — Effect of moisture content during pre-treatment or storage on the germination response of alder, birch, and oak seeds.

In the orchard setting, QA was presented from the perspective of having a good cone crop – What next? What considerations are important? Chris covered a variety of considerations and I'll underline the main activities he presented. The importance of <u>regular orchard maintenance</u> from the standpoint of safety and increased efficiency was emphasized. <u>Maintaining tree health</u> and <u>protection from cone and seed pests</u> were topics discussed. During crop development, having an <u>estimate of crop size</u> aids in the <u>administration</u> and organization required to run a biologically sound and efficient collection. Having a <u>cone collection strategy</u> in place involves identification of crop trees; a strategy for picking (most BC collections are ***picked*** on a clonal basis) and ensuring all equipment is ready. The <u>timing of collection</u> is critical and varies by clone. An overview of the indicator system used at Kalamalka seed orchards was reviewed *(see News Bulletin #38 for additional details on harvest efficiency and the cone axis test*). <u>Cone collection methods</u> and <u>cone storage and shipment</u> methods were reviewed. The bottom line was that vigilance pays dividends!!

Al discussed the Ontario Seed Plant and his responsibility in organizing cone collections for the province. This is currently difficult as the province's seed inventory is distributed at various locations and the full inventory of seed is unknown. This situation will probably change in the near future. Ontario is still performing a large amount of aerial seeding and this demand accounts for a large proportion of the cone collections. Seed processing for direct seeding need not be to the same high standards as seed used for container sowing. The importance of cash flow from the sale of spent cones was discussed. This raised one of our ***lively discussions*** concerning diligence in ensuring that insects are not being exported with cones. Al indicated that an 80°C treatment was being used to eradicate pests.

Seed testing was reviewed by Dale with respect to the International Seed Testing Association (ISTA) rules. Emphasis was on using techniques that give results that are as repeatable as possible. The initial critical step is to obtain a random, representative sample of the seed lot. The sample is used to determine moisture content (destructively), purity, 1000-seed weight, and germination. Germination tests are conducted to estimate the proportion of seed that will produce a normal seedling. Depending on species, seeds that are dormant need to be treated, generally by a period of moist chilling, before being germination tested. Viability tests are also useful to quickly indicate if seeds are capable of germinating.

Donna pointed out that the main functions of seed storage are to: provide favourable conditions to minimize loss of seed quality, maintain correct identity and location, and protect from harm and/or loss. The Alberta Public Lands and Forests Division currently has about 1 700 individual seed lots for a total of 42 000 kg of seed in storage. The underground seed storage facility at the Alberta Tree Improvement and Seed Centre (ATISC) is maintained at -18ºC and is monitored 24/7 for temperature fluctuations, mechanical failure, power loss, and unauthorized entry. Seed lots are tested prior to storage for moisture content, germination, 1000-seed weight, and purity. Ideally, seed lots should have moisture contents between 4 and 8%. Seed is stored in heat-sealed, 5-mil plastic bags and placed in waxed cardboard boxes. Bags are labeled inside and out and replaced every 10 years. Boxes are labeled on the outside. Government seed lots are retested every 4 to 5 years for germination and moisture content.

ATISC has a research seed germplasm conservation program, which is mainly comprised of single tree collections used for tree improvement research. There are 5 500 seed lots with the average lot size being about 20 grams. The bulk of this seed is stored in a walk-in cooler in the ATISC technical building. Where amounts allow, duplicate storage of irreplaceable lots is maintained in the underground storage facility.

Seed pretreatments were reviewed by Dave in terms of the seed requirements for germination: adequate moisture, alleviating dormancy, and at the nursery temperature sums. If adequate moisture is not contained in seeds, stratification will not be effective and germination will not occur. Species differ in their maximum and 'optimal' stratification moisture contents. If surface drying is employed following soaking it is very important that only moisture on the seed coat is removed. This is to ensure seed flows freely (a seeder requirement), pathogen build-up is limited, and oxygen is readily accessible to the embryo. Another one of our lively discussions followed regarding soak durations, surface drying, and the time it takes for moisture to reach the embryo. If in doubt, dissect some seeds (without destroying the embryo) – do the embryos bend (imbibed) or break (dry)? Additional work is needed on tracking the uptake of moisture into seeds in terms of the actual route and the rate of uptake under operational conditions. Stratification requirements can vary greatly by species, individual seed lots, and even families. More work is needed in quantifying this variability. Operators should understand the benefits of stratification – not only in overcoming dormancy, but also in speeding up germination (and decreasing the window for pests to attack the succulent tissues of germinants), increasing uniformity and the vigour of the seed. Vigour is the ability of the seed to germinate under sub-optimal conditions.

Susan and Fernando covered nursery aspects. Inputs of concern to the nursery are seed quality and quantity, seed cost, and seed condition (cleanliness, presence of insect/disease damage). Some nurseries also pretreat their sowing requests and may employ seed cleaning (sink:float separations) during seed imbibition. The nursery is concerned about and must consider: sowing equipment and performance monitoring, amount and type of grit, handling of seeded blocks, and temperatures to use for germination. Susan discussed the key performance indicators for Canadian Forest Products Ltd. including cavity fill, seed use efficiency, thinning labour costs, and sowing labour costs. Impressive gains (6% in germination) were illustrated through the use of styrogrit versus convential grit material. Fernando discussed the "order'-based information management system (PREMIS) that is being used at Pelton Reforestation Ltd.. This records pertinent seed information and is a tool used to increase the seed to seedling efficiency ratio.

George presented work on thermo-kinetics of water absorption. The three phases of water absorption and the impact of temperature on the rate and maximum level of imbibition were explained. Results on Noble fir indicate that although faster imbibition is accomplished with higher water temperatures a greater maximum moisture content is achieved with lower water temperatures. Differences between filled and empty seeds were discussed. For more details refer to the original paper (Edwards, D.G.W. 1971. The kinetics of water absorption in stratifying and nonstratifying noble fir (*Abies procera* Rehd.) seeds. Can. J. For. Res. 1:235–240).

The final speaker of the day was Conor O'Reilly who presented the paper indicated on the agenda. Two co-authors should also be recognized here – Norberta Atrip and Colin Doody. Conor traveled all the way from Ireland and I think it is appropriate to include the entire abstract here.

Alder (*Alnus glutinosa* (L.) Gaertn.), birch (*Betula pubescens* Ehrh.), and pedunculate oak *(Quercus robur* L.) now form an important part of the planting program in Ireland. However, seed germination in the nursery bed is often low and/or slow. To this end, the effect of seed moisture content (MC) during pre-treatment on the germination performance of these species was investigated. Alder and birch have orthodox seed characteristics (easy to store provided MC <12%), whereas oak is difficult to store successfully (relatively high MC levels must be maintained during storage). Seeds of each species were adjusted to various MC levels and stored at 4°C for various periods. Acorns were also stored at -3 and 1 °C. The optimum or target MC (TMC) during prechilling was about 30% in alder and 35% in birch. Germination increased following up to 24 weeks of prechilling for seeds at TMC levels in alder and 12 weeks in birch, significantly better than achieved using the standard method (fully imbibed for shorter periods). Thereafter, germination remained almost unchanged for seeds that received up to 36 weeks of prechilling, whereas the fully imbibed seeds deteriorated or germinated prematurely. In oak, acorns adjusted to the highest MC level (46%) and stored for 4–6 months at -3°C had the highest germination (76%) while the non-soaked controls and those adjusted to 37% MC has the lowest values (17% for both treatments). Interestingly, the control acorns had significantly lower germination than seeds that were soaked and then dried back to similar levels.

Thank you for everyone who participated. We are currently looking at the East Coast for 2006 and Tannis Beardmore and myself are hoping to co-ordinate a joint workshop with the IUFRO Seed Physiology and Technology research group. It is never too early to start putting the buzz in your manager,s ears for travel requests ☺.

I'll leave you with my favourite little desktop saying:

Data is not Information
Information is not Knowledge
Knowledge is not Wisdom

Dave Kolotelo

IS WESTERN LARCH A POOR SEED PRODUCER IN NATURAL STANDS AND SEED ORCHARDS?

Introduction

Western larch (*Larix occidentalis* Nutt.) is the largest of the North American larches and has limited distributions growing at moderate elevations in the dry interior of the Pacific Northwest. Its distribution extends from SE British Columbia (BC) southward in the Cascade Mountains of Washington and Oregon and in the Inland Empire forest regions of NE Washington, Northern Idaho, Northwest Montana and in the NE corner of Oregon (Fowells 1965, Schmidt and Shearer 1995). It is considered to be a pioneer species that becomes established, before other more shade tolerant species, in areas where there has been fire, slides, volcanic activity, glaciation or other disturbances. Most disturbances appear very quickly whereas glaciation may take centuries, but all leave very similar and dramatic landscapes.

Glaciation has been part of the Pacific Northwest ecology for the last 16 000 years with the last glaciers about 12 000 years ago after which the areas became colonized by herb-dominated species and sub-alpine forests. The presence of summer rains may have prevented fires and favored *Pseudotsuga*, but not larch. However, we cannot be sure which species dominated because recent fossil evidence is based on pollen deposits and fossil *Pseudotsuga* and larch pollen are not distinguishable (Owens and Simpson 1986, Whitlock 1995). Presently, western larch thrives in relatively cool temperatures averaging 7°C annually but ranging from 47°C to -37°C with a frost-free period of 60 to 160 days and an annual rainfall averaging 760 mm, only 20% of which falls during the active growing season (Schmidt and Shearer 1995).

Western larch is a seral rather than a climax species because of its intolerance for shade. It usually grows in mixed stands that include Douglas-fir (*Pseudotsuga menziesii*), lodgepole pine (*Pinus contorta*), Interior spruce (*Picea glauca* x *engelmannii*), sub-alpine fir (*Abies lasiocarpa*), and, on drier sites, ponderosa pine (*P. ponderosa*) (Fowells 1965, Schmidt and Shearer 1995). Several studies in the past have suggested that western larch may be a poor seed producer but this conclusion may have resulted from studies being done on rather isolated individuals or those growing in harsh environments (Owens and Molder 1979a). Other studies of trees in potted seed orchards have also concluded that western larch, under those conditions, produces few filled seeds, often only 10 to 20 per cone (Owens et al. 1994) and as low as 2 to 3 filled seeds per cone (Webber and Ross 1995). Similar low filled seeds per cone have been observed in potted orchards in western Oregon (Richard Snedzko USFS, Cottage Grove, Oregon, pers.com.). The generalization that larch is a low seed producer also comes from research on other species including *L. laricina* (tamarack), *L. decidua* (European), *L. kaempferi* (Japanese), and *L. siberica* (Siberian). In most of these studies the main factors believed to cause loss of filled seeds were the infrequency of successful pollination, low pollen fertility, and early embryo abortion. In contrast, another report indicated that western larch was one of the best seed producers in the Mountain West (Schmidt and Shearer 1995).

Conflicting results, such as these, have caused concern about establishing western larch seed orchards that may have unacceptably low filled seed production. In order to address these concerns, two studies were done in 2003, one in an established clonal soil-based seed orchard in the Okanagan Valley of BC and another in five natural stands in the Inland Empire in the Northwestern U.S. The purposes of the studies were to compare cone morphology, determine the seed potential per cone, the filled and empty seeds per cone, and the possible causes of filled seed loss in clonal seed orchards and natural stands of western larch.

Methods and Materials

Western larch genetics research and tree improvement programs began in the United States in the mid-1970's and in BC in 1987. A western larch seed orchard was established near Lewiston, Idaho in the mid-1970's and another at the Kalamalka Seed Orchard (KSO) near Vernon, BC in 1987 (Jaquish et al. 1995). Both began to produce some seed cones after about 6 years and are now coming into full production. Both orchards are reasonably well isolated from natural stands of western larch.

At the KSO in the late summer of 2003, five mature cones were sampled from each of 10 clones that were open pollinated by orchard pollen. Each cone was sealed in a coin envelope and the cones from each clone were sealed in a paper bag. Most cones opened in the envelopes and seeds from that cone remained in the envelope. Data were taken on cone morphology, including cone length, cone weight, total cone scales, sterile cone scales (those lacking any winged structures), fertile scales (those bearing winged structures), and seed potential (fertile scales x 2). After weighing each cone and its seeds, the numbers of fertile scales and sterile scales were counted and the seeds of various sizes were placed on the sticky surface of strapping tape attached to a small board. All but the smallest seeds were then sliced longitudinally with a double-edged razor blade (broken in half for safety) and the cut seeds were observed using a dissecting microscope. The number of filled seeds, empty seeds, early-aborted seeds, late-aborted seeds, and insect or disease damaged seeds were counted and the seed efficiency was calculated (SEF, the number of filled seeds/seed potential).

In the late summer of 2003, at least five cones from each of five trees were collected from five different natural stands in the Inland Empire. Unfortunately, cone collections were done differently by those doing the collections at the different sites. Consequently, the number of cones collected per tree varied and in most cases all of the cones were placed in one bag resulting in a mix of seeds from many cones. This did not allow careful analysis of seeds relating to a particular cone nor statistical analysis as was intended. All seeds from the cones of one tree were placed on the strapping tape, sliced, observed, counted and categorized in the same manner as those from KSO.

The data for cone size and morphology and categories of seeds from natural stands and KSO were tabulated and the standard deviation determined.

Results

Cones from KSO averaged 3.5 cm (2.6–4.2 cm) and cone weight averaged 3.4 g (2.3–4.5 g). Total scales averaged 69 (50–82), sterile scales 7 (2–10), and fertile scales 67 (52–115). The seed potential averaged 123 (103–148), filled seeds per cone 55 (16–90), empty seeds 63 (29–104), early-aborted seeds 0.4 (0–1.8), late-aborted seeds 5 (1–10), insect- and disease-damaged seeds 0.1 (0–0.4), and rudimentary seeds 0.4 (0–1.8). The SEF averaged 44% (15–70%), meaning that 44% of the ovules on the fertile scales were successfully pollinated, fertilized and developed into viable seeds (Table 1).

Cones from the five natural stands in the Inland Empire averaged 3 cm (2.7–3.4 mm) and cone weight averaged 2.8 g (2–3.2 g), but this was minus many of the seeds that had been shed into the common bag. Total scales averaged 62 (56–73), sterile scales 16 (9–25), and fertile scales 46 (39–64). The seed potential averaged 90 (69–127), filled seeds 38 (23–64), empty seeds 45 (37–61), early-aborted seeds 3 (0.4–9), late-aborted seeds 6 (3–11), insect- and disease-damaged seeds 4 (1–10), and rudimentary seeds 3 (0.4–8.8). The SEF was 42% (31–52%), meaning that about 42% of ovules on the fertile scales were successfully pollinated, fertilized, and developed into viable seeds (Table 2).

Discussion

Two conclusions seem evident at a glance, and without elaborate statistics: firstly, western larch can be a good seed producer in natural stands (38 filled seeds per cone, 42% SEF) and secondly, it can be a better seed producer (54 seeds per cone, 44% SEF) when placed in a well managed soil-based seed orchard in a suitable dry environment.

For most members of the Pinaceae, 30 to 50 seeds per cone and a SEF of over 30% is average or better than average and is much better than species in most other conifer families (Owens 1991). However, in western larch, the number of cones per tree may fall short of other species in the Pinaceae and certainly fall short of some in other families, such as the Cupressaceae. The reproductive success of a tree is a combination of the number of filled seeds per cone times the

Table 1. Cone and seed analysis of five cones from each of ten western larch clones at Kalamalka Seed Orchard in 2003

Clone	Cone length (cm)	Cone weight (g)	Total scales	Sterile scales	Fertile scales	Seed potential	Filled seeds	Empty seeds	Rudi-mentory	Early abortion	Late abortion	Insect/ disease	Total seeds	SEF %
A	4.2	4.5	73	8	64	127	60	65	0	0	2	0	127	47
B	3.6	3.8	79	6	74	148	50	96	0.4	0.2	2	0.2	147	34
C	4.0	4.2	74	5	69	138	74	58	0.2	0.2	6	0	133	54
D	3.9	4.2	69	10	56	112	63	39	0.2	0	10	0.4	113	56
E	3.4	3.1	68	4	61	121	43	77	0.4	0.4	1	0	121	30
F	3.4	3.0	72	8	115	129	90	29	0	1.8	8	0.2	129	70
G	3.0	2.3	59	10	53	106	51	46	1.2	0.4	8	0	107	48
H	3.6	3.3	73	2	71	141	34	104	1.8	0	3	0	143	24
I	2.7	2.2	64	10	53	106	16	88	0	1.2	1	0	106	15
J	3.5	3.2	62	9	52	103	62	31	0	0	10	0.2	103	60
Avg.	3.53	3.38	69.3	7.2	66.8	123.1	54.3	63.3	0.42	0.42	5.1	0.1	122.9	43.8
SE	0.38	0.68	6.01	2.81	18.7	16.03	20.63	27.2	0.59	0.59	3.53	0.137	15.49	17.4

Table 2. Cone and seed analysis of 29 trees from five Western Larch sites in the Inland Empire in 2003.

Site	No. trees	No. cones	Cone length (cm)	Cone weight (g)	Total scales	Sterile scales	Fertile scales	Seed potential	Filled seeds	Empty seeds	Rudi-mentory	Early abort.	Late abort.	Insect/ disease	Total seed	SEF %
PC	5	50	3.0	2.6	62	12	50	99	47	43	2.1	2.0	6	3.0	103	52
IEPC	5	50	3.4	3.1	73	9	64	127	64	61	8.8	9.0	11	10.0	164	51
Goos	5	25	2.8	2.0	60	25	35	69	25	37	0.4	0.4	5	1.4	69	32
BCC	9	47	2.7	3.2	56	14	42	76	31	39	1.7	2.0	3	1.0	78	42
HE	5	70	3.1	3.0	59	20	39	77	23	43	2.0	2.0	4	5.0	79	31
Total	29	242														
Avg.			3.0	2.78	62	16	46	89.6	38	44.6	3.0	3.08	5.8	4.08	99	42
SD			2.74	0.49	6.52	6.44	11.47	23.74	17.3	9.53	3.31	3.38	3.11	3.67	38.7	10

PC, Plum Creek, Montana; IEPC, Idaho; Goos, Goosman, Washington; BCC, Idaho; HE, High Elevation, Washington

number of cones per tree (Owens 1991). Fewer cones per tree in larch results from the cone position and method of cone initiation – they normally occur on dwarf or short shoots that are one or more years old, thus limited by previous vegetative growth (Owens and Molder 1979 a, b). This differs from many other conifers in which most cones develop from newly initiated axillary apices (Owens and Blake 1985). We may enhance cone production in seed orchards and sometimes in seed-production areas in young natural stands by using cultural treatments and application of plant growth regulators (Bonnet-Masembert 1982, Ross 1991, Philipson 1996). Cones are also lost during development due to frost, insects, disease, and other less obvious reasons. These factors can usually be managed in seed orchards.

Cone morphology is important in seed production. In natural stands and seed orchards, larger cones tend to have more scales, more fertile scales, and fewer sterile scales resulting in a higher seed potential. This commonly results in more filled seed per cone, although not necessarily a greater SEF. Therefore, simply collecting larger cones may result in collecting more filled seed. Since cone size was a clonal trait in this study, there may be some gain in seed production by selecting seed orchard clones having larger cones. The number of fertile scales averaged about 67 and was over 50 for all 10 seed orchard clones but averaged only 46 in natural stands. Larger cones that bear more fertile scales may partly result from better cultural treatments in seed orchards. This increased the seed potential in seed orchard clones (123 compared to 90 in natural stands) and was probably the most important factor in increasing filled seeds in seed orchard trees.

The increase in fertile scales was partly due to an increase in total cone scales but also due to a decrease in number of sterile scales. Ovule initiation and early development occur in the late summer and fall before pollination (Owens and Molder 1979 c) and physiological or environmental conditions acting at that time may promote more ovule initiation and more pre-dormancy ovule development producing more fertile ovules per cone at pollination.

The causes for seed losses are varied and depend on the species, its reproductive biology, and the site in which the trees are grown. Like most other genera in the Pinaceae, western larch has a one-year reproductive cycle (actually about 17 months from cone initiation until seed maturity) in which pollination, and cone and seed maturity occur within 5 to 6 months – from about April until September (Owens 1995). During this short time, fertile ovules within a cone may be pollinated, fertilized and develop into filled, viable seed or form a variety of non-viable seeds. Some of the causes for the loss of filled seeds have been determined for western larch (Owens et al. 1994) and were used in this study to estimate the relative importance of different causes in reducing filled seed production.

A filled seed is a fully developed and rounded seed with a well-developed seed coat. When sliced the seed contains a yellow embryo that fills about 80% of the shiny white megagametophyte. These are likely viable seeds (Kolotelo 1979).

Empty seeds are very common and may result in the loss of about half of the seeds in natural stands (50% of seed potential) and in seed orchard (51% of seed potential). Empty seeds appear the same as filled seeds externally but when sliced, empty seeds contain only a collapsed brown sac, which is the aborted megagametophyte. Empty seeds in larch may result from two different causes: 1) Ovules that were not pollinated develop normally until the time of fertilization. At fertilization both the seed coat and megagametophyte are well developed and the megagametophyte has a very high water content. This development occurs whether the ovule was or was not pollinated. In larch (Owens et al. 1994) and Douglas-fir (Orr-Ewing 1957) pollinated and non-pollinated ovules develop normally until about the time of fertilization then non-pollinated ovules that are therefore not fertilized abort and the megagametophyte dehydrates leaving only the collapsed sac, which is the fibrous megaspore membrane (Owens et al. 1994). In contrast, the megagmetophyte in a fertilized ovule converts its watery contents mostly into storage proteins and lipids that fill the megagametophyte cells enclosed by the megaspore membrane and the embryo develops within the megagametophyte (Owens et al. 1994). 2) A second cause of empty seeds is that most megagametophytes, in which the eggs are self-pollinated, abort about the time of fertilization and result in empty seeds that appear the same as those that were not pollinated (Orr-Ewing 1957, Hagman and Mikkola 1963, Mergen et al. 1965, Owens et al. 2004).

Wings may develop on scales without a visible ovule attached. These may have had tiny ovules but they aborted before or about the time of pollination. Other wings may bear small ovules that aborted at later stages. Rudimentary seeds are ovules that aborted soon after the time of pollination and remained as a very small aborted ovule (“seed”) attached to a well-developed seed wing. Early ovule abortion results in small “seeds” with a thin, partially developed seed coat attached to a well-developed seed wing. The above structures form a gradient from undeveloped ovules to partially developed “seeds” for which there may be different causes, such as frost damage at pollination or early pre-fertilization self-incompatibility (Owens et al. 1994). We know very little about the latter in larch but it appears to occur in other conifers.

Late abortion occurs during embryo development and may result in seeds with poorly developed embryos and shrunken megagametophytes. These are thought to result form late acting self-incompatibility (self-inviability) and the seeds are usually not viable. All of these causes result in small amounts of potentially filled seeds being lost during development, in total usually only 5 to 10% of seeds. In seed orchards, these losses may be reduced by increasing cross-pollination through supplemental pollinations, reducing the risk of frost damage by sprinkling with water, and maintaining good soils and nutrition of trees.

Cone and seed insects and diseases are not well documented for larch (Hedlin et al. 1980, Sutherland et al. 1987). Damage was slight at KSO but accounted for about 4% of filled seed loss in the natural stands. It varied considerably among stands and this was more than the tabulated data show since in some stands some cones could not be analyzed because they were nearly destroyed by insects feeding on cones and seeds. In seed orchards losses may be reduced through spraying of insecticides as was done at KSO. However, in seed orchards insect pests and diseases, that we do not yet associate with larch, may appear and spread from other species in the orchard, especially those having very similar seed cones and reproductive biology, such as Douglas-fir.

Summary

Western larch cones, although small, have many scales and a high proportion of fertile scales thus a high filled seed potential (over 120 seeds per cone). The filled seeds per cone and SEF are quite high in both natural stands and the clonal seed orchard compared to many other genera within the Pinaceae. The high number of filled seeds per cone may be increased in seed orchards over natural stands by about 40% as a result of good orchard management. Empty seeds are the main cause for loss of filled seeds in western larch and they may result from the lack of successful pollination by fertile pollen or self-pollination, both of which result in megagametophyte abortion about the time of fertilization. The empty seeds resulting from both causes look the same externally and internally. These causes may be reduced in seed orchards by supplemental pollinations using a polymix of highly fertile pollen. With the high potential for filled seed production in conventional soil-based seed orchards, it is hard to recommend potted orchards for western larch, as the latter have had a short history of low filled seed production with no definitive known causes.

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SEED PRODUCTION IN 2004 FROM J.D. IRVING, LIMITED'S PARKINDALE SEED ORCHARD

This has been our best year for seed production at the Parkindale Seed Orchard. The following quantities of cones were collected with the approximate expected seed yield: second-generation black spruce – 95 hL / 23 million seed, first-generation white spruce – 185 hL / 40 million seed (we only picked 40% of the cones), first-generation Norway spruce – 126 hL / 15 million seed, and first-generation white pine – 14 hL / 750 000 seed which was picked from ramets that are less than 5 years old.

This was the first year to use lift equipment to collect Norway spruce cones. Ladders were not tall enough to reach the top of these trees. All of the older first-generation blocks will require lift equipment within the next few years. The ramets are 10+ m tall as we do not top the trees.

We began extracting the seed from these cones in late October and should be done by the end of January 2005. We will also do some custom cleaning (210 hL), for the P.E.I. Provincial Government and Nexfor Fraser Papers. The extracting and cleaning process will keep 2 hourly employees busy until the end of March 2005.

On the nursery side, we struck 2 million Norway and white spruce cuttings this year. These came from potted hedges, grown from crosses made in the top 20% of our first- generation white spruce clones and weevil resistant Norway spruce. We also transplanted over 100 000 somatic embryos this year. Emblings could replace most of the rooted cutting production within the next few years.

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NON-SPECIFIC POLLINATION MECHANISMS MAY CREATE OBSTACLES FOR PROPER SEEDING IN *JUNIPERUS*

Angiosperms have a specialized receptive area for pollen receptivity because pollen does not have direct access to the ovules. In contrast, gymnosperm ovules are exposed to the environment and pollen lands on the micropyle where it is drawn in to the ovule by the micropylar drop as it is reabsorbed. The micropylar fluid is a dilute sugar solution with the presence of proteins. The composition of the micropylar fluid has been analysed only in a limited number of species.

Under natural conditions, pollen from various species as well as insect eggs and abiotic material (dust, etc.) may land on the micropylar drop. The effect of particles of different sizes on the pollination mechanism of *Juniperus oxycedrus* ssp. *oxycedrus* was investigated within a research project funded by APAT (Italian Environmental Protection Agency). The study dealt with the reproductive efficiency of three *Juniperus* species growing in the Mediterranean environment of Italy.

Branches with mature female cones were cut and placed in vases of water and kept at room temperature. When micropylar drops appeared on the cones, pollination trials were conducted with: 1) viable pollen of the same species, 2) non-viable pollen of the same species killed by exposure to 120°C for 1 hour, and 3) silica gel dust of different sizes (10–15, 40–63, and 63–200 µm).

The diameter of the micropylar drops (assumed to be spherical) was measured under a stereo microscope before and at different times after pollination and volumes were calculated. The deposition of pollen as well as any kind of powdery material lead to a decrease in size of the pollination drop. Variation in diameter is to be interpreted as partial or total reabsorption and thus the '*recognition*' of pollen by the micropylar drop. Unpollinated micropylar drops usually persist in the branches kept in the laboratory for 12 days with few changes in volume due to temperature and other environmental factors.

Results indicated a non-significant variation in unpollinated drops, while a significant decrease in volume was observed when viable pollen, non-viable pollen, silica gel 10-15 µm, and silica gel 40-63 µm were applied on the micropylar drops. In the case of viable and non-viable pollen, the volume of the micropylar drop decreased to 0 (total reabsorption) while there was only a partial reabsorption in the case of silica gel.

These studies reveal that the pollination mechanism in *Juniperus oxycedrus* ssp. *oxycedrus* is highly non-specific. The micropylar drop is reabsorbed into the ovule even in response to non-viable pollen and inorganic particles of similar size to *Juniperus* pollen. Since deposition of non-viable pollen induced total reabsorption of the micropylar drop, it led to a decrease in the length of the period of female receptivity. In fact, the pollination drop is normally secreted again the next day. Silica gel particles caused partial reabsorption of the drop, thus decreasing the probability of pollen capture. The combination of these two effects may explain the poor viable seed production observed in different populations of junipers.

This lack of specific capacity to recognize its own pollen is a detriment to the pollination process of *Juniperus*, especially if the plants are exposed to sources of particulate matter, dust, etc. The ecological implications are more evident if we consider that many airborne inorganic particles arise from human activities such as vehicles traffic, industry and crushing or grinding operations. Knowledge of the existence of this obstacle can, however, be helpful to overcome the problem itself.

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IUFRO SEED PHYSIOLOGY AND TECHNOLOGY RESEARCH GROUP MEETING

The IUFRO (International Union of Forest Research Organizations) Seed Physiology and Technology Research Group (2.09.00) meeting was held the 19 to 24 September at Nanjing Forestry University, Nanjing, Peoples Republic of China. The Nanjing Forestry University and the Southern Tree Seed Inspection Center, State Forestry Administration hosted this meeting. The Nanjing Forestry University is a rapidly growing institution with interests in both seed technology and research.

The meeting was divided into two components; one where general presentations were made and the other consisted of field trips. The research presented was broad covering genetics, physiology, biochemistry, ecology, morphology, and innovative seed dissemination and germination technologies. The proceeding were published and a few copies are still available upon request to Dr Yu Fangyuan (fyyu@njfu.edu.cn). The field trips took us through the area surrounding Nanjing. We visited ancient burial tombs, shrines, temples, and monuments to Sun Yet Sen.

The next IUFRO Seed Physiology and Technology Research Group will be held in conjunction with the 2006 CTIA meeting.

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NATIONAL TREE SEED CENTRE

Seed Centre staff have been involved in a number of activities and projects over the past year. A large investigatory experiment was initiated the fall of 2003 to evaluate various combinations and durations of warm and cold to promote growth of the immature embryo and alleviate dormancy of black ash (*Fraxinus nigra*) seed. One series had varying durations of warm followed by cold and a second series had varying durations of three temperature treatments; cold followed by warm followed by cold. The experiment also examined the use of peat and kimpak to evaluate their impact as stratification media and ability to limit growth of mold on the seed. Data have been collected and await analyses. Another experiment focused on sugar maple (*Acer saccharum*) to evaluate the impact of seed source on germination of seed subjected to several soaking times, chilling durations, and germination temperatures. Part one of this experiment was conducted in 2003 using seed collected the fall of 2002 and Part two used the same seed lots after the seed had been in storage at -20°C for 12 months to evaluate the impact storage may have on alleviating dormancy. The data have been collected and await analyses.

This was a good seed production year and the months of September and October were busily spent making collections. Collections are now focused on obtaining seed from at least 15 well spaced individual trees in a population. These type of collections are more useful for researchers and take into account tree to tree variation in seed yield and quality. Over 500 collections were made which is a huge effort considering the small number of staff. The winter months will be more challenging in order to process and test all this material in a timely fashion. A number of collections were made in cooperation with several research projects. Species involved were: choke cherry (*Punus virginiana* var. *virginiana*), pin cherry (*P. pensylvanica*), mountain maple (*A. spicatum*), striped maple (*A. pensylvanicum*), white spruce (*Picea glauca*), black ash, red ash (F. pensylvanica), and white ash (*F. americana*).

Elongation of Black Ash Embryos

Although black ash embryos are morphologically complete at the time of seed dispersal, seeds will not germinate at this time because the embryo is both immature and dormant. The embryo must double in length prior to germination. A number of treatments were evaluated, for a senior undergraduate forestry thesis, to determine which was most effective in promoting embryo elongation.

Two seed lots were used to evaluate the impact of three treatments: 1) presence or absence of the pericarp, 2) duration of incubation, and 3) incubation temperature. The pericarp was removed from one-half of the seeds. Seeds were soaked for 24 hours prior to incubation for 2, 4, 6, 8, 10, 12, and 15 weeks under constant temperatures of 15°C and 20°C in Conviron germination cabinets.

Of the three treatments, pericarp removal had a significant impact on embryo elongation. Incubation for 12 weeks was sufficient for promoting maximum embryo elongation for seeds with no pericarp while 15 weeks was insufficient for seeds with the pericarp intact. Embryo elongation at 15°C was generally greater for both pericarp treatments. A longer period of time was required for maximum embryo elongation at 20°C. The overall results showed that removing the pericarp and incubating the seeds for 12 weeks at 15°C produced an average embryo length of 16 mm. Leaving the pericarp intact and incubating the seeds for 15 weeks at both temperatures produced an average embryo length of 12–13 mm (Horsman 2004).

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WOODY PLANT SEED MANUAL

A number of years ago Frank Bonner, formerly of the USDA in Starkville, Mississippi, undertook the task to completely update "Seeds of woody plants in the United States" published in 1974. Well it has been a monumental piece of work as you can imagine. In the interim, until the new manual is published, completed genera have been made available on the web. The most reliable site to use in order to download these is from the National Tree Seed Laboratory's web site http://www.ntsl.fs.fed.us/ . Follow the link from the main page.

Frank informed me that the manual has been completed and is currently in layout. He expects the galleys to be proofed soon and the book to be published in 2005. This should prove to be a popular publication and I expect all of you will want to purchase a copy for your bookshelf. I know I can hardly wait!

Dale Simpson

UPCOMING MEETINGS

ISTA Ordinary Meeting 2005
April 25-28, 2005 Bangkok, Thailand
Contact: www.seedtest.org

8th International Workshop on Seeds
"Germinating New Ideas"
May 8-13, 2005 Brisbane, Australia
Contact: info@seedbio2005.asn.au

AOSA/SCST Annual Meeting
June 15-22, 2005 Saskatoon, Saskatchewan
Contact: aosaoffice@earthlink.net

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