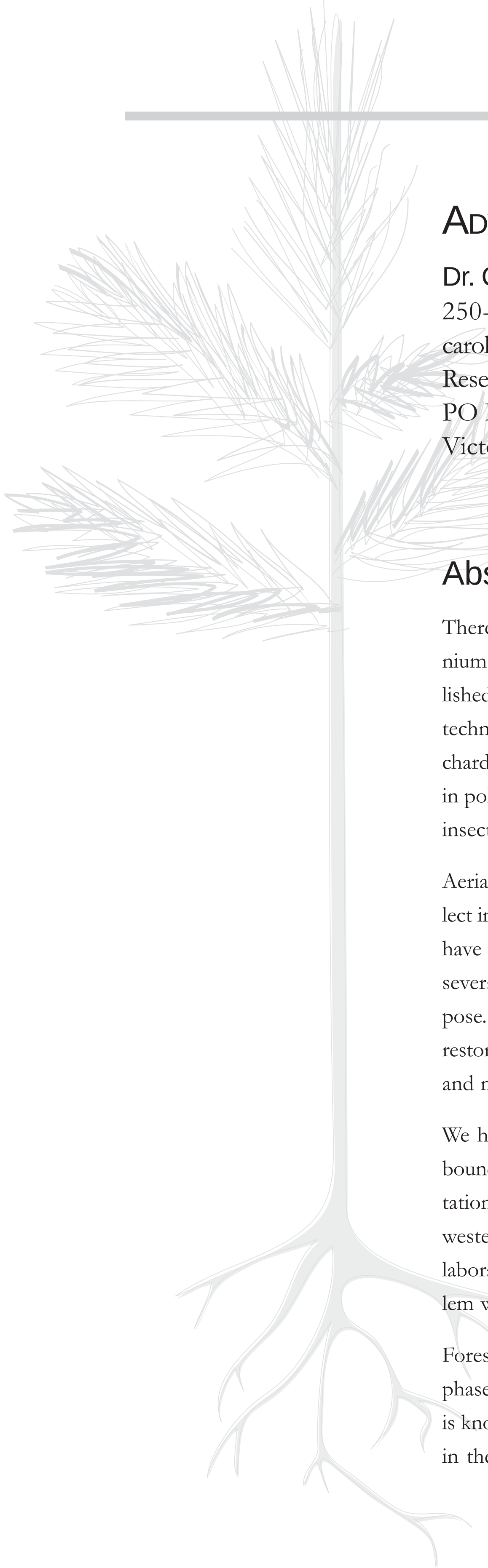

ADVANCES AND CHALLENGES IN SEED BIOLOGY

Dr. Carole L. Leadem
250-387-7038
carole.leadem2gems2.gov.bc.ca
Research Branch, British Columbia Ministry of Forests
PO Box 9519, Stn. Provincial Govt.
Victoria, B.C. V8W 9C2

Abstract

There have been significance advances in seed biology as we enter the new millennium. The production of genetically improved seeds in seed orchards is an established practice throughout North America, and the use of isozyme analysis and other techniques has greatly improved our ability to assess biodiversity of trees in seed orchards and wild stands. We can now directly identify male parents by analyzing DNA in pollen chloroplasts, and researchers are developing antibody markers to determine insect damage to developing seeds.

Aerial helicopter collection of cones in wild stands has improved our ability to collect in remote, formerly inaccessible locations. The higher costs of seed orchard seeds have prompted public and private nurseries to upgrade seedlot quality; variations of several specific gravity separation techniques have shown much promise for this purpose. The greatly expanded use of native species, about which we know little, for restoration of public lands has placed great demands on staff in seed processing plants and nurseries.

We have no lack of challenges for the future. On what basis do we determine the boundaries of seed zones, and how should seed orchard progeny be deployed in plantations? Breaking dormancy remains a problem in species such as yellow-cedar and western white pine, and better testing methods are needed to more closely correlate laboratory tests with nursery germination. Variability between seed sources is a problem with all species.

Forestry (at least in the U.S. and Canada) is currently at an important transitional phase in determining the objectives for managing our forestry resources. The struggle is knowing whether or not today’s objectives will reflect what society wants (or needs) in the future.

Keywords

production, collection, processing, storage, dormancy, germination, testing

Introduction

There have been significance advances in seed biology as we enter the new millennium, and it has been a challenge just to summarize them. Desiring to present a balanced view of these advances and challenges, I sought the opinions of a number of individuals in a variety of seed-related fields. In this synopsis, I have not distinguished between minor or major advancements and challenges, but have relied on upon the judgement of others as to their significance. Often, in practical application, even minor advancements can have significant impacts. The content is somewhat biased to British Columbia, but I think that the trends are similar enough in western parts of Canada and the United States (as well as the National Tree Seed Laboratory) to capture the essence of current practices in seed biology, and the potential challenges for the future.

Seed Production—Advances and Challenges

Wild stands

Production of forest tree seeds is unpredictable--most seeds come from wild plants, and good crops do not occur every year. Collecting seeds is also problematic, because of the size of mature trees, and because crops develop in remote locations. A significant advance in this regard is aerial collection of seeds by helicopter, which has greatly improved our ability to collect in formerly inaccessible locations. This is especially important to species such as *Abies* where crops develop in the top third of crown. There are safety concerns with this method, and it is expensive, due to the cost of helicopter time. However, compared to on-the-ground collection, aerial collection is competitive with other methods, as more bushels can be collected in a shorter time, and seeds can be collected to within 3 days of maturation.

We are beginning to understand the causes of regeneration failure in species such as western larch (*Larix occidentalis*). In British Columbia (B.C.), western larch is at the northern edge of its distribution, and generally a poor seed producer. A study of several wild stands showed a high frequency of empty seeds, probably as a result of pollination failure, and large losses due to predation by rodents and birds. To enhance natural regeneration in larch, it is important that foresters appreciate the reproductive biology of the species, and schedule harvesting and site preparation to coincide with the occurrence of good seed years.

There are still no cost-effective methods for stimulating seed production, or controlling insects and fungal infestations in natural stands, nor can we predict the occurrence of potential crops with any certainty. Most conifers do not produce collectable seed crops every year, with the time between good years varying from 2 to 15 years. Angiosperms characteristically produce seeds annually, but production can vary considerably from year to year. Several attempts have been made to correlate weather variables with good crop years, and dry summers appear to be favourable to initiate floral buds in Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), white spruce (*Picea glauca*), larch, and ponderosa pine (*Pinus ponderosa*) (Eremko et al. 1989).

Seed orchards

Seed orchards and seed production areas have been developed for many economically-important forest species. We are able to manage developing crops with watering, fertilization, and insect and disease control. In yellow-cedar (*Chamaecyparis nootkatensis*), the more favourable conditions in orchards has succeeded in reducing the reproductive cycle from 3 to 2 years!

Currently B.C. is in transition from reliance on seeds from wild stands to seed production in orchards. The amount varies by species, but 34% of seeds now sown in B.C. are from seed orchards. However, seed orchards will not solve all our production problems. In species such as lodgepole pine (*Pinus contorta*), orchards are not meeting the demands for A-class seeds. Lodgepole pine suffers from low seed yield, possibly due to insect predation (*Leptoglossus*), and to poor pollen drop production in dry areas where many orchards are located.

We are also now beginning to appreciate that environmental conditions in orchards may have unforeseen after-effects on the seeds that are produced there. In B.C., Norway, and Sweden, orchards are generally located in milder climates that favor seed production. However, it has been found that trees from northern seed sources that are bred in southern latitudes may produce progeny that are maladapted to the northern environment of their parents. Characteristics such as germination, seedling height and frost hardiness have been shown to be affected by environment in which the parents were bred (Stoehr et al. 1998).

A basic assumption of orchard management is that all parent trees contribute equally to seed crop production, i.e., that all trees have the same ability to produce flowers, and there is random mating among all males and females. However, trees do not flower at the same rate, and some trees flower either earlier or later than the majority of the trees in the orchard. Both these factors have the potential to create genetic bias in orchard crops. Nonetheless, genetic diversity in seed orchards has been found to be as great or greater as that found in wild stands, most likely due to the fact that orchard parents are brought into closer proximity than would occur in the natural stands.

Biochemical monitoring

Many advances have resulted from the application of biochemical techniques to forestry. Isozyme analysis has been primarily applied to identify genetic parents in seed orchards and wild stands. However, because it uses female megagametophyte tissue, isozyme analysis can only directly identify female parent. Recently, by analyzing the DNA in pollen chloroplasts, we can now directly identify male parents. At the present time, polymorphic markers are available for lodgepole pine, Douglas-fir, and white spruce. Other notable developments are the use of antibody markers for seed predators. Dr. John Borden, Simon Fraser University, is using these antibodies to determine the cause of seed losses in orchards. In an intriguing application to forestry forensics, Dr. Eleanor White, Canadian Forest Service, Victoria, B.C. has developed an operational technique to identify rustled logs by matching DNA from stumps with that from the stolen logs.

Genetic deployment

An integral aspect of deployment is defining seed zone boundaries. Other than lengthy testing of phenotypes, one can infer the relative size of seed zones by considering whether the species is broadly or narrowly distributed, or whether the flowers are pollinated by animals or by wind. Although pollen is distributed by wind, and theoretically more widely distributed, pollen does not appear to travel very far in natural stands. *Abies* pollen, for example, is dispersed only 60 m from the parent tree. For the present, at least, it appears that we cannot shortcut the phenotype testing process.

Another issue is the conservation of genetic material beyond the boundaries of naturally occurring populations (ex situ conservation). In B.C., ex situ conservation is not a problem for most conifers, except for minor species such as limber pine (*Pinus flexilis*), whitebark pine (*Pinus albicaulis*), western white pine (*Pinus monticola*), Sitka spruce (*Picea sitchensis*), subalpine larch (*Larix lyallii*), and jack pine (*Pinus banksiana*). Shrubs and hardwood species, however, generally have not received much attention.

In the United States, private companies are deploying by families to manage for particular traits in bare root beds and in plantations. This avoids inter-family competition in the nursery that may lead to culling and loss of genotype from the population. Although there are advantages to such segregation, it does increase seed and seedling costs. Presently in B.C., the official policy on deployment is to maintain a minimum level of biodiversity (measured using a weighted gamete contribution of breeding seed orchard parents).

Seed Processing—Advances and Challenges

Processing conifer and hardwood seeds

Processing methods for most coniferous species forest tree seeds now yield very high levels of filled seeds, free from debris. In general, modified equipment from agriculture continues to be used for seed extraction, and there have been no recent developments in this area. At the National Tree Seed Laboratory, progress has been made in processing hardwood seeds, which are typically fairly low quality, and contain wings and other seed attachments. An agricultural machine for processing grass seeds has been found to effectively break up seed clusters and remove seed attachments. For removing sticks and similar debris, the indent cylinder has been very successful.

Upgrading seed quality

Many existing USDA-FS seed stores are getting older (some are 20 years or older) and declining in quality. Seed owners would undoubtedly welcome upgrading of these stores, even if many seeds were discarded, since they are responsible for storage costs. The higher cost of orchard seeds also has prompted public and private nurseries to upgrade seedlots.

Variations of several specific gravity separation techniques have shown much promise for upgrading seed quality. Most notable is Incubation-Drying-Separation (IDS), a water separation technique developed in Sweden. IDS has not been widely accepted in North America. IDS is effectively for lodgepole and Scots pine seeds but it does not work consistently on other species, probably due to differences in seed coat characteristics. There is considerable interest in extending the IDS technique to more conifers and also to angiosperms, but there has not been much progress to date. The basic IDS technique has been variously modified from the original method, which was to soak dry seeds, then dry them for short periods (8 hours) at room temperature. It is now common practice to separate seeds after stratification. For lodgepole pine and *Abies* species, seeds are separated mid-way through the stratification process.

The sedimentation flume method (also from Sweden) is similar in concept to the IDS specific gravity separation, with the difference that water streams of various speeds are used to effect more precise separation. To my knowledge, there is no direct experience with this method in North America.

Seed Storage—Advances and Challenges

Orthodox and recalcitrant seeds

Depending on their storage behaviour, seeds traditionally have been classified as “orthodox” or “recalcitrant”. Orthodox seeds can be dried to low levels around 5% moisture content (mc), chilled to below freezing (-20 °C), and stored for many years. Most recalcitrant seeds will not tolerate dehydration below 25% and many require 40-75% mc. They can be very sensitive to chilling which causes severe damage, and cannot be stored for only short periods.

The Tree Seed Centre stores seeds of a variety of conifer (about 25 native species) and hardwoods (about 20 native species). All are orthodox in behaviour, and can be stored at 5-10% mc, usually for 10 years or more. The National Tree Seed Laboratory is storing many more species (conifers, hardwoods, shrubs, grasses) than in the past, but most seem to behave in orthodox manner. Some species, such as the high oil seeds of hickory (*Carya*), cannot be stored below 0 °C.

Survival curves are needed for tree seeds, such as those that have been developed for agricultural seeds. The ability to predict storage life has considerable implications for long-term storage and germplasm conservation. Seed survival data are slowly accumulating for orthodox species, both temperate and tropical, and it appears at present that there are little differences in the longevity coefficients among species (except for some oil seeds).

Intermediate seeds

About a decade ago, researchers became aware of a third class that appears to have storage characteristics midway between orthodox and recalcitrant seeds. These are referred to as “intermediate” seeds. Intermediate seeds withstand some dehydration to fairly low mc (some to 10%), but are damaged if dried lower than this. They can be cold stored for short periods, but lose viability over several weeks to months.

Seeds of temperate trees such as oak (*Quercus*), chestnut (*Castanea*), and sycamore (*Platanus*), and seeds with high lipid contents, such as walnut (*Juglans*) and hickory, appear to be intermediate in nature. Most cannot be stored longer than weeks to months without significant deterioration. Some advances in storing intermediate seeds have been made in Europe where protocols have been developed to allow large-scale storage of acorns for 2 to 6 years (Suszka et al. 1996). Using ethylene and gibberellins, non-dormant seeds of beech (*Fagus sylvatica*), cherry (*Prunus avium*), and ash (*Fraxinus excelsior*), can be stored at low temperatures up to 6 years.

Seed Treatments—Advances and Challenges

Seed sanitation

Greater attention is being given to seed sanitation to reduce seed-borne pathogens. In B.C., seeds of all species are soaked in running water prior to stratification. Western larch and Douglas-fir are soaked in hydrogen peroxide at some nurseries to reduce infestations of *Fusarium*. Seedlots are routinely tested for several seed-borne pathogens, and test results (percent contamination) are printed on seed labels to alert nursery growers of potential problems.

Dormancy-Breaking Treatments—Advances and Challenges

Deep or variable dormancy

We have made great strides in improving the stratification of conifers (Edwards 1981), and some European hardwoods (Suszka et al. 1996), which, depending upon the species, can be conveniently grouped into five major dormancy categories (Leadem 1997, Leadem et al. 1997). Some progress has been made with soaking yellow cedar seeds in GA_3 and propanol prior to stratification, reducing the chilling requirement from three to two months (Dr. Alison Kermode, Simon Fraser University). Species such as southern pines [longleaf (*Pinus palustris*), slash (*Pinus elliotti*) and loblolly (*Pinus taeda*)], that were previously considered to be non-dormant, seem to respond well to stratification, sometimes as long as 60 days.

Seed moisture

We have gained a better appreciation of the role of seed moisture. The control of seed moisture during stratification not only results in improved germination speed and completeness (Jones and Gosling 1994), but allows seeds to be stored in a non-dormant condition until used. Reducing the seed moisture enabled storage of seeds in the nondormant state for 9 months for ponderosa pine and Douglas-fir (Danielson and Tanaka 1978), and for one year for *Abies amabalis*, *A. grandis*, and *A. lasiocarpa* (Edwards 1980). Reduction of moisture content is an essential element of the statification-redry technique for *Abies* seeds (Edwards 1981). Yellow cedar, on the other hand, seems to require very high moisture environments during stratification, even small amounts of standing water (D.G.W. Edwards, pers. comm. 1996).

Seed Testing—Advances and Challenges

Biochemical Indicators

In regard to traditional germination testing; we appear to be stuck in time. On the other hand, current biochemical research is offering new tools to assess the efficacy of seed treatments without the need for germination tests. In intact seeds, the physical restraint of the embryo root cap, megagametophyte, testa, and nucellus present major obstacles to germination. Degradation of these tissues is an essential event in early germination. Several enzymes that degrade cell walls, pectin methyl esterase and malate dehydrogenase, potentially could be developed as germination indicators (Dr. Alison Kermode, Simon Fraser University; Dr. Bruce Downie, University of Kentucky). Similarly, late embryogenesis abundant (LEA) proteins, a group of compounds appearing in the final stages of seed development, have been proposed as seed maturity indicators (Dr. Santosh Mishra, University of Victoria).

Correlation between laboratory tests and nursery performance

Better methods are needed to improve the correspondence between laboratory germination and nursery performance, especially for yellow-cypress and western white pine. Several approaches have been proposed, such as altering test conditions (i.e., comparison of double tests at warm and cold temperatures), or developing mathematical correction factors.

Seed viability and vigor

We still need quick and reliable assessment methods that are suitable in a production environment requiring large numbers of tests. Subjective seed quality tests, such as tetrazolium chloride (TTZ) staining, and X-ray analysis are difficult to standardize, and many analysts use them reluctantly. X-ray analysis is very rapid, but equipment for taking and developing X-rays is expensive. Tetrazolium chloride (TTZ) is a sound method based on physiological and anatomical characteristics, and inexpensive to conduct, but it is often avoided because it is time consuming and difficult to learn.

Vigor tests attempt to predict seed performance under various conditions. Vigor tests are potentially a more sensitive indicator of performance because vigor declines more rapidly than viability. How to measure seed vigor has remained a challenge for many years, due to our inability to clarify what seed vigor is. Various indices have been proposed, but with little success: germination rate, germination under very high or low temperatures, and measuring respiratory activity in the embryonic axis and in whole seeds.

Nursery Production—Advances and Challenges

Nursery growers have always been innovative and practical in their approach to problems. One grower is using an in apartment-size washer-spin dryer to centrifuge seeds and remove free water from the seed surfaces prior to sowing. Seeds are placed in a mesh bag, and centrifuged about one minute. Afterwards, seeds require only about 2-3 minutes of air-drying on a bench top to become free-flowing and easy to handle.

The higher costs of orchard seeds have prompted private B.C. nurseries to find ways to enable them to sow one seed per cavity. Some nurseries are using pneumatic air separators to upgrade seeds after stratification, capitalizing on the fact that stratification increases the weight differential between filled and damaged or empty seeds. In another B.C. nursery, fluid drilling, a method in which single pre-germinated seeds are placed into a gel medium, is being used to mechanically sow western larch and yellow pine. Several nurseries are maintaining their greenhouses at a constant 30 °C for the first 2 to 3 weeks after sowing to achieve much faster germination, and a more uniform crop. The additional heating expense has shown to be cost-effective.

Nursery practices and biodiversity

Maintaining biodiversity is an essential tenet of reforestation, but recently it has been shown that some nursery practices can alter the genetic representation of seedlings. An examination of isozymes from plantation trees grown from seed orchard seeds showed reduced heterozygosity (genetic diversity) in the plantation trees. The reduction was not due to the mortality of the trees in the plantation, but resulted from culling practices in the nursery. The reason is that seed lots do not germinate at the same rate, and later germinants tend to be smaller. Later germinants are more likely to be culled during thinning, with the resultant loss of their genetic contribution to the population.

Native (non-tree) Species—Advances and Challenges

Seed production

A significant trend in the past decade is the increased use of native, non-tree species for the restoration of public lands. The National Tree Seed Laboratory is processing and storing native plants needed for revegetation and restoration of roads, while in California, native species are used in the reclamation of mines and other sites. The individual species vary, but on average, the Moran Reforestation Center is dealing with about 150 different species each year. They include conifers, hardwoods, shrubs, grasses, perennials, annuals, and sometimes even bulbs. In British Columbia, native species, mostly wildflowers, remain a minor component of seed dealer collections and sales. Seed characteristics (storage, dormancy requirements, germination conditions) of native species are virtually unknown.

Native non-tree species typically produce seeds each year, so there is always a collectable crop. We know little, however, about the details of natural reproductive cycles. The reproductive strategies of many species appear to be spread over time and space, e.g., the patterns observed in desert wildflowers, wherein some plants flower every year, but the species producing the flowers are not always the same.

Seed processing and storage

Starting with a blank slate, seed processing and nurseries staff made great progress. Native seed collections are difficult to clean; characteristically, seeds are small with low numbers of filled seeds. Although screens are fairly effective for removing debris from seeds of native species, other standard methods such as air separation and indent cylinders have not been very useful. Storability of native non-tree species is similar to that of tree seeds, falling into orthodox (store), recalcitrant (don't store) or intermediate (short storage) storage classes.

Seed dormancy treatment

In California, boiling water dips or hot water soaks are used to release dormancy of hard-coated seeds. The Moran Reforestation Center has totally eliminated the use of acids because of the dangers associated with chemical use. Mechanical scarification (abrasion) is also avoided due to the potential for seed damage and introduction of pathogens. Outdoor stratification seems to work well for many native species. Seeds are sown directly into containers in the fall, watered well, and stored in unheated greenhouses over the winter. The containers are checked periodically to ensure that the soil remains moist. A similar practice is used for native species in B.C.

In summary, the biggest constraint with native species is Biology vs. Bureaucracy; i.e., seeds don't always cooperate when they are needed. The challenge is how to develop prescriptions when species requirements are totally unknown. One possible approach is what I refer to as the "silver platter" (Leadem 1997), which is to make an educated guess based on seed characteristics such as coat appearance and habitat of origin (i.e., looks like…., so treat like …..). In the interim, sharing anecdotal information about native species can help compensate for our lack of knowledge. This could be accomplished in a variety of ways--public presentations, newsletters, or even a cooperative information databank.

Field Germination—Advances and Challenges

Progress has been made in understanding seed biology under natural conditions. More researchers are incorporating field germination studies into integrated research projects, and developing techniques for monitoring seed production, losses, and germination under field conditions. Such projects will ultimately reveal the complexity of the factors (and their interactions) that influence germination in the field. The challenge, as always, is how to secure long-term commitment (money, resources) for field studies, and how to integrate new knowledge into forest management practices.

Extension and Communications—Advances and Challenges

For many practical foresters, tail-gate, one-on-one, extension is still the preferred method of communication. Many practitioners don't read technical papers or use the WWW to access information, but they still need to consult with knowledgeable people. This is becoming increasingly difficult, because people have less time (and money!) to travel to technical meetings. In future, video conferencing may be more widely used to exchange information between small groups with common interests.

In the realm of the printed word, we have been more successful in getting the message out. Newsletters such as the Canadian Tree Improvement Association Seed and Seedling Extension Newsletter, and IUFRO Tree Seed Working Group Newsbulletin keep readers informed about reforestation throughout the year. Nursery Notes, published by the USDA-FS, conveys technical information in a form more easily assimilated by practicing foresters and nursery workers. Proceedings of technical meetings also disseminate highlights quickly to a wide audience. Every effort should be made to retain these important communication vehicles.

Summary--Future challenges

We have no lack of challenges for the future, but the most important challenge is deciding where we are headed. What traits do we want to select, and what do we manage for? Should we select for rapid tree growth, or to maintain biodiversity? In northern latitudes, frost hardiness may be a more important trait for selection. Variability between seed sources is a problem with all species, and contributes to the lack of success of many treatments and test methods. At present there is no way a priori to determine what specific seed sources require. This problem may not have a solution, for even half-sib material in seed orchards responds quite variably to seed treatments.

Forestry (at least in the U.S. and Canada) is currently at an important transitional phase in determining the objectives for managing our forestry resources. Although we have made many advances in the last decade, our present challenge is knowing whether or not today's objectives will reflect what society wants (or needs) in the future.

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References

Danielson, H.R. and Y. Tanaka. 1978. Drying and storing stratified ponderosa pine and Douglas-fir seeds. For. Sci. 24: 11-16.

Edwards, D.G.W. 1980. Storage on prechilled *Abies* seeds. In Proc., IUFRO International Symposium on Forest Tree Seed Storage, September 23-27, 1980, Petawawa National Forestry Institute, Chalk River, Ont. Edited by B.S.P. Wang and J.A. Pitel. Canadian Forestry Service, Ottawa, Ont. pp. 195-203.

Edwards, D.G.W. 1981. Improving seed germination in *Abies*. Proc. Int. Plant Propag. Soc. 31: 69-78.

Eremko, R.D., Edwards, D.G.W., and D. Wallinger. 1989. A guide to collecting cones of British Columbia conifers. Canada/British Columbia Forest Resource Development Agreement Report 055. Publ. British Columbia Ministry of Forests, Research Branch, Victoria, B.C. V8W 3E7. 114 p.

Jones, S.F. and P.G.Gosling. 1994. "Target moisture content" prechill overcomes the dormancy of temperate conifer seeds. New For. 8: 309-321.

Leadem, C.L. 1997. Dormancy—Unlocking seed secrets. In: Landis, T.D., Thompson, J.R., tech. coords. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-GTR-419. Portland, OR: US. Department of Agriculture, Forest Service, Pacific Northwest Research Station: 43-52.

Leadem, C.L., Gilles, S.L., Yearsley, H.K., Sit, V., Spittlehouse, D. and P.J. Burton. 1997b. Field studies of seed biology. Land Management Handbook 40, British Columbia Ministry of Forests, Victoria, B.C. 196 p.

Stoehr, M.U., L'Hirondelle, S.J., Binder, W.D., and J.E. Webber. 1998. Parental environmental aftereffects on germination, growth, and adaptive traits in selected white spruce families. Can. J. For. Res. 28 (3): 418-426.

Suszka, B., C. Muller, and M. Bonnet-Masimbert. 1996. Seeds of forest broadleaves. From harvest to sowing. Trans. by A. Gordon. Institute National de la Recherche Agronomique, Paris. 297 p.