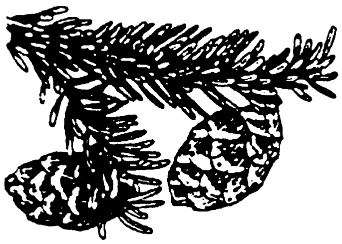
CANADIAN TREE IMPROVEMENT ASSOCIATION/
ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES



Tree Seed Working Group

NEWS BULLETIN

No. 46 December 2007

SEED TRANSFER

THIS ISSUE AT A GLANCE

CHAIR'S 'ARMCHAIR' REPORT

Happy Holidays to all. As 2007 draws to a close we can look forward to a wide variety of meetings and extension events in 2008. The CTIA will be having its meeting in Quebec City August 25th to 28th with a special tour of the Berthier nursery and tree seed centre on Sunday August 24th just before the CTIA. Please see Fabienne Colas's note on the topic and look for updates on the conference web site: http://www.iufro-ctia2008.ca/index.php

In January, our Northern Silviculture Committee in BC is addressing "Changing Northern BC Forests – Silvicultural Challenges and Implications" and this meeting will explore many of the options and information available regarding strategies to deal with the post-Mountain pine beetle and climate change scenarios that this portion of BC needs to deal with now. Nationally, a meeting was held in November in Sault Ste. Marie "Adapting to Change: Managing Tree Seed Under an Uncertain Climate" and Kathleen Brosemer has provided an overview of the meeting and indicated that proceedings should be available in the spring of 2008.

We at the BC Ministry of Forests and Range Tree Seed Centre are celebrating 50 years in operation this year and are planning several activities for clients, neighbours, and staff. I am also hoping to incorporate a technical session in the mix and this will likely take place prior to the next News Bulletin. We will be advertising heavily in BC, but if others would like to be made aware of upcoming activities, please send me an e-mail and I'll include you in our announcement distribution list.

Last, but certainly not least is the upcoming Tree Seeds 2008 meeting with the theme of 'Trees, Seeds and a Changing Climate" that is being cosponsored by the International Union of Forest Research Organizations (IUFRO), International Tree Foundation, and the International Seed Testing Association (ISTA). The meeting will be held September 22–25 at the Kew Royal Botanical Gardens and University of Sussex. For more information, please see the following webpage http://www.kew.org/msbp/tree-seeds-2008/ . This should be an interesting and exciting meeting and a great opportunity to discuss tree seed issues among our widespread and diverse colleagues.

Climate change is factoring more prominently in all of our lives and the decisions we make regarding our future forests. In BC, there is lots of momentum to put together a plan for moving forward with the basic question of how do we regenerate our forests in light of climate change. See Greg O'Neill's article for his perspective on moving forward with seed transfer. Myself, I've been quite involved in the basic questions regarding the provincial seed supply and especially with lodgepole pine where the mountain pine beetle is liquidating our canopy seed bank produced over many decades. For more information regarding seed supplies and the Mountain pine beetle outbreak, see the following bulletins at this link: http://www.for.gov.bc.ca/hti/pinebeetle/index.htm

Thank you to everyone who has contributed articles to this edition. I was hoping that this edition could serve as a summary of seed transfer systems or protocols in place across the country, but you don't always get what you want. For those interested in the BC perspective, Cheng Ying and Alvin Yanchuk have put together an excellent review of the current system in BC – " The development of BC's tree seed transfer guidelines: purpose, concept, methodology, and implementation" in Forest Ecology and Management 227: 1–13. The other selected reference is the paper by Andreas Hamann and Tongli Wang "Potential effects of climate change on ecosystem and tree species distribution in BC" in Ecology 87: 2773–2786. Both are worthwhile reads, especially if you can get a colour copy of the latter. Our next News Bulletin will be an open forum for seed topics, so anything goes – please contribute. That's it for 2007 - wishing everyone the best and look forward to seeing many of you in 2008.

Dave Kolotelo
Chairperson

EDITOR'S NOTES

Climate change is a topic that we are being continually bombarded with or maybe more appropriately beaten into our thick skulls. There are lots of things that we can do to try to mitigate climate change but forest managers are being faced with having to manage the forest now so that in the future it will continue to supply all the products that are needed. This is not a trivial challenge. Just look at the variation among the outputs from the various climate change models. Which scenario might be the closest one to the future? On the other hand, managers need to manage and not incorporating climate change into planning is probably worse than making an informed decision and trying something.

There are some basic 'things' that can be done such as seed transfer. That is the theme of this News Bulletin and several articles address this. This idea is definitely not new and we are probably faced with having to transfer seed further than we are comfortable with. So, I hope that you will find something of interest that will stimulate your intellect.

I wish you all a very Merry Christmas and a prosperous New Year!

Dale Simpson
Editor

TREE SEED WORKING GROUP

Chairperson
Dave Kolotelo
BC Ministry of Forests and Range
Tree Seed Centre
18793 - 32nd Avenue
Surrey, BC V3F 0L5
Tel.: (604) 541-1683 x 228
Fax.: (604) 541-1685
E-mail: Dave.Kolotelo@gov.bc.ca

Editor
Dale Simpson
Natural Resources Canada
Canadian Forest Service
Atlantic Forestry Centre
P.O. Box 4000
Fredericton, NB E3B 5P7
Tel.: (506) 452-3530
Fax.: (506) 452-3525
E-mail: Dale.Simpson@nrcan.gc.ca

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

ONTARIO TREE SEED CONFERENCE A SUCCESS

A conference this past November, “Adapting to Change: Managing Tree Seed Under an Uncertain Climate” drew about 80 people to Sault Ste. Marie, from as far away as New Brunswick, British Columbia and the Great Lakes States. The talks were thought-provoking and overall drew many compliments from attendees. While the papers and discussion were framed in a North American context, it is hoped that the various jurisdictions, especially Ontario, will incorporate some of the conference conclusions into action plans and policies yet to be developed.

Topics and speakers included:

- Climate Trends and Projections: Implications for Managing Tree Seed? - Dan McKenney, Canadian Forest Service
- Adaptation, Migration or Extirpation: Effects of Climate Change on Native Tree Populations - Sally Aitken, University of British Columbia
- Genetic Effects of Rapid Climate Change After the Last Ice Age - Jason McLachlan, Notre Dame University
- Brave New Breeding Zones - Greg O’Neill, B.C. Ministry of Forests and Range
- Comparison of Seed Transfer Function and Focal Point Seed Zone Approaches in Present and Future Climates - Bill Parker, Lakehead University
- Natural Migration, Assisted Migration, and Climate Change. What Will Nature Do and What Will We Have to Do For Nature? - Dan McKenney, Canadian Forest Service
- The Reserve Selection Problem in an Environment of Uncertain Climate Change - Kevin Crowe, Lakehead University
- Coordinating Gene Conservation for Climate Change Action Plans: A Role for CON**FOR**GEN - Judy Loo, Natural Resources Canada
- Developing a Comprehensive Change-Management Plan - Jack Woods, Forest Genetics Council of British Columbia

Highlights from the talks, question and answer sessions, a pre-conference survey of attitudes and practices, and the interactive session at the conference are being compiled into a Proceedings which will be co-published by the two conference sponsors. Ontario Ministry of Natural Resources Climate Change Initiative will publish the proceedings as part of its climate change series and Forest Genetics Ontario will make the proceedings available on its website, www.fgo.ca, as a pdf file to download. It’s expected that the proceedings will be available in the spring of 2008.

Some of the conference highlights include:

- There was consensus among the speakers, that there is a huge amount of uncertainty associated with climate change, and taking action is a risky business. However, failing to act may be the worst thing we could do. Many agreed that actions should start small (e.g., start slowly shifting seed zones), give plenty of warning, and monitor effects of changes. One speaker said, "In science it's rare to have the opportunity to take action before the train wreck. Let's take advantage of it."

- The threat of species extinction is real. In B.C., all of the climate habitat of subalpine larch (*Larix lyallii*) is predicted to disappear by the year 2100 under the most common global circulation model scenarios. However, Ontario will likely lose fewer species than many other jurisdictions in North America because of relatively little elevation change and other factors. Many Ontario species have southern provenances in the US which may be adapted to the climates that Ontario will experience in the future.

- Suitable climate habitats for tree species will change, but that doesn't mean tree species will follow at the same pace or at all. When tree species migrate, they must compete with species already established in that location, whose ability to compete may or may not be compromised by climate change. Also, today's ecological zones will have limited utility in the future.

- The issue of assisted migration has enormous implications. How do we decide whether to assist tree species to migrate as the climate changes? Will our objective be conservation or economics? What about phenology - day length is important to optimal growth and to reproductive success, and that will not be changing with the climate. In Ontario we may be wise to look at moving species east-west as well as north, as we have strong east/west climatic gradients due to the influence of Hudson Bay. In addition, will we need to assist the entire ecosystem community in moving, including soil organisms which may be essential yet don’t occur in the new climate? And when does an assisted migrant become an introduced exotic? We have no guidelines for making good decisions, yet people are beginning to make these decisions without them.

- A new term for some attendees was

epigenetics, the heritable changes in gene function that occur without the DNA changing (maternal effects). Epigenetics research could help reveal new ways to manage tree seed and forests in general to adapt better to climate change. According to Ontario's research geneticist Pengxin Lu, "In forest trees, significant epigenetic effects (sometimes referred to as maternal environment effects) were reported from a study of Norway spruce (*Picea abies*), in which seeds collected from ramets of the same clone growing in a northern orchard and a southern orchard performed/adapted differently on the same planting site. More research is needed because the existence or absence of epigenetic effects has important implications to the interpretation of observed differences among tree populations in provenance trials (e.g., are the differences due to genetic differences or due to the differences of the maternal environments?) and the locations of seed orchards in tree improvement."

- Jason McLachlan from the University of Notre Dame gave a fascinating talk on how tree species have moved northward in eastern North America since the last ice age and how fossil pollen data appear not to reliably record range dynamics. He also provided an excellent outline of the pros and cons of assisted migration.

- Today we have little policy for managing forests under climate change. British Columbia, who expects to have greater changes in climate than other parts of Canada due to elevation and coastal effects, is planning to have new policy over the next 4–5 years. It was pointed out that it is important to be cautious because policy changes could lead to huge costs for those managing forestry on the ground. But there is also a cost of not changing policy. B.C.'s Jack Woods gave an excellent talk on planning for change; among his points: Inertia is a problem when it comes to realigning resources and any plan will have errors but having no plan will result in more errors

- Ecosystems are complex and this complexity must be factored into our decision making. Many participants called for a decision support system to help guide our efforts. There is a great need to ensure that the many variables at play - those we have information on and those we don't - are included in decisions.

Last but not least was the talk by Sean Thomas from the University of Toronto, who at the evening banquet gave a fascinating and very entertaining tour through the history of clashes between those who wish to conserve and those who wish to make use of forest products. It had been a long day, yet after his presentation people stayed at the tables and discussion was lively.

Kathleen M. Brosemer
Forest Genetics Ontario
510 Queen Street East, Suite 24
Sault Ste. Marie, ON P6A 2A1
E-mail: kbrosemer@fgo.ca

SEED TRANSFER 101

Seed Transfer and Maladaptation

Trees get homesick! Despite the large distribution range of most tree species, the growth and health of individual trees can be greatly affected when they are moved to new climates (or the climate changes). Movement can result in maladaptation which can manifest as cold injury, slow growth, snowbreak, low wood density, large branches, stem defects, pest attack, disease, etc. - which reduces recoverable volume and wood quality (Campbell 1979; Zobel and Talbert 1984).

Populations of forest trees become adapted to their native environments through natural selection. Over the generations, populations of trees synchronize their seasonal growth patterns with their local growing season conditions (e.g., average date of first frost) (Simpson 1994). When trees are transferred to a colder environment (i.e., upward in elevation or poleward) than that in which they evolved, they may grow for a longer portion of the year because they are genetically 'programmed' to do so. However, in doing so, they may suffer cold injury in those years when early fall frosts or late spring frosts occur. In contrast, trees transferred from colder climates to warmer environments are more conservative in their growth timing than 'local' trees from warm environments, predisposing the transferred trees from cold environments to suppression by local populations.

Similarly, trees from wetter environments typically grow longer into the summer than trees from drier environments. When planted in environments that are drier than those in which they evolved, their genetic programming causes them to grow longer into the summer than local trees from dry environments. Consquently, the transferred trees from wet environments will be taller than the local trees adapted to the dry environment. However, movement beyond a given precipitation distance will result in cold injury as the transferred trees will be slow to develop sufficient cold hardiness in the fall. Trees are also adapted to the daylength in which they evolved; moving seedlings to a different latitude from that in which they originated can affect their timing of budset and result in cold injury (Campbell and Sorensen 1973).

Tree populations are also adapted to many biotic factors, often displaying increased resistance or tolerance to those insects and pathogens to which they are most frequently exposed (Ying and Liang 1994; Alfaro et al. 2000; O'Neill et al. 2002). For example, movement of populations to warmer environments results in a greater incidence of pitch moth attack and gall rust in lodgepole pine (Ying and Liang 1994), *Meria* needle cast in western larch (Rehfeldt 1992), and white pine weevil attack in Sitka spruce (Ying 1991).

Identifying Appropriate Seed Transfer Distances

Two features characterize seed transfer: calculation of maximum seed transfer distances and the system used to guide or regulate the transfer of seed. The following section summarizes the two most common methods used to calculate maximum seed transfer distances – i.e., the "genetic similarity" and "transfer function" approaches.

Genetic similarity

In most locations, natural selection has sculpted the trees' genetic composition such that the local provenances are the fastest growing and healthiest at a site. In addition, trees from similar climates tend to be similar genetically. Consequently, planting trees that come from climates similar to the planting site helps to ensure good growth and health.

The genetic similarity approach to determining maximum seed transfer distance seeks to ensure that trees from the seed source location and the planting site are genetically similar. Maximum seed transfer distances are calculated as the geographic or climatic distance between populations that differ genetically by a specified limit. For example, maximum transfer distances are determined from the overlap of the frequency distributions of phenotypes of seed sources from different climates ("mismatch index") (Campbell 1986), or by calculating the climatic or geographic distance needed to distinguish between populations based on their average phenotypes (the population "least significant difference") (Rehfeldt 1994; Parker and van Niejenhuis 1996).

Data for these analyses are obtained from provenance tests that are usually conducted in nursery beds to provide rapid and inexpensive results. The analysis can exploit a wide range of adaptive traits to detect subtle differences among populations. However, its main limitation is that it can be difficult to quantify growth impacts associated with these calculations and results are subject to the size and design of the provenance test. Furthermore, patterns of variation within and among populations in nursery tests may not be the same as those observed in older field tests.

Transfer functions

A second approach to determining maximum seed transfer distances uses transfer functions in which growth or other adaptive traits are plotted as the response variable, and transfer distances (i.e., the value of the climatic or geographic variable of the planting site minus that of the seed source) as the independent variable (Raymond and Lindgren 1990; Rehfeldt et al. 1999). The resulting scatterplot usually shows a peak close to the zero transfer distance, suggesting that seed from locations climatically or geographically proximal to the plantation grows fastest.

By relating growth to transfer distance, the impact of seed transfer can be directly interpreted from the transfer function and transfer distances that result in acceptable growth impacts can be readily identified. However, tests should be sufficiently old (e.g., approximately 15 years of age) to ensure that trees have been exposed to the range of climates expected at each test site, otherwise growth impacts associated with seed transfer may be under-estimated (Ying and Yanchuk 2006). The main limitation to this approach is the high cost of establishment and maintenance of long-term field tests.

Transfer curves sometimes suggest that populations transferred a short distance will out-perform local populations at a given site. These situations may represent an evolutionary lag in which the best adapted populations for a site have not had sufficient time to migrate or adapt to that site since the last glaciation, or they may reflect recent climate change (Namkoong 1969; Rehfeldt et al. 1999; Wang et al. 2006). In these cases, assymetric transfer guidelines are appropriate. For example, one may wish to limit seed movement to locations 2°C (mean annual temperature) colder or 3°C warmer than the seed source location (Fig. 1).

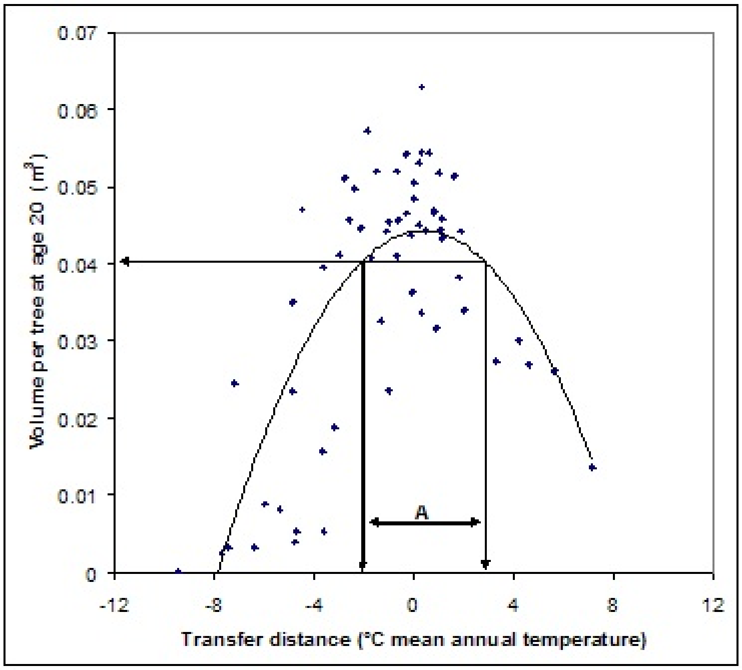


Figure 1. Transfer function showing a maximum seed transfer distance (A). Negative transfer distance indicates seed moved to colder locations. Seed moved to sites 2°C mean annual temperature (MAT) colder or to sites 3°C MAT warmer than the seed source location can be expected to yield tree volumes 90% of that expected of the most productive provenance.

Seed Transfer Systems

Fixed Zones

Seed transfer systems can be categorized into two main approaches. Fixed systems attempt to delineate jurisdictions into environmentally or genetically uniform seed zones – areas within which seed can move freely, but across which seed may not move. Hence, the label 'discrete' is often applied to these systems. The recurrence of similar environments across the landscape often results in fixed zones being non-contiguous.

Zone delineation is performed on the basis of geographic (latitudinal, longitudinal, or elevational) bands (e.g., from 600 to 1000 m elevation) or climate bands (e.g., from 3 to 5°C mean annual temperature), or some combination of these parameters, with band widths equal to maximum seed transfer distances calculated using one of the two methods described above. Fixed zones can also be delineated on the basis of ecosystem classification units, if the climatic widths of ecosystem units are similar to the calculated maximum seed transfer distance. When some environments are more frequent than others in a jurisdiction, use of narrower band widths for the most frequent environments can minimize total transfer distance, and therefore minimize total maladaptation (O'Neill and Aitken 2004).

Fixed zone systems are easy to implement and use. Transferability of seed from any location can be determined simply by consulting a seed zone map. However, the inability to move seed located near a zone boundary into an adjacent zone reduces seed deployability to half of that of floating seed transfer systems (see below).

Floating Zones

Floating zone (a.k.a. focal point) seed transfer systems use maps of continuous geographic or climatic variables, or contours of estimated genetic variation to identify a unique deployment zone for each seedlot, or a unique seed procurement zone for each planting site. Ecosystem units do not lend themselves to use in the floating zone system because they are seldom delineated on quantitative variables. Deployment (or procurement) zones identify areas that are genetically (Parker and van Niejenhuis 1996), geographically, or climatically (McKenney et al. 1999) similar to the seed source (or planting) location. Where maximum seed transfer distances are applied symmetrically (e.g., seed can be moved to locations either 1.5°C MAT warmer or colder), the deployment and procurement zones are congruent and delineate the area bounded in both directions from the seed source or planting location by the maximum seed transfer distance.

Floating zone systems have several advantages over fixed zone systems. Foremost, deployability (the number of hectares to which each seedlot can be transferred) is twice that of the fixed zone system because seed can be moved the full maximum seed transfer distance in either direction from the seed source location, whereas in the fixed zone system, total zone width must be no greater than the maximum seed transfer distance. Second, in a floating zone system, asymmetric transfer distances can be implemented easily as part of a facilitated migration program to help ensure that planted trees are well-adapted throughout the rotation. (In this situation, deployment and procurement zones for a common location are not congruent.) Facilitated migration cannot be applied in a fixed zone system because seed cannot be moved outside of each fixed zone.

The main disadvantage of the floating zone system is that accurate identification of deployment or procurement zones is difficult to accomplish without a geographic information system and each seed source or planting location requires delineation of a unique deployment or procurement zone.

Many seed transfer systems, both fixed and floating, delineate zones based on geographic variables, either for simplicity of use, or because of the absence of a fine resolution climate model. Where geographic and climatic variables are not well correlated, some acceptable transfers may not be well adapted and some unacceptable transfers may be well adapted.

References

Alfaro, R.I.; Lewis, K.G.; King, J.N.; El-Kassaby, Y.A.; Brown, G.; Smith, L.D. 2000. Budburst phenology of Sitka spruce and its relationship to white pine weevil attack. For. Ecol. Manage. 127: 19–29.

Campbell, R.K. 1979. Genecology of Douglas-fir in a watershed in the Oregon Cascades. Ecology 60: 1036–1050.

Campbell, R.K. 1986. Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. Silvae Genet. 35: 85–96.

Campbell, R.K.; Sorensen, F.C. 1973. Cold acclimation in seedling Douglas-fir related to phenology and provenance. Ecology 54: 1148–1151.

McKenney, D.W.; Mackey, B.G.; Joyce, D. 1999. Seedwhere: a computer tool to support seed transfer and ecological restoration decisions. Environ. Model. Software 14: 589–595.

Namkoong, G. 1969. Nonoptimality of local races. Pages 149–153. *In* Proc. 10th South. Conf. For. Tree Imp. Texas A&M University Press, College Station, Texas.

O'Neill, G.A.; Aitken, S.N. 2004. Area-based breeding zones to minimize maladaptation. Can. J. For. Res. 34: 695–704.

O'Neill, G.A.; Aitken, S.N.; King, J.N.; Alfaro, R.I. 2002. Geographic variation in resin canal defences in seedlings from the Sitka spruce x white spruce introgression zone. Can. J. For. Res. 32: 390–400.

Parker, W.H.; van Niejenhuis, A. 1996. Regression-based focal point seed zones for *Picea mariana* from northwestern Ontario. Can. J. Bot. 74: 1227–1235.

Raymond, C.A.; Lindgren, D. 1990. Genetic flexibility – a model for determining the range of suitable environments for a seed source. Silvae Genet. 39: 112–120.

Rehfeldt, G.E. 1992. Breeding strategies for *Larix occidentalis*: adaptations to the biotic and abiotic environment in relation to improving growth. Can. J. For. Res. 22: 5–13.

Rehfeldt, G.E. 1994. Adaptation of *Picea englemannii* populations to the heterogeneous environments of the Intermountain West. Can. J. Bot. 72: 1197–1208.

Rehfeldt, G.E.; Ying, C.C.; Spittlehouse, D.L.; Hamilton, D.A., Jr. 1999. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation. Ecol. Monogr. 69: 375–407.

Simpson, D. 1994. Seasonal and geographic origin effects on cold hardiness of white spruce buds, foliage and stems. Can. J. For. Res. 24: 1066–1070.

Wang, T.; Hamann, A.; Yanchuk, A.; O'Neill, G.A.; Aitken, S.N. 2006. Use of response functions in selecting lodgepole pine populations for future climates. Global Change Biol. 12: 2404–2416.

Ying, C.C. 1991. Genetic resistance to the white pine weevil in Sitka spruce. BC Min. For., Res. Note.

Ying, C.C.; Liang, Q. 1994. Geographic pattern of adaptive variation of lodgepole pine (*Pinus contorta* Dougl.) within the species' coastal range: field performance at age 20 years. For. Ecol. Manage. 67: 281–298.

Ying, C.C.; Yanchuk, A.D. 2006. The development of British Columbia's tree seed transfer guidelines: purpose, concept, methodology, and implementation. For. Ecol. Manage. 227: 1–13.

Zobel, B.J.; Talbert, J.T. 1984. Applied Tree Improvement. John Wiley and Sons, New York.

Greg O'Neill
Ministry of Forests and Range
3401 Reservoir Road
Vernon, BC V1B 2C7
E-mail: Greg.ONeill@gov.bc.ca

SEED TRANSFER IN QUEBEC

The main concern for tree breeders and reforestation managers is to provide seedlings of good genetic and morphological quality, that are well adapted to a variety of growing conditions, so their genetic potential can be realized. To achieve this goal, the Ministère des Ressources naturelles et de la Faune du Québec (MRNF) has established seed transfer rules across the province, that take into account the genetic quality of the material.

Since 1985, Quebec has established seed transfer rules for every species reforested, using primarily the hierarchical system of ecological classification (Thibault 1985; Saucier et al. 1998). In order to meet reforestation needs of a specific region, preference was given to transfers within the same ecological unit moving to the largest ones. Maximum limits were fixed at 300 m elevation, 0.5 degrees latitude to the south and 1 degree latitude to the north. These rules still apply to unimproved sources, which represent 20% of the seedlings used for reforestation.

Over the years, studies on patterns of genetic variation were conducted by geneticists for the most important conifer species. These studies led to the development of mathematical models for connecting patterns of variation observed between sources with geographic coordinates of their origin as well as variables representing the climatic conditions of the reforestation sites (Mátyás and Yeatman 1992; Li et al. 1997a, b; Beaulieu et al. 2004). Variables such as latitude, longitude, elevation, aridity index, vapor pressure deficit, precipitation, and number of degree-days are considered in the models. Software was also developed to automate the calculation of risk associated with the transfer of a provenance within a region. Using Arcview 3.2 in conjunction with ecological region boundaries, a map is created that represents the region in which a specific source can be used and the risk associated to every location on the map. These are powerful tools to guide seed transfer decisions in Quebec (Beaulieu et al. 2003); they were used to refine seed zones for first-generation seed orchards, primarily established in the 80's. For second-generation seed orchards, seed zones correspond to breeding zones, as defined by tree improvement programs.

While a seed zone is determined for every seed source, the notion of risk associated to that seed zone could still be interpreted differently according to the reforestation manager in each region. It was thus necessary to establish standards for the management of risk. Since 1999, the MRNF has set up management information system databases called "SEMENCES" (for seeds) and "PLANTS" (for seedlings), to which every manager has access. This software is the main tool for managing the overall operations, from cone collection to seedling production to reforestation.

The next challenge is to incorporate impacts of climate change into seed transfer rules. The model developed for white spruce in Quebec (Andalo et al. 2005) could be used as a reference for other species. Also, monitoring the performance of white spruce seed sources transferred according to this model, within provenance tests replicated on a large number of sites (Beaulieu and Rainville 2005), is desirable to support growth expectations.

References

Andalo, C.; Beaulieu, J.; Bousquet, J. 2005. The impact of climate change on growth of local white spruce populations in Quebec, Canada. For. Ecol. Manage. 205: 169–182.

Beaulieu, J.; Bousquet, J.; Rainville, A.; Zhang, S.Y. 2003. Le Québec à l'aube d'une révolution en génétique forestière. Carrefour de la recherche forestière 2003. Actes des Colloques. pp 5–16.

Beaulieu, J.; Perron M.; Bousquet, J. 2004. Multivariate patterns of adaptive genetic variation and seed source transfer in *Picea mariana*. Can. J. For. Res. 34: 532–545.

Beaulieu, J.; Rainville A. 2005. Adaptation to climate change: genetic variation is both a short- and long-term solution. For. Chron. 81: 704–709.

Mátyás, C.; Yeatman C.W. 1992. Effect of geographical transfer on growth and survival of jack pine (*Pinus banksiana* Lamb.) populations. Silvae Genet. 41: 370–376.

Li P.; Beaulieu J.; Bousquet J. 1997a. Genetic structure and patterns of genetic variation among populations in eastern white spruce (*Picea glauca*). Can. J. For. Res. 27: 189–198.

Li, P.; Beaulieu, J.; Daoust, G.; Plourde, A. 1997b. Patterns of adaptive genetic variation in eastern white pine (*Pinus strobus*) from Quebec. Can. J. For. Res. 27: 199–206.

Saucier, J.-P.; Bergeron, J.-F; Grondin, P.; Robitaille, A. 1998. Les régions écologiques du Québec méridional, 3e version : Un des éléments du système hiérarchique de classification écologique du territoire mis au point par le ministère des Ressources naturelles du Québec. Ordre des ingénieurs forestiers du Québec. Encart de L'Aubelle no 124, 12 pages.

Thibault, M. 1985. Les régions écologiques du Québec méridional, deuxième approximation : carte en couleurs à l'échelle 1/1 250 000. Serv. De la rech. Appliquée, Min. Énerg. et Ress., Quebec.

André Deshaies
Direction générale des pépinières et des stations piscicoles
Ministère des Ressources naturelles et de la Faune
880 chemin Ste-Foy
Québec, QC G1S 4X4
E-mail: andre.deshaies@mrnf.gouv.qc.ca

André Rainville
Direction de la recherche forestière
Ministère des Ressources naturelles et de la Faune
2700 rue Einstein
Québec, QC G1P 3W8

TRANSFER OF SEED IN NEW BRUNSWICK

Almost half of the land area, or approximately three million ha of New Brunswick forest land, is Crown Land. Under the Crown Land and Forests Act, the Province is responsible for reforestation of all Crown land. Kingsclear Provincial Nursery grows 20 million seedlings per year that are planted on harvested sites. The main species are black spruce (*Picea mariana*), white spruce (*P. glauca*), red spruce (*P. rubens*), and Norway spruce (*P. abies*) which make up ~85% of the planting program. The remaining species planted include jack pine (*Pinus banksiana*), white pine (*P. strobus*) and small quantities of red pine (*P. resinosa*) and eastern white cedar (*Thuja occidentalis*).

Although forest management *per se* is under legislation there are no legislated seed transfer regulations. The Tree Improvement Unit is responsible for providing the seed to the provincial nursery for seedling production. So the seed is "regulated" at least on Crown land. There are no regulations or legislation for private land although many of the companies and private woodlot owners do plant the best possible seed sources on their lands.

As New Brunswick is one of the smallest provinces we are fortunate that we do not have many seed zones. Based on testing conducted by the NB Tree Improvement Council we have determined that for most species the entire province can be treated as one seed zone. An exception to this is red spruce where we have a southern and a northern seed source.

For the major tree species that are planted such as black spruce, white spruce, Norway spruce, and jack pine all the seed originates from seed orchards. Some improved seed is also planted for the southern source of red spruce. For the other species, seed is collected from good quality natural stands.

We are fortunate to have one seed zone as it has allowed us to focus on many species. For black spruce and jack pine we are close to starting third-generation programs and have started collecting seed from second-generation white spruce orchards. Red spruce orchards are just starting to produce and we will be establishing white pine orchards in the next couple of years.

Kathy Tosh
NB Dept. Natural Resources
Tree Improvement Unit
3732 Route 102
Island View, NB E3E 1G3
E-mail: kathy.tosh@gnb.ca

WATER ACTIVITY: A NEW PARADIGM FOR SEED AND POLLEN MANAGEMENT

A Concept Developed in the Food Industry

Water activity (Aw) measurement is a concept developed, and mainly used, by food-processing and pharmaceutical industries for "Intermediate moisture products". Contrary to water measurements such as moisture content (MC) that quantify the total amount of water in a product (quantitative analysis), the measurement of Aw pictures the intensity of the connections of water with other molecules such as carbohydrates, lipids and proteins; it is a qualitative analysis.

Water activity illustrates the energy status of water and consequently its degree of availability and mobility in a product. Aw is a ratio of vapour pressures between the measured sample and pure water. It ranges from 0 to 1, and corresponds to an equilibrium relative humidity from 0 to 100 %. As Aw increases, the energy that binds water with other molecules decreases so the water becomes more and more available for unwanted chemical and biotic reactions in the product such as oxidation of the molecules and development of bacteria or fungi (Fig. 1). These reactions cause the degradation or aging of the product. Thus, Aw is a more reliable predictor of the organic product's stability than the total water amount.

The determination of Aw consists of measuring the equilibrium relative humidity generated by a sample placed in a tight vial or container at a given temperature; Aw meters are consequently some kind of hygrometers fitted with specific probes.

A Fast, Simple and Non-destructive Measure

Water activity measurement has two major operational advantages:

- The data are obtained very quickly. Depending on the samples analysed and the accuracy required, the result is obtained in less than 30 seconds to approximately 20 minutes.
- The sample is not destroyed and can be used again. This is of great interest for very small or precious samples such as pollen or seeds related to specific crossings or samples devoted to *ex situ* conservation programs.

Water activity measurement also has additional advantages. It works by itself and no additional equipment such as an oven or weighing scale is needed. Routine or analytic use requires a short training period and the portable apparatus can be used anywhere. Thanks to recent technologies, the Aw equipment is affordable and obtains reliable, high precision measurements.

Aw is Suitable for Forest Reproductive Material Moisture Management

Thanks to major R&D work done since 2000, the Forest Ecosystems research team of Cemagref located at Nogent-sur-Vernisson, France determined that moisture management of pollen and seeds can be monitored via Aw measures (Philippe et al. 2006). However, the measure only applies to orthodox seeds because the high MC of recalcitrant seeds during storage exceeds the range of the Aw scale.

The work was carried out using seeds of the two main coniferous species used in France, *Pseudotsuga menziesii* (Mirb.) Franco. and *Pinus pinaster* Ait.; broadleaved species were primarily represented by *Fagus sylvatica* L. and *Fraxinus exelsior* L. with a few experiments done on *Ulmus minor* Mill. samaras. Some R&D work was done on pollen of the three main coniferous species involved in controlled pollination in France: *Pseudotsuga menziesii*, *Larix kaempferi* Carr., and *Pinus pinaster*. This work, based on construction and interpretation of sorption isotherms, allowed for the identification of the ideal water activity for a given species. Adopting the optimal Aw value minimizes the deterioration processes and contributes to maintaining the viability of seeds or pollen. Fortunately, optimal Aw values determined from the sorption isotherms agreed with storage MC recommendations found in the literature. Optimal recommended storage MCs are more often expressed as a range of values rather than a single one; this demonstrates that MC is not a single factor but a consequence of the combination of the Aw value, composition, and structure of seeds or pollen. By managing seedlots in these optimal conditions defined with Aw from their harvest to their final use, it is now possible to better preserve them over time.

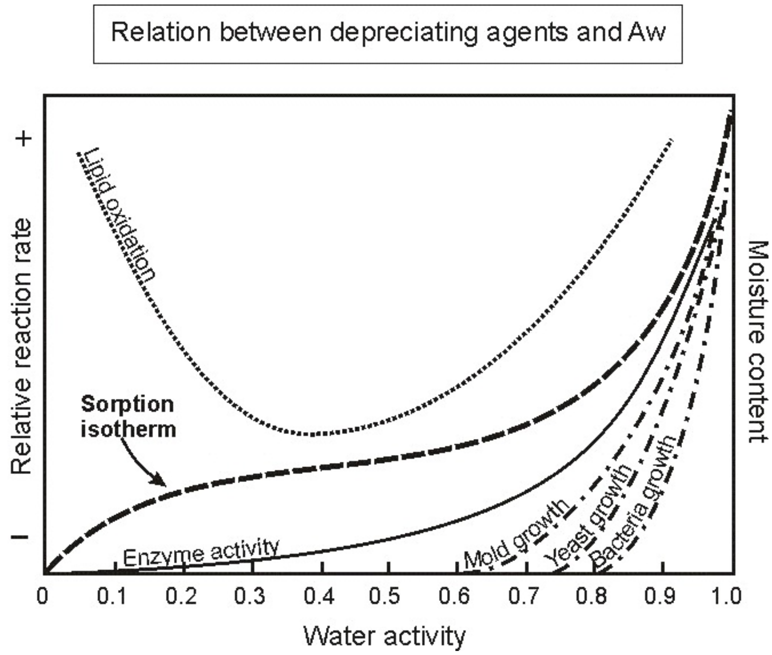


Figure 1. Relative activity of deterioration agents to different water activity. Adapted from Labuza T.P., L. Mc Nally, D. Gallagher, J. Hawkes et F. Hurtado, 1972. Stability of intermediate moisture foods. 1: lipid oxidation. J. Food Sci. 37: 154–159 and other sources.

Aw is not Affected by the Diversity of Seed and Pollen Sources

The characteristics of forest reproductive material for a given species are essentially variable due to the parental genetic diversity and uncontrolled environmental conditions during seed and pollen formation. Aw measurement is not dependent on the phenotypic variability of forest reproductive materials including difference in size, ripeness or percentage of empty or dead seeds. As a result, Cemagref demonstrated that the same moisture content of pollen or seed samples within a species may correspond to significantly different Aw values (Baldet and Verger 2004). This makes water content a weak predictor of pollen and seed stability during all of the moisture management phases for short- or long-term storage. Aw provides better information than MC for predicting seed or pollen conservation because Aw describes the global biological and chemical potential of water of an organic compound as a whole.

A Technique Currently Routinely Used in France

In 2003, the two main French tree seed centres (Office national des Forêts [La Joux] and the Vilmorin Company [Angers]) cooperated with Cemagref and INRA in a joint development project. They quickly decided to adopt, on an operational basis, this fast and non-destructive technique for the moisture management of forest tree seedlots. French State forest seed orchards also routinely use the Aw measure for managing larch pollen used for artificial pollination.

A Research and Technology Transfer Project Between France and Quebec

Since spring 2007, with the collaboration of Cemagref, the Direction de la Recherche Forestière and the Centre de semences forestières de Berthier, both from the Quebec Ministry of Natural Resources, a project was initiated using Aw as a new criterion for seed and pollen management in the province. The project was funded by a Quebec-France program (61[e] Commission de la coopération franco-québécoise). The work was divided into two parts:

- R&D is performed at the Direction de la Recherche and primarily consists of determinating the best Aw values for the conservation of seed and pollen of major reforestation species in Quebec.
- Operational integration of the Aw criteria as a quality test during the extraction process and the conservation of seedlots at the

Berthier seed centre.

Work started with black spruce (*Picea mariana*), which is the major reforestation species (Baldet et al. 2007, in French). After seven months, the main techniques have been transferred to Quebec and the best stability determined for black spruce seeds. These results are consistent with models adopted in France and confirm the interest of Aw measurement on forest seeds when applied to this boreal species. At the same time, the CSFB collected information on Aw from each seed quality test. These data will be of great interest to better characterize the tree seed bank with the Aw criterion in terms of mean values and variability for a given species. Over the next months, we will continue to describe the water dynamics of other species used in Quebec as well as complete the operational implementation of Aw at the seed centre in Berthier. A more complete picture of our results will be presented at the CTIA meeting in Quebec in August 2008.

Aw, a Valuable Tool for Pollen and Seed Managers

Cemagref and DRF think that Aw is of great interest for seed and pollen management. Monitoring the moisture status and deterioration of seed and pollen lots with such a biological indicator rather than a physical one is a good direction to take. We are convinced that many other tree seed centres that store orthodox seeds can implement this measure successfully without a significant cost. We are ready for new collaborations; mutual benefits can be expected by improving general knowledge of forest reproductive material water dynamics prior to and during storage.

Different applications such as planned monitoring of Aw during seed conservation may also be of great interest to prevent sudden detrimental seed deterioration. Monitoring the stability of seeds during storage with Aw could lead to a reduction in frequency of germination tests and, therefore, generate recurrent savings without loss of information.

References

Baldet, P.; Verger, M. 2004. Water activity as a more reliable method than moisture content applied to pollen and seeds moisture management. Page 456. *In* B. Li and S. McKeand, eds. Proc. Forest Genetics and Tree Breeding in the Age of Genomics: Progress and Future. IUFRO Joint Conference of Division 2. http://www.ces.ncsu.edu/nreos/forest/feop/Agenda2004/iufro_genetics2004/proceedings.pdf

Baldet, P.; Colas, F.; Bettez, M. 2007. Mesure de l'activité de l'eau : intégration d'une nouvelle technologie pour le contrôle de la qualité des semences et des pollens. Colloque de transfert de connaissances : Des plants aux plantations; techniques, technologies et performances. Ministère des Ressources naturelles et de la Faune, Direction de la recherche forestière, Direction générale des pépinières et des stations piscicoles. Carrefour de la recherche 2007 (Québec), 19 septembre 2007, Recueil des résumés. p. 33-35. (http://www.mrnf.gouv.qc.ca/carrefour/document/reboisement-resume.pdf) Conference file : http://www.mrnf.gouv.qc.ca/carrefour/document/baldet-p.pdf

Philippe G.; Baldet, P.; Héois, B.; Ginisty, C. 2006. Reproduction sexuée des conifères et production de semences en vergers à graines [Sexual reproduction and seed production in seed orchards.]. Collection Synthèses, Cemagref, 570 p.

For more information on the collaboration project between France and Quebec : http://www.cemagref.fr/Informations/Presse/InfMediaEV/infomedia82EV/im82PollensEV.htm

Patrick Baldet
Cemagref
Unité de recherche Écosystèmes forestiers
Domaine des Barres
45290 Nogent-sur-Vernisson
France
E-mail: patrick.baldet@cemagref.fr

Fabienne Colas
Ministère des Ressources naturelles et de la Faune
Direction de la Recherche forestière
2700, rue Einstein
Quebec, QC G1P 3W8

Michèle Bettez
Ministère des Ressources naturelles et de la Faune
Direction générale des pépinières et des stations piscicoles
Centre de semences forestières de Berthier
1690, chemin Grande Côte
Berthier, QC J0K 1A0

NATIONAL TREE SEED CENTER TURNS 40

2007 was a year for the Seed Centre to celebrate its 40th birthday. The Centre was initially established at the Petawawa Research Forest in 1967 at a time when tree genetics and breeding research was expanding across Canada. Demand was high for small quantities of seed of known origin and quality for research, especially provenance testing. As well, reforestation programs were expanding in Canada and there was a need for research and training on seed collection, processing, testing, and storage. Providing seed for research is still a priority for the Seed Centre. Long-term storage of seed for *ex situ* gene conservation is also important. With forest genetic resources threatened by climate change and invasive alien species, it is important to conserve samples of the gene pool that may prove invaluable for future restoration and research.

To celebrate the Seed Centre's success, staff were involved in a number of activities. New seed display panels were developed for the conifer and deciduous species in storage. Each species is represented by a seed sample encased in resin supplemented by a color picture of its flower or cone or leaf and a silhouette of the tree's form. A new, color Impact Note was produced that summarizes what the Seed Centre is all about. A poster was created to highlight the primary roles of the Centre: collection, conservation, and collaboration. A paper, highlighting accomplishments of the Centre, was published in the Forestry Chronicle (Simpson and Wang 2007). As well, staff participated in various trade shows, tours, symposia, and workshops to promote the Seed Centre. Copies of the Impact Note and Poster can be obtained upon request.

The seed crop was light to non-existent in the Maritimes. This afforded us the opportunity to travel to Newfoundland to collect some seed there. Over 100 collections were made from 10 species. This greatly expands the number of accessions from this province. We have started to repeat our cycle of germination testing. With the number of available staff it is only practical to test seed every 10 years although there are exceptions. Seedlots are tested for moisture content as well in order to identify instances where conditioning is required and to track moisture content and germination together over time.

An undergraduate forestry student conducted her senior thesis on a study of embryo elongation and germination of black ash (*Fraxinus nigra*) seed. Black ash seed are immature and dormant when shed. Seed must be first treated in order for the embryo to grow then receive another treatment to alleviate dormancy. Three single-tree collections from each of two locations were used. Various durations of cold-warm-cold treatments were applied to the seed. She found that there was no relationship between embryo elongation and germination. Seed germination was maximized at 58% after a treatment of 60 days of cold (3°C), 120 days of warm (22°C) and 210 days of cold (3°C) (O'Donnell 2007).

References

O'Donnell, S.B. 2007. Embryo elongation and germination f *Fraxinus nigra* seeds. Univ. New Brunswick, Faculty For. Environ. Manage, Unpbl. BScF thesis. 39 p.

Simpson, J.D.; Wang, B.S.P. 2007. The National Tree Seed Centre celebrates 40 years. For. Chron. 83: 719–722.

Dale Simpson and Bernard Daigle
Natural Resources Canada
Canadian Forest Service
Atlantic Forestry Centre
P.O. Box 4000
Fredericton, NB E3B 5P7
E-mail: Dale.Simpson@nrcan.gc.ca

CTIA TREE SEED WORKING GROUP TOUR AND WORKSHOP

Members of the Tree Seed Working Group are organizing a tour to be held prior to the joint CTIA-IUFRO Conference scheduled for Quebec City August 25–28, 2008. The tour is planned for Sunday, August 24 and will be held at Berthier, located about mid way between Montreal and Quebec City. The nursery and tree seed centre at Berthier will be celebrating their 100th anniversary! As well as the nursery and seed centre, other stops scheduled in Berthier include an indoor larch seed orchard, a white spruce seed orchard, and production of white spruce from cuttings. Transportation will be provided from both Quebec City and Montreal. The site visit will begin at 10 am and finish around 3 pm with a scheduled arrival time of 5 pm in Quebec City.

The Tree Seed Workshop will be held the following day, August 25. The organizers are currently working to develop a theme and topics to be addressed by speakers. For further information please contact:

Fabienne Colas
Direction de la Recherche forestière
2700, rue Einstein
Quebec, QC G1P 3W8
E-mail: Fabienne.Colas@mrnf.gouv.qc.ca

UPCOMING MEETINGS

NSC Winter Workshop
"Changing Northern BC Forests: Silvicultural Challenges and Implications"
Jan. 21–23, 2008 Prince George, BC
Contact: Rob Bryce 250-960-5982

ISTA Tropical Tree and Shrub Seed Workshop – Purity, Moisture Content, Germination, Storage., Tetrazolium
April 7–10, 2008 Curitiba, Brazil
Contact: Antonio Medeiros
medeiros@cnpf.embrapa.br

ISTA Temperate Tree and Shrub Seed Workshop – Tetrazolium, Sampling, Moisture Content
June 13–15, 2008 Peri, Italy
Contact: Fabio Gorian fabio.gorian@libero.it

CTIA Tree Seed Working Group Tour and Workshop
August 24/25, 2008 Berthier and Quebec City
Contact: Fabienne Colas
Fabienne.Colas@mrnf.gouv.qc.ca

Canadian Tree Improvement Association
joint with IUFRO Work. Grps. 2.04.01 & 2.04.10
"Adaptation, Breeding and Conservation in the Era of Forest Tree Genomics and Environmental Change"
Aug 25–28, 2008 Quebec City, QC
Contact: Jean Beaulieu
Jean.Beaulieu@nrcan.gc.ca

IUFRO Tree Seed Symposium
"Tree Seeds 2008 – Trees, Seeds and a Changing Climate"
Sep 22–25, 2008 Wakehurst Place, UK
Contact: Matt Daws m.daws@kew.org

RECENT PUBLICATIONS

Bhandari, R.S.; Dobhal, B.; Pande, S. 2006. Effect of cone worm, *Dioryctria abietella* Denis and Schiffermuller (Lepidopteira : Pyralidae) on the seed efficiency of *Pinus wallichiana*. Indian For. 132(11): 1493–1498.

Cherry, M.L.; Anekonda, T.S.; Albrecht, M.J.; Howe, G.T. 2007. Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*). Can. J. For. Res. 37(1): 1–10.

Choi, C.H.; Cho, K.J.; Tak, W.S. 2007. Seed characteristics and germination properties according to change of cone production in *Pinus densiflora* stands. J. Korean For. Soc. 96(3): 317–324.

Cochrane, J.A.; Crawford, A.D.; Monks, L.T. 2007. The significance of *ex situ* seed conservation to reintroduction of threatened plants. Aust. J. Bot. 55(3): 356–361.

De Atrip, N.; O'Reilly, C. 2007. Effect of seed coverings and seed pretreatments on the germination response of *Alnus glutinosa* and *Betula pubescens* seeds. Eur. J. For. Res. 126: 271–278.

De Atrip, N.; O'Reilly, C. 2007. Germination response of alder and birch seeds to applied gibberellic acid and priming treatments in combination with chilling. Ann. For. Sci. 64: 385–394.

El-Kassaby, Y.A.; Prescher, F.; Lindgren, D. 2007. Advanced generation seed orchards' turnover as affected by breeding advance, time to sexual maturity and costs, with special reference to *Pinus sylvestris* in Sweden. Scand. J. For. Res. 22(2): 88–98.

El-Kassaby, Y.A.; Stoehr, M.U.; Reid, D.; Walsh, C.G.; Lee, T.E. 2007. Clonal-row versus random seed orchard designs: interior spruce mating system evaluation. Can. J. For. Res. 37(3): 690–696.

Ghildiyal, S.K.; Sharma, C.M.; Khanduri, V.P. 2007. Improvement in germination of chirpine (*Pinus roxburghii*) by a presowing treatment with hydrogen peroxide. J. Trop. For. Sci. 19(2): 113–118.

Iwaizumi, M.G.; Watanabe, A.; Ubukata, M. 2007. Use of different seed tissues for separate biparentage identification of dispersed seeds in conifers: confirmations and practices for gene flow in *Pinus densiflora*. Can. J. For. Res. 37: 2022–2030.

Jinks, R.L.; Willoughby, I.; Baker, C. 2006. Direct seeding of ash (*Fraxinus excelsior* L.) and sycamore (*Acer pseudoplatanus* L.): The effects of sowing date, pre-emergent herbicides, cultivation, and protection on seedling emergence and survival. For. Ecol. Manage. 237(1-3): 373–386.

Kang, K.S.; Mullin, T.J. 2007. Variation in clone fertility and its effect on the gene diversity of seeds from a seed orchard of *Chamaecyparis obtusa* in Korea. Silvae Genet. 56(3-4): 134–137.

Keyes, C.R.; Maguire, D.A. 2007. Seed rain of ponderosa pine beneath partial overstories. New Forests 34(2): 107–114.

Kulcu, R.; Yaldiz, O. 2007. Composting of goat manure and wheat straw using pine cones as a bulking agent. Bioresour. Technol. 98(14): 2700–2704.

Mapula-Larreta, M.; Lopez-Upton, J.; Vargas-Hernandez, J.J.; Hernandez-Livera, A. 2007. Reproductive indicators in natural populations of Douglas-fir in Mexico. Biodivers. Conserv. 16(3): 727–742.

Meunier, C.; Sirois, L.; Begin, Y. 2007. Climate and *Picea mariana* seed maturation relationships: a multi-scale perspective. Ecol. Monogr. 77(3): 361–376.

Miller, D.R. 2007. Limonene: Attractant kairomone for white pine cone beetles (Coleoptera : Scolytidae) in an eastern white pine seed orchard in western North Carolina. J. Econ. Ent. 100(3): 815– 822.

Mimura, M.; Aitken, S.N. 2007. Adaptive gradients and isolation-by-distance with postglacial migration in *Picea sitchensis*. Heredity 99(2): 224–232.

Murray, M.P. 2007. Cone collecting techniques for whitebark pine. West. J. Appl. For. 22(3): 153–155.

O'Reilly, C.; DeAtrip, N. 2007. Seed moisture content during chilling and heat stress effects after chilling on the germination of common alder and downy birch seeds. Silva Fenn. 41(2): 235–246.

Owens, J.N.; Fernando, D.D. 2007. Pollination and seed production in western white pine. Can. J. For. Res. 37(2): 260–275.

Pawlowski, T.A. 2007. Proteomics of European beech (*Fagus sylvatica* L.) seed dormancy breaking: Influence of abscisic and gibberellic acids. Proteomics 7(13): 2246–2257.

Savva, Y.; Denneler, B.; Koubaa, A.; Tremblay, F.; Bergeron, Y.; Tjoelker, M.G. 2007. Seed transfer and climate change effects on radial growth of jack pine populations in a common garden in Petawawa, Ontario, Canada. For. Ecol. Manage. 242(2-3): 636–647.

Sharma, R. 2007. Variation studies in provenances and plus trees of *Pinus roxburghii* Sarg. Indian For. 133(4): 519–526.

Shibata, E. 2007. Effects of deer debarking on masting and trunk radial growth of Hondo spruce, *Picea jezoensis* var. *hondoensis*, in a subalpine forest of central Japan. For. Ecol. Manage. 252: 159–164.

Stevens, A.D. 2007. Botanical gardens and their role in *ex situ* conservation and research. Phyton (Horn) 46(2): 211–214.

Strong, W.B. 2006. Seasonal changes in seed reduction in lodgepole pine cones caused by feeding of *Leptoglossus occidentalis* (Hemiptera : Coreidae). Can. Ent. 138(6): 888– 896.

Tereso, S.; Zoglauer, K.; Milhinhos, A.; Miguel, C.; Oliveira, M.M. 2007. Zygotic and somatic embryo morphogenesis in *Pinus pinaster*: comparative histological and histochemical study. Tree Physiol. 27: 661–669.