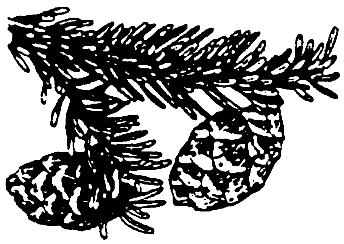
CANADIAN FOREST GENETICS ASSOCIATION
ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE

Tree Seed Working Group



NEWS BULLETIN

No. 57 August 2013

THIS ISSUE AT A GLANCE

CHAIR'S 'ARMCHAIR' REPORT

Hello! I hope everyone is having a great summer. For many parts of BC it has been our driest July ever with no recorded precipitation in the Vancouver area and beyond. Initial indications are that it certainly hasn't helped our lodgepole pine seed set issues in the north Okanagan and our falldown appears to be occurring earlier in this hot dry summer. This is our most serious seed set issue in BC (by far) and was the subject for the majority of our Tree Seed Working Group workshop in Surrey on July 22nd. This dry weather may also have implications for reproductive bud determination as most of our species are differentiating their meristematic tissues at that stage and hot, dry weather has often been a positive factor in the promotion of reproductive bud formation.

I'd like to thank all of the speakers who presented at our Tree Seed Working Group workshop at the BC Tree Seed Centre. The majority of the content for this News Bulletin edition is the abstracts from the various presentations. Thank you to Lindsay Robb for her article on testing seed storage containers. In addition, a webpage has been set up that provides a pdf version of the presentations as well as the abstracts (https://www.for.gov.bc.ca/hti/treeseedcentre/tsc/workshops&presentations/tswg_2013/index.htm). The workshop went smoothly (further details inside), although a sombre element was present with the recent passing of Tim Lee who managed the Vernon Seed Orchard Company. Tim was a strong tree improvement advocate, friend to many, and one who could always be counted on to do the right thing. He will surely be missed and I send my deepest sympathies to family, friends and colleagues. The workshop was dedicated to the memory of Tim Lee.

In trying to promote submitting articles for the News Bulletin we have been having a lottery at our workshop for everyone who has contributed an article since the last TSWG workshop. This year's grand prize winner was Fabienne Colas, who received a copy of Claire Williams' book "Conifer Reproductive Biology". Other prizes were awarded to Al Foley, Michele Fullarton, and Michael Stoehr.

There has not been a dedicated theme for the last few Newsbulletins and I thought it's time to try and reintroduce that concept. Articles do not need to be on the indicated subject, but it does offer a cohesive tie to the other submitted articles. The theme for the December 2013 News Bulletin # 58 will be "From Seed Collection to Seed Storage" and is intended to provide a forum for processing both conifer and angiosperm tree species. It will also be an opportunity for those to sound in on storage practices prior to propagule processing. Do you process a unique species? Do you have a unique processing method for our conventional reforestation species or have specific processing problems? News Bulletin # 58 may provide the venue for introducing the topic to a wide audience? or it may win you a cool prize in the future. Please consider contributing an article to the TSWG News Bulletin – Thank you.

Dave Kolotelo
TSWG Chair

EDITOR'S NOTES

The normal June issue was delayed in order to publish abstracts of speakers from the seed workshop that was held at the BC Tree Seed Centre in Surrey prior to the Canadian Forest Genetics Association conference. The workshop was very well attended and all the presentations were excellent. Kudos go to Dave for organizing the workshop and to his staff for setting up the venue.

Lindsay Robb has provided a very interesting article about testing storage containers for long-term storage of small quantities of seed. This should be helpful to anyone who may want to revisit the type of containers that they are using.

I hope that you enjoy the remainder of the summer and look forward to a great fall. May the seed gods be kind to you!

Dale Simpson
Editor

TREE SEED WORKING GROUP

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

All issues of the News Bulletin are available at: www.for.gov.bc.ca/hti/treeseedcentre/tsc/tswg.htm

TREE SEED WORKSHOP OVERVIEW

On July 22nd, 2013 the BC Tree Seed Centre hosted the TSWG workshop with the overall theme of "Reproductive Biology". There were 48 people in attendance and the agenda below illustrates the speakers and their topics. The day started with a general review of Reproductive Biology topics and then after our morning refreshment break switched to focusing in on our lodgepole pine seed set problems in north Okanagan seed orchards. There was an open Q&A session in the afternoon followed by a tour of the Tree Seed Centre. Some were fortunate to then be able to go to Whistler for the Forest Genetics 2013 meeting.

Abstracts for most talks are included in this edition, but holidays complicated that for a few individuals and the Abstracts and pdf versions of the Powerpoint presentations will be uploaded to this webpage for future reference https://www.for.gov.bc.ca/hti/treeseedcentre/tsc/workshops&presentations/tswg_2013/index.htm.

Welcome	Heather Rooke
Conifer Reproductive Biology evolutionary pathways	Dave Kolotelo
Broadleaf Reproductive Biology	Dale Simpson
Reproductive Biology System and Its Impact on Genetic Architecture	Jodie Krakowski
Conifer Pollination Mechanisms Revisited	Dr. Patrick von Aderkas
Seed Production in Lodgepole pine	Dr. Joe Webber
Variation in reproductive traits of lodgepole pine in British Columbia	Anne Berland
Lodgepole pine crop statistics from Select Seed Orchards	Jack Woods
Questions About the Role of Seed Bug Herbivory in Seed Production Coming from Interior Lodgepole Pine Orchards	Jim Corrigan
Lepto-cams and Thermocouples: Why do Insect Bags Prevent August Pli Seed Loss in the Interior of BC?	Dr. Ward Strong
Seed Shortfall in Lodgepole Pine	Dr. Lisheng Kong
Methodology to Organize Operational Pli Cone Harvests by Using Observations of Seed Set Declines	Gary Giampa

I'd like to thank everyone who contributed to the workshop – speakers for their time and effort – you all did a great job, staff at the TSC who helped with planning and organizing and especially Aggie Ellis who organized most of the catering, and Michael Postma and Diane Douglas who took time out of their Sunday to help set up and then help with audiovisual and registration logistics. Finally I'd like to thank the Vernon Seed Orchard Company and SelectSeed Ltd. for their financial contributions towards the workshop.

Dave Kolotelo

CONIFER REPRODUCTIVE BIOLOGY EVOLUTIONARY PATHWAYS

The intent of this talk was to provide a baseline for conifer reproductive biology from its early beginnings to the current diversity found among north-temperate conifers. I consider it a work in progress. Time estimates of significant events are primarily based on fossil evidence and are therefore restricted to samples which have been 'fossilized' under appropriate conditions and have been found. Land plants appeared approximately 450 million years ago (MYA) and coincided with the appearance of mycorrhizal associations. These early land plants reproduced like ferns and were dependent on water for spore dissemination. Xylem evolution preceded seeds and this allowed plants to reduce potential for embolism under freezing or drought conditions, in addition to significant height increases and an increased competitive ability for sunlight.

The evolution of seeds is generally considered in terms of three large-scale evolutionary changes:

1) Heterospory – The differentiation of two separate spore types differing in size and sex. Considered to have occurred multiple times in plant evolution.
2) Megaspore Reduction / Retention in Sporophyte – The reduction to one female sprore (megaspore) from many and maintenance of the structure within the body of the parent plant (endospory).
3) Integument Evolution – The evolution of protective tissues to protect the innermost structures.

In conifers, a variety of pollen reception mechanisms evolved and these finalized the conversion to the water independence of spores for movement and fertilization. Seeds, in addition to mycorrhiza and xylem, are considered the major evolutionary factors allowing conifers to dominate large land areas, especially those with stressful or sub-optimal environments. Seeds can be thought of as "concentrated life" as the seed contains the future diploid tree, the initial food reserves, and the seed coat for protection. Seeds are the dispersal package and they also 'allowed' for the development of seed dormancy to synchronize germination with the appropriate environmental conditions to maximize survival and growth.

The two main groups of seed plants, Angiosperms (about 250,000 species) and Gymnosperms (600−700 species) diverged approximately 310 MYA. Inverted ovules, pointing towards the central axis, is a characteristic of most conifers and evolved approximately 265 MYA. The inverted ovules' orientation (up or downwards pointing) is related to the type of pollen present (saccate or non-saccate). Subsequent to ovule inversion there was also a division of the Pangeaen supercontinent about 200 MYA resulting in species isolation and evolution of some highly variable reproductive biology mechanisms among conifers. One third of all conifers are attributed to two genera: *Pinus* in the Northern hemisphere and *Podocarpus* in the Southern hemisphere.

Conifer cones are basically structures that house ovules, allow for pollen to enter, and then close to protect the embryo. In many species this is a woody structure, but *Taxus* and *Podacarpus* are examples of conifer genera with fleshy structures to house the seed. In general, pollen cones (microsporangia arranged spirally along a central axis) are relatively simple and each microsporangium is considered to be a modified leaf. These pollen cones do not show a great deal of diversification among genera. The seed cones (bract scale complexes arranged spirally along a central axis) are considered to be more complicated as each bract-scale complex is considered to be a modified shoot vs. a modified leaf as for pollen cones. In contract, seed cones have differentiated greatly in terms of gross morphology, ovule orientation, and type of ovular secretion mechanism.

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BROADLEAF REPRODUCTIVE BIOLOGY

Seed plants started to evolve 340 million years ago with Angiosperms arising about 130 million years ago, but it was about 100 million years ago that the number of species began to rapidly evolve. Angiosperms produce true flowers which can be either perfect or imperfect. Perfect flowers contain both male and female reproductive structures whereas imperfect flowers are either sex. Flowers occur in a variety of arrangements such as: spike, raceme, panicle, corymb, cyme, umbel, catkin, and head. Some genera or species are monoecious where both males and females occur on the same tree as opposed to dioecious species where males and females occur on separate trees. Pollen that is dispersed by wind (anemophilous) is small and is dispersed over great distances. Pollen from species that produce showy, fragrant flowers is generally dispersed by insects (entomophilous). This pollen is larger and its dispersal is generally limited. Broadleaved species produce fruit that contains the seed. The type of fruit produced is species-dependent. Examples include: achene – sycamore (*Platanus occidentalis*); double samara – maple (*Acer* spp.); single samara – elm (*Ulmus* spp.), ash (*Fraxinus* spp.); acorn – oak (*Quercus* spp.); nut – hickory (*Carya* spp.), butternut (*Juglans cinerea*); legume - Kentucky coffeetree (*Gymnocladus dioicus*); capsule – poplar (*Populus* spp.); pome – apple (*Malus* spp.), mountain ash (*Sorbus* spp.); and drupe – cherry (*Prunus* spp.). Fruits are shed by most species in late summer and fall and are primarily dispersed by wind. Dispersal by animals is important for species such as mountain ash, cherries, oaks, and butternut. Seed of broadleaved species often exhibits dormancy due to seed coat, embryo, morphology or a combination. Seed coat dormancy can be alleviated by soaking seed in cold or hot water or treating the seed with acid to allow moisture to enter. Moist chilling or combinations of warm, moist incubation and moist chilling alleviate embryo and morphological dormancy.

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REPRODUCTIVE BIOLOGY SYSTEM AND ITS IMPACT ON GENETIC ARCHITECTURE

Since the days of Darwin, scientists have observed that life history traits are associated with patterns of population adaptation and diversity. Significant relationships between genotypic and adaptive diversity and genetic architecture have been demonstrated for numerous life history traits, the most consistent being: mating system, taxonomy, life form, successional status, range extent and contiguity, and dispersal mechanism of seed and pollen. In nature, many of these traits are correlated and few studies have evaluated the individual factors. Generally, longer-lived, late-successional, outcrossing, animal-dispersed perennials that are not endemic have higher expected and observed heterozygosity than annual, endemic or narrowly distributed, gravity- or wind-dispersed, pioneer species. The former group generally have less differentiated populations than the latter. However, there is wide variation among species. In addition to life history traits, many studies have quantified equally strong − sometimes even stronger − external influences on plant genetic architecture. Dominant influences include: refugia and post-glacial range expansion, spatial and temporal disturbance patterns, soil and geology, hybridization, management interventions, sample breadth and size for genetic studies, and type of genetic marker used. Adding to the complexity, these factors can also have significant interactions with life history traits. While general patterns of total heterozygosity, population differentiation, and heterozygote deficiency are associated with life history characteristics, species-specific data based on a carefully designed study with clear objectives are needed to answer questions about any particular species.

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CONIFER POLLINATION MECHANISMS REVISITED

Pollination mechanisms in Gymnosperms can be divided into types, depending on the shape of pollen and ovule micropyle and whether pollination drops are present. Two general types exist: pollen capture mechanisms (PCM) and extra-ovular capture and germination (ECG). Of these, PCM has the greatest diversity, as these show six different sub-types. Phylogenetic analysis of modern and extinct gymnosperms reveals that pollination drops were fundamental to pollination capture mechanisms of the earliest seed plants. The most derived types of PCM are found within Pinaceae. ECG, also derived, is restricted to a few conifer families and genera. Pollination drops provide a nectar function in gnetophytes and possibly in cycads. This provides an independent origin to study convergent evolutionary patterns in plant-insect relations.

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SEED PRODUCTION IN LODGEPOLE PINE

Seed yields (filled seeds per cone) from north Okanagan (NO) lodgepole pine (*Pinus contorta* var. *latifolia*) orchards have not met expectations compared to seed yields routinely realized at Prince George (PG). Since the NO is substantially hotter and drier than PG, early efforts focused on better irrigation systems (broad cast sprayers) and crown cooling (misting). Data collected from Kalamalka Seed Orchard 307 over the period of 2000 to 2005 did not show any significant improvement in either cone numbers or seed yields. However, cones protected by insect bags had consistently more seed.

Since most NO orchards were experiencing poor seed production, in 2006 we began to collect standardized orchard statistics from eight orchards on four NO sites and two orchards at the PG Tree Improvement site. All 10 orchards produce seed for three PG and three southern interior seed planning units. We also compared production from two orchards of the same provenance base at PG and NO.

Over the period of 2006 to 2012, the trend for higher seed yields per cone but fewer cones at PG continued. Since the number of filled seed per cone (FSPC) was high and remained fairly consistent (20–25 FSPC) at PG, variation in the number of seed per tree principally resulted from variation in the number of cones per tree (100–300 cones per tree). Year-to-year variation occurred in all orchards but those NO orchards producing seed for the PG area (with one notable exception) consistently produced lower yields than those NO orchards producing seed for the southern interior. Orchard site also was important. Southern interior orchards in Armstrong, BC (Pacific Regeneration Technology Inc.) (PRT) equalled or exceeded that from PG and for the last two years have consistently out-produced all other NO orchards.

Over the seven years of observation, the loss of seed from un-bagged cones in all NO orchards ranged from about 2 to 11 FSPC. There was no seed loss from un-bagged cones at PG. Of the four NO orchard sites, Kalamalka and Tolko had the greatest losses (about 10) with the two PRT and Vernon Seed Orchard Company orchards each losing about 5 and 4 FSPC, respectively. The greatest loss of seed from un-bagged cones appeared to occur in August. Cones exposed for a two week period from April to the end of July were not significantly affected but cones exposed in August had significantly fewer seed per cone. Again, bagged and un-bagged cones from PG did not show any differences in seed yields.

There is no general agreement about the bagging affect. One side of the argument suggests the losses are too large to be caused by insects. However, bagged cones and insect management (spraying) all show better seed yields than untreated cones. Insect-protected cones can account for some of the losses but on average, we expect seed yields from lodgepole pine to be in the order of 20–25 FSPC. However, production from NO orchards ranges from 10–15 FSPC. If we account for 5–10 FSPC losses from unprotected cones, we still must account for the other 5–10 FSPC that may not be attributed to insect predation.

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VARIATION IN REPRODUCTIVE CAPACITY OF LODGEPOLE PINE (*Pinus contorta* var. *latifolia*) IN BRITISH COLUMBIA

Lodgepole pine (*Pinus contorta* var. *latifolia*) is the most wide-ranging pine in North America. Populations in British Columbia vary widely in phenotypic and genotypic characteristics and differences between populations can be linked to local climate or to geographic predictors. The effect of climate on variation in reproductive characteristics has never been examined, yet is vital to the production of seed necessary for reforestation. This study aims to determine the relationship between climate and variation in female cone and seed characteristics. The study makes use of the Illingworth provenance trial, sixty common garden plots that are distributed throughout British Columbia. Female cones from seven source populations were collected at 22 sites during the summer of 2012. Data processing to date includes measuring cone length, and determining the number of scales per cone. Initial results indicate wide variation between sites for both variables. Further data processing will include determining the number of seeds per cone. The final product of this study will be a response function relating climate variables to cone morphology and seed yield.

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LODGEPOLE PINE CROP STATISTICS FROM SELECTSEED LTD. ORCHARDS

SelectSeed Ltd. is wholly owned by the Forest Genetics Council of BC, a government – industry cooperative. It's mandate is the production of genetically selected tree seed within a financial objective of long-term sustainable profit and a modest return on capital employed. Nine of the 14 seed orchards operated by SelectSeed under contract are lodgepole pine (Pli) (*Pinus contorta* var. *latifolia*) (about 29 of 35 thousand ramets). As a result, Pli seed production and sales are key to the SelectSeed's long-term business prospects.

The original seed production and cash-flow expectations used for SelectSeed's business plan were based on the small amount of information available in about 2000. More recent information clearly shows issues with low seed production (filled seeds per cone) from Pli orchards located in the north Okanagan valley of south-central British Columbia relative to orchards located near Prince George in central BC. Using seed production statistics from the nine SelectSeed orchards, comparisons are made between forecast and actual seed production statistics, with breakdowns for Pli derived from naturally occurring stands from seed planning units (SPU) located in southern BC vs. central BC, and between north Okanagan orchards vs. those located in slightly cooler environments just outside the Okanagan valley. A SPU is a geographic area where tree populations are genetically similar enough to allow seed transfer within the geographic and elevation boundaries of the SPU.

Overall seed production across the nine SelectSeed Pli orchards does not vary substantially from initial forecasts (Fig. 1). Orchards with selected trees from SPU in central BC, however, have had poorer seed production than those from SPU in southern BC. There is a confounding of SPU origin and orchard location that makes direct comparison difficult, but there is evidence that Pli populations from central BC SPU, planted in orchard locations cooler than the Okanagan valley but not cool enough to be within naturally occurring lodgepole pine forests, are producing more seed than the same populations in north Okanagan orchard locations. Reduced seed production relative to potential is a significant financial concern to the company, even though actual production levels are close to initial forecasts.

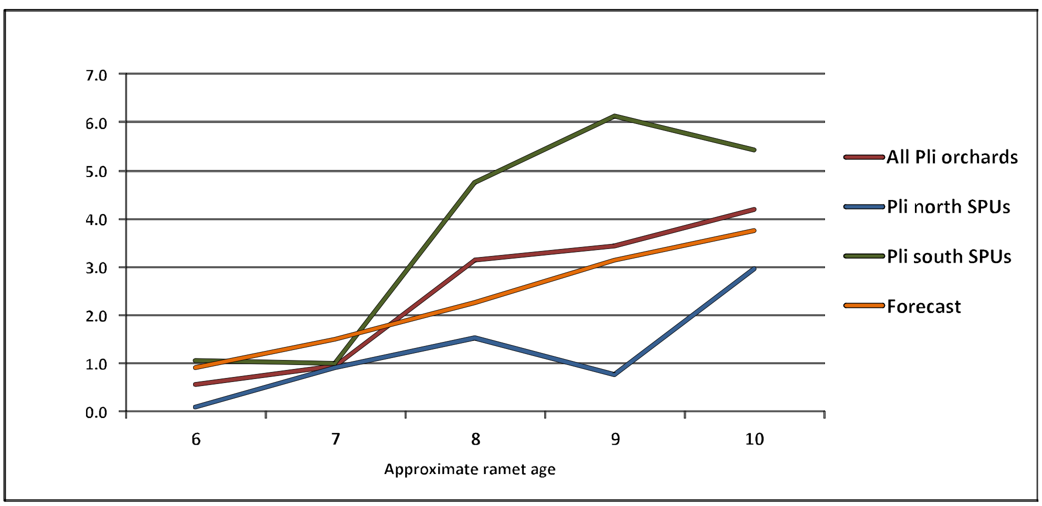

Figure 1. Age-adjusted grams of lodgepole pine seed produced per orchard position for selected trees from northern and southern seed planning units (SPU).

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QUESTIONS ABOUT THE ROLE OF SEED BUG HERBIVORY IN PRODUCTION COMING FROM INTERIOR LODGEPOLE PINE SEED ORCHARDS

Herbivory by the western conifer seed bug (*Leptoglossus occidentalis*) has been assumed to be the principal cause of substantial annual shortfalls of seed coming from lodgepole pine orchards located in the interior of British Columbia. The difference between the number of filled seeds per cone (FSPC) produced in cones protected from seed bug attacks (by being enclosed inside nylon-mesh bags) and the numbers of viable seeds coming from cones left open in the environment has been used as an indicator of the amount of seed bug herbivory taking place in these orchards. This is the 'Seed Bug Model' for losses occurring in this cropping system. Operational production records from Kalamalka lodgepole pine Seed Orchard 307 for 2001 to 2010, taken with bagging trial results from this orchard for the same years, allow us to make numerical estimates of whole-orchard FSPC shortcomings occurring annually during the past decade. These results are discussed in the context of whether they are, or are not, consistent with a Seed Bug Model.

For over fifteen years, concerted efforts have been made to establish a relationship between the number of seed bugs observed in Orchard 307 and the FSPC losses attributed to seed bug herbivory. These efforts have not been successful as no seed bug density:crop damage relationship and no economic threshold have ever been established for this cropping system. Without an economic threshold, pesticide treatments for seed bugs have never been conducted in a manner consistent with the modern practice of Integrated Pest Management. Beyond this consideration, comparisons of FSPC values derived from annual crop production statistics with FSPC values for cones held in bags show that applications of the pesticide Sevin for seed bug control never have raised operational seed yields to the levels obtained inside the bags. In fact, seed deficits of one-third or more of the crop occurred in each of eight years in which pesticides were applied for seed bug control. These results were not consistent with expectations coming from the Seed Bug Model. If FSPC losses were being caused by insect herbivory, why did treatments by an insecticide fail to increase FSPC numbers close to those seen inside the bags?

Work done by Dr. Joe Webber and colleagues in 2010 showed that major FSPC losses occurred in cones that were not enclosed inside bags during the month of August. Being exposed to the environment through any two-week period from the beginning of May to the end of July did not significantly reduce FSPC values. Timing of harvest trials done in 2011 showed a similar temporal profile for FSPC declines. However, none of the cones used in the 2011 trial were held in bags during any point of the growing season. Versions of both experiments have been replicated across multiple years at several locations. Now, it is recognized that the majority of the FSPC losses observed in north Okanagan lodgepole pine orchards occur in August.

By using the FSPC deficits derived from bagging trials and multiplying this by the number of cones harvested in any given year (derived from crop production statistics), we can calculate the number of seeds 'lost' during a growing season. For 2010, this number was estimated to be about 5.4 million seeds. Bagging experiments indicate that these FSPC shortcomings occurred in August. Using this information, we can calculate the number of seed bugs needed to cause the FSPC deficits observed for Orchard 307 in 2010. Over the course of 30 days, 5,374,000 seeds were lost, eaten by seed bugs with a maximum individual daily herbivory rate of 5 seeds per day. This loss would require a minimum of 35,826 seed bugs to be actively feeding, each eating their daily maximum number of seeds on every day of the month.

Orchard 307 was harvested through August 2010. Staff members spent a minimum of 175 person-hours per week in the orchard in August and they physically handled every cone that was picked. Although workers reported seeing seed bugs, we never received any reports of seed bugs being seen in anything approaching the numbers that would be required to cause these FSPC losses under the Seed Bug Model.

In summary, there never has been a relationship established between seed bug population sizes and the FSPC damage that they reputedly cause. Pesticide treatments have never raised FSPC levels in the operational harvests to those predicted (from bagging trials) to be possible if seed bugs were causing the FSPC losses. With the recently acquired knowledge of when the FSPC declines occur during the growing season, we can calculate how many seed bugs must be present in Orchard 307 to cause the losses attributed to them by the Seed Bug Model. Nothing even close to the number of seed bugs required to cause these deficits was observed in 2010, nor have they been observed in any other year during the past decade. It is for these reasons that I question the Seed Bug Model. I do not believe that the differences in FSPC observed between cones held inside bags and cones left exposed in the environment can be solely attributed to herbivory by seed bugs.

Currently, there are a number of investigations that are examining other aspects of this problem:
1) seed samples taken through the decline period are being examined microscopically to observe the physiological aspects of losses of filled seed,
2) the relationship between seed set, area of origin of the orchards, temperature and seed declines is being investigated in field trials,
3) in-cone temperatures and seed bug herbivory are being monitored on selected cones throughout the growing season,
4) harvesting schedules that incorporate the timing of FSPC declines by clone are being developed to maximize the yields coming from interior lodgepole pine seed orchards, and
5) a method to detect seed bug herbivory on individual lodgepole pine seeds is being developed.

It is hoped that this multi-faceted approach will help to address the unanswered questions that remain about seed losses occurring in interior lodgepole pine orchards.

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LEPTO-CAMS AND THERMOCOUPLES: WHY DO INSECT BAGS PREVENT AUGUST Pli SEED LOSS IN THE INTERIOR OF BC?

Lodgepole pine (*Pinus contorta* var. *latifolia*) seed orchards in the Southern Interior of BC have suffered from low seedset for many years. The causes largely remain a mystery, despite years of research. In 2011 and 2012, it was discovered through bagging studies that much of the seed loss occured in August. The August seed loss is entirely preventable by enclosing the cones in mesh bags, while seed loss earlier in the season was not affected by mesh bags.

Only two theories exist as to why mesh bags prevent seed loss. One is that the bags alter the microclimate, protecting the cones from, for example, extreme heat events. The other is that the bags exclude *Leptoglossus occidentalis*, the western conifer seedbug, which might feed on seeds during August. Both theories were tested during the summer of 2013.

The microclimate theory was tested by choosing 4 cones on each of 10 lodgepole pine trees in commercial seed orchards in Vernon, BC. Cones were close together, with similar aspect and tree height. A one millimetre hole was drilled into the base of each cone, and a small K-type thermocouple was inserted and held in place with zip-ties. All four thermocouples on each tree were connected to a 4-channel datalogger that recorded the internal cone temperatures at 5 minute intervals. Two of the cones on each tree were enclosed in a mesh bag; the other two were left unbagged. Internal cone temperatures were compared between bagged and unbagged cones, examining maximum temperatures and times spent above various temperature thresholds.

The seedbug theory was tested by installing one time-lapse camera in each of the 10 trees used in the thermocouple experiment. Each camera was aimed at a cluster of 3–4 cones and set to take photos at 5 minute intervals. A similar cone cluster on a nearby branch, with the same aspect and tree height, was enclosed in a mesh bag. Photos were examined for the presence of seedbugs, which were categorized by activity: feeding, walking, mating, etc. At season's end, bagged and unbagged cones will be harvested and seeds extracted to determine the extent of filled seed loss.

As of July 3, 79,206 cone temperature measurements had been taken. Mean cone temperatures were always slightly higher in bagged than in unbagged cones. Bagged cones also had more hours above any given threshold temperature, such as 35°C. There is more variation in cone temperatures between trees and between cones within a treatment, than between treatments. No statistical analysis has been conducted yet, but it appears that cone temperatures are unlikely to be the cause of the August seed loss.

By July 3, 81,425 images had been collected and examined. Of these, 1,703 images showed seedbugs on or near the cones. Feeding was a dominant activity. Each cone cluster was fed upon from 0 to 19.7 hours. Seedbugs were never observed feeding on two cone clusters. These sightings confirm previous evidence that there is a clonal difference in seedbug host preference. It also confirms the likelihood that seedbugs are responsible for the August seed loss.

However, these data only record events up to July 3. Things might change during the month of August. Bags might shield cones from extreme heat events not experienced during May or June. Seedbugs may not feed upon cones during August. Though this evidence is suggestive, we must wait until our August data have been collected before drawing conclusions.

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SEED SHORTFALL IN LODGEPOLE PINE

Samples for histological study were collected from a seed orchard located at Kalamalka Research Station, Vernon, BC. Weekly sample collections were completed between mid-July and mid-September, 2012. A big increase in damaged seed was confirmed during August. The percentage of filled seed per cone (FSPC) diminished progressively over the course of the first two weeks, reaching levels of under 50% FSPC, where it stayed until mid-September. Under a dissecting microscope, seed death occurred in two steps: death of the megagametophyte was followed by that of the embryo. Dying tissues ranged in colour from yellowish-brown to dark brown. Both gametophyte and sporophyte, i.e., embryo, were soft in texture, unlike their healthy counterparts, which were not only firm, but of much lighter hue.

Histological analysis revealed tissue degeneration in the seed. Healthy tissue was composed of storage cells that had abundant reserves of protein bodies, starch grains, and lipid bodies. The cells were tightly appressed to one another. Tissue degeneration was categorized as three types: Type I began with the appearance of tiny intercellular spaces. These spaces increased gradually in size and then the tissue developed large holes. Fungal hyphae were frequently observed in cells as well as intercellular spaces. Type II degeneration had the appearance of cell liquidization. Cell walls were dissolved and cell contents were amorphously coagulated. Yellow particulate structures were frequently observed. Type III was progressive loss of cell contents until only cell walls remained. Protein body breakdown was followed by vacuolation and nuclear disintegration. Tissue integrity failed with cells showing signs of cytoplasmic collapse and cell wall rupture. Types I and II occurred randomly at multiple loci in a megagametophyte. Different stages of Type I degeneration were sometimes present in the same tissue. Types I and II were found in samples from all collection dates, whereas Type III was observed in all dying megagametophytes that had softened tissue.

Our histological study does not support the possibility of *Leptoglossus occidentalis*-related seed loss. However, our study did not exclude the possibility of other organisms that might contribute to seed loss. The presence of yellow particles found in many of the samples suggests the presence of an unidentified organism or an unknown aggregation phenomenon. A major reason for degeneration in seed was the presence of a fungus. Hyphae were widespread in holes that developed in the degenerating megagametophyte in many of the samples. In conclusion, seed shortfall in lodgepole pine shows a progressive degeneration of the seed and there are different types of tissue degeneration which can occur in the absence or presence of a fungus and/or some unidentified particles implying that there is more than one biotic or abiotic factor responsible for seed death. High temperatures in August may accelerate the process of seed degeneration.

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METHODOLOGY TO ORGANIZE OPERATIONAL LODGEPOLE PINE CONE HARVESTS BY USING OBSERVATIONS OF SEED SET DECLINES

Poor seed set in North Okanagan lodgepole pine (*Pinus contorta* var. *latifolia*) seed orchards has been an issue for a number of years. Results from numerous trials indicate that seed set levels are acceptable until early in August. After this point seed set declines rapidly. Each lodgepole pine clone experiences this sudden loss of seed at a different point in time. We relate this period to growing degree days.

In order to increase seed production, each clone must be harvested at the correct time. If the cones from a particular clone are harvested too early seed quality is poor. If the same clone is picked after its unique seed decline period has started seed production is poor.

We are working on developing a method to identify the best time to harvest each clone. We collect weekly cone samples from throughout the summer and x-ray the seed in order to identify the onset of sudden seed decline. Relating this information to degree days will allow us to schedule our cone harvest timing so that each clone is collected when seed yield and seed quality are at acceptable levels.

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TESTING CONTAINERS FOR RESEARCH AND CONSERVATION SEED STORAGE

Once dried, seed is best stored in hermetically-sealed containers (Manger *et al.* 2003). If containers are not effectively sealed, seed will gradually absorb moisture and storage life will be reduced (Yanping et al. 1999). The seal that can be achieved is just as important as the container material. Freire and Mumford (1986), Gómez-Campo (2002) and Manger et al. (2003) have shown that many containers do not seal effectively.

There are advantages and disadvantages to the types of containers, their lids and materials such as cost, weight, and reusability. Screw lids for rigid containers allow easy access but may occasionally loosen during freezing or thawing. Much more effective are lids with natural rubber or bromobutyl seals that can be clamped or crimped onto the container. Permanently sealed containers, such as metal cans or glass vials, are not desirable when frequent access to the seeds is required. There are glass sealing jars that have been shown to seal effectively and are used in seed banks. However, these pose issues with weight and the necessity to match the quantity of seeds to the size of the jar requiring different sizes to be available, in order to minimize oxidation and ageing. Other options used by seed banks are plastic and foil bags. The bags can be cut to fit seed quantities and the air inside expelled. The bags are heat sealed and can be cut open and reused. There are also laminated types of foil bags that help to prevent punctures and leakage.

At Alberta Tree Improvement and Seed Centre (ATISC), a range of glass and plastic containers has been used for long-term storage of seed for conservation or research purposes. Glass bottles with 3 different types of screw lids and three types of plastic bottle of various sizes have been used. These containers were not designed specifically for cold storage and many do not claim to seal even in normal conditions.

This trial was set up to test the seal effectiveness of a variety of containers currently being used and being considered to store valuable and irreplaceable research and conservation seedlots at the ATISC. This test looked at ten glass and plastic containers currently used at ATISC for storage, two types of static shield plastic bags, and three types of foil bags, as well as the 'zip-loc' type bags often used for seed transportation.

Methods

The container testing method used was the same method employed by the Millennium Seed Bank, at the Royal Botanic Gardens, Kew and was originally adapted from a technique developed by Gómez-Campo (2002).

Freshly dried self-indicating silica gel was placed into test containers at 1 g per 100 ml volume. This is a small enough volume to change colour at the earliest signs of leakage and not just absorb any added moisture but large enough to be easily visible (Fig 1).

Figure 1. Example of indicator silica gel in 'zip-loc' type plastic bag showing before and after colour change from dry orange to humid green.

Nine glass and plastic bottles already in use in the research and conservation seed bank were tested (Fig. 2), as well as the heat sealed 5 mil polyethylene bags used in the Reforestation Seed Program (RSP).

Figure 2. Nine types of bottles currently being used for long-term seed storage in the conservation & research seed bank

Six other bags were also tested (Fig. 3). All bags were filled 50–70% with inert Styrofoam to help 'bulk out' the volume. When calculating the amount of silica to add, the Styrofoam was considered to be 50% air, e.g., a 200 ml bag with 100 ml of Styrofoam would receive 1.5 g of silica. The containers were sealed in the usual way at ambient conditions of 8.6–15.9% RH and 17.8–21.7°C (Fig. 4). Ten replicates were set up for each container, with the exception of the 3M foil bag which had only two reps (Table 1).

Figure 3. Seven bag types tested, including those currently used in the reforestation seed bank (140) and the 'zip-loc' type bags used in the seed lab for temporary storage (130).

Figure 4. Method of sealing bottles involved tightening of lids and wrapping the join with either electrical tape or pipe joint tape (far left).

Once sealed, the containers were transferred to high humidity conditions of 96–98% RH at ambient laboratory temperature. The colour of the silica gel was checked every two weeks for changes. Once changes in colour from orange to green were detected, indicating the seal had failed, the container was removed from the test. Before removal, verification that the container had been 'sealed' appropriately was confirmed. Opaque foil bags were not checked but were left to the end of the test.

After 4 weeks at ambient temperatures, any remaining containers were then moved to -18°C and the silica gel checked periodically for changes. The test was terminated after 70 days in -18°C.

Results

Most containers that failed did so while at ambient air temperatures (Table 1). Of the ten containers currently used for long-term seed storage, three passed through to -18°C and one with only a 90% pass.

At the end of the test, the glass bottles, two plastic bottle types and all three foil bags had passed with all replicates still indicating a hermetic seal.

Conclusion

Five types of containers currently being used in the research and conservation seed bank cannot be hermetically sealed and of the ones that passed, all are rigid container types. From a long-term conservation perspective, switching from solid containers to bags that can be cut to fit seed quantities and therefore reduce oxidation should lengthen seed life. Moving to a foil bagging system will also provide extra space and reduce weight for cold storage flooring and shelves, as well as for staff carrying seed trays.

There were four main differences between the three foil bag types tested. These were lamination, size availability, gusseting, and the ability to obtain stock. The foil bags obtained from Flair Flexible Packaging Corporation, Calgary were gusseted and after testing with small seed, it was decided that the gussets made it too difficult to remove seed from the bag. There was also a very limited size range and the largest size available was not suitable for our larger pine collections. Therefore, the Flair foil bags were eliminated.

The 3M Canada foil bag was not gusseted and did provide a larger size than Flair, although there were not many sizes overall and the large bags would need to be cut to suit with more sealed edges. However, obtaining even a few samples from 3M for use in the test proved extremely difficult and time consuming. This poor customer service plus the risk of extra sealed edges eliminated the 3M bags from consideration.

The Baltimore foil bags proved to be the best choice for many reasons in the end. In addition to passing the test, the tri-laminate provides protection for the foil from sharp seeds. The bags are not gusseted, making seed removal easy. There are four sizes available ranging from the 75 ml volume used in this test to the largest at approximately 4 L (the largest available of any tested foil bags). In addition, these bags are used by other seed banks and the company is very knowledgeable about this type of use. The only negative aspect is that these bags are manufactured in Europe. Costs were calculated including shipping for an initial large order to repackage all ~7000 research and conservation seed collections plus enough extra foil bags to last for a few years of new storage. This worked out to 18.4¢ per bag and was deemed an acceptable and competitive cost.

Update

The bags were ordered and the repackaging project was completed for all research and conservation seed collections in May 2013. All new seed collections stored in the conservation & research seed bank will be packaged in foil bags. Quality testing will be done on each new order using the above method.

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Table 1. Description and results of containers tested for seal effectiveness. Days in light green were held in high humidity and ambient lab temperature. Days in light blue were held at -18°C.

Container #	Container Type	Volume (mL)	Description	Comments	Approx. Styrofoam (mL)	Approx. silica gel (mg)	Day 12 % passed	Day 27 % passed	Day 20 % passed	Day 35 % passed	Day 70 % passed
1-10	bottle	500	Plastic, type 1	Current use	n/a	5.00	100	100	100	100	100
11-20	bottle	250	Plastic, type 1	Current use	n/a	2.50	100	90	90	90	90
21-30	bottle	125	Plastic, type 1	Current use	n/a	1.25	100	10	0		
31-40	bottle	125	Plastic, type 1	Current use	n/a	1.25	80	0			
41-50	bottle	125	glass/plastic lid	Current use	n/a	1.25	100	100	100	100	100
51-60	bottle	60	plastic, type 2	Current use	n/a	0.60	100	100	100	100	100
61-70	bottle	60	plastic, type 1	Current use	n/a	0.60	0				
71-80	bottle	30	plastic, type 2	Current use	n/a	0.30	100	0			
81-90	bottle	25	plastic, type 3	Current use	n/a	0.25	80	0			
91-100	bag	75	Baltimore heat sealed trilaminate foil bag	New	50	0.50	n/a	n/a	n/a	n/a	100
101-110	bag	75	3M Static Shield plastic bag SCC1000 - constant heater sealed	New - No instructions on sealing were provided.	50	0.50	0				
161-170	bag	75	3M Static Shield plastic bag SCC1000 - plastic heater sealed	New - No instructions on sealing were provided.	50	0.50	0				
111-120	bag	800	3M Static Shield plastic bag SCC1000 – zip-lock type closure	New	400	6.00	0				
121-130	bag	75	2 mil polyethylene bag – 'zip-loc' closure	Temp. use	50	0.50	0				
131-140	bag	800	5 mil polyethylene bag - plastic heater sealed	Storage use for RSP.	400	6.00	0				
141-150	bag	75	Flair 2oz foil bag - constant heater sealed	New	75	0.50	n/a	n/a	n/a	n/a	100
151-152	bag	250	3M Military spec foil bag - constant heater sealed	New	150	1.75	n/a	n/a	n/a	n/a	100

Container Manufacturers
Passing containers only. Prices were quoted 2011 and do not include taxes or shipping.

Baltimore Innovations Ltd.
http://www.baltimoreinnovations.co.uk/packaging-sector/barrier-foils/
Email: sales@baltimoreinnovations.co.uk
Jackson's Business Park, Wessex Rd, Bourne End, BUCKS SL8 5DT, United Kingdom
$64.00/1000

Flair Flexible Packaging Corp.
http://www.flairpackaging.com
Email: cscanada@flairpackaging.com
4100 72 Avenue SE, Calgary, AB T2C 2C1 Canada
Stand up, pouch style, bottom gusset, without zip-lock, gloss, 2oz
$73.79/1000

3M Canada
EMX Enterprises Limited (western Canada distributor)
250 Granton Drive, Richmond Hill, ON L4B 1H7 Canada
Email: info@emx.ca

Moisture Barrier Bag MILpac 131
Pricing followed up over 5 months – never received.

I can provide no ordering details for the solid containers that passed, beyond that they were ordered from Fisher Scientific. The specification and manufacture of these types of containers have changed frequently in the history of our seed bank, e.g. container shape, metal vs plastic lids, plastic lid inserts vs paper seals. The exact containers tested, although they are the most recent ordered and used at ATISC, are no longer available from Fisher.

Foil Bag Heat Sealer

Pal Distributors Inc.
http://www.palgroup.ca/distributors/machines/sealers.html
Email: calgary@palgroup.ca
3N, 5550-36 St. SE, Calgary, AB T2C 1P1, Canada
Constant Heat Foot Sealer, 12" 300CFN
$560.00

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UPCOMING MEETINGS

42nd Atlantic Forest Nursery Conference and Seed Orchard Workshop
October 1–3, 2013 Debert, NS
Contact: Blair Andres
Blair.Andres@Northernpulp.com

ISTA Annual Meeting
June 16–19, 2014 Edinburgh, UK
www.seedtest.org

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