



Tree Seed Working Group News Bulletin

76

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2025

Canadian Forest Genetics Association
Association canadienne de génétique forestière



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ArmChair Report No 76

I'd generally start out with a Happy New Year, but have cause to pause due to the redheaded elephant that is no longer in the room. It is still painful and surreal to think that Melissa is no longer with us. I lost a colleague, friend and in many ways the energizer bunny for this working group. Melissa really expanded the scope of the working group through her gregarious, answer-seeking, people connecting, friendly, intelligent and fun demeanor. Some descriptions that others have used are bright light, *force majeure* and firecracker – she was all of those and so much more. Many of you have reached out with your condolences and questions – I have no answers and am still personally drawn to the possible accidental nature of this tragedy.

Memorial services were held in both Lindsay, Ontario and Fredericton, New Brunswick. An obituary can be found at this [link](#) with a memory wall to post condolences, memories and photos. We are dedicating this News Bulletin issue to Melissa as well as our upcoming TSWG workshop this summer. Melissa was always trying to expand our working groups scope, membership and significance and she would have liked you to keep providing content for our News Bulletins and workshops. In our 75th News Bulletin Melissa thanked all the women who had helped and inspired her. She was passionate about the “[Women in Wood](#)” organization and they rebranded their logo to honour her for distribution at the hometown memorial service. We are using that as our memorial logo and graciously thank the Women in Wood organization for allowing us to use it.

Melissa visited me last June on her west coast 40th birthday trip. We went hiking to Kennedy Falls and passed this massive western redcedar en route. Always prepared, Melissa was able to find the nearby geocache and left a Tree Seed Centre pen behind in place of a coin she took for her brother. I have lots of great memories of Melissa which I will cherish.



The show must go on and that is certainly what Melissa would have wanted. I am extremely grateful to Fabienne Colas who agreed to do the desktop publishing for this edition – Thank you Fabienne. Unfortunately, we lost

another retiree on February 4th, when Dr. Cheng Ying passed away. He was the BC provenance forester who was instrumental in early seed transfer guidelines and previously worked at Petawawa National Forestry Institute.

Planning is underway for this year CFGA meeting in Ottawa and our associated workshop. This year we are jointly putting together a workshop with Rob Keen and the [Canadian Tree Nursery Association](#). It makes sense to join forces versus having two separate sessions with widely overlapping audiences. The title of the workshop is “**Growing Resilience: The Power of Seeds and Seedlings**” and planning is underway. Updates for the workshop and conference can be found at this [website](#). Or use the following QR Codes.



English



Français

In this time of fiscal uncertainty, it would be helpful for planning purposes to have an indication of people planning to attend the TSWG/CTNA workshop or CFGA conference. It won't be written in stone, but very helpful as planning progresses. You are also welcome to suggest topics of interest you would like to see covered this summer or in future News Bulletin editions.

Part of workshop the day will be a member only CTNA Annual General Meeting for approximately two hours. The TSWG will use this timeslot to do a bit of navel gazing, draft a course for our future and possibly focus on some seed specific topics. I've served as Chair of this Working group for the past 25 years and plan on retiring within the next year. I expect to stay involved with tree seed in some capacity, but will not be dedicating as much time to this working group or the CFGA in the future.

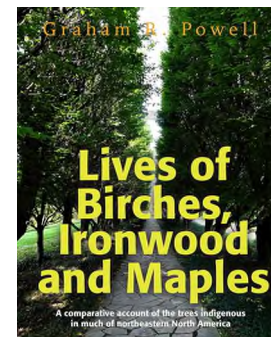
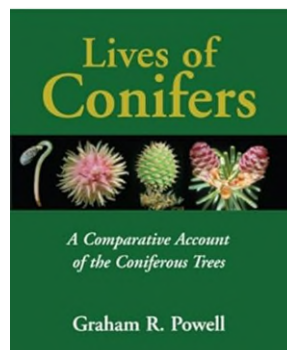
Last, but certainly not least I'd like to thank everyone who has contributed to this News Bulletin and encourage you to contribute your unpublished research, your unsolved problems, meeting summaries, acknowledgements and obituaries – really anything to do with tree seed. It's a stormy period for forestry in Canada, so anything we can

do to help increase knowledge and efficiencies brings some sun rays through the clouds. The deadline for our next News Bulletin will be shortly after our workshop in Ottawa on August 11th. Any questions related to content, please contact me. Thank you.

Dave Kolotelo

TSWG Chair

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The Passing of Dr. Graham Powell

It is with great sadness to be informing you of the passing of Dr. Graham Powell on February 28, 2025. You can find an obituary at this [link](#). Graham was a Professor of Forestry at the University of New Brunswick and chair of our Tree Seed Working Group from 1987 to 1991, spanning News Bulletins 8 to 15. At our 2023 workshop in Vernon, Graham, at age 88, virtually presented on [Reproduction in Balsam fir](#). The presentation was recorded by Melissa as they had developed a friendship as part of Melissa's search for knowledge, which Graham still had much to share. It is weirdly and sadly coincidental to be paying tribute to both of them, although there was a 50-year age gap.

There is a more personal connection as Graham was the first person to hire me as a summer student, primarily as a research assistant to his graduate students Kathy Tosh and Guy Caron (who also was a former TSWG chair). That was my beginning in conifer reproductive biology and my interest in that topic has been maintained and even increased lately with the lack of individuals invested in this topic. Graham was one of our most revered professors due to his exacting, intense and demanding demeanor. I still have his classic second year "Tree Development" and "Dendrology and Silvics" notes from 1982. For those lucky enough to have him as a teacher I know you all have special memories. For those not so lucky he did leave a legacy with his 2009 book, *Lives of Conifers: A Comparative Account of the Coniferous Trees* initially published in hardcover, but now also available in a softcover version.

On October 1, 2023 his second book was published – *Lives of Birches, Ironwood and Maples: A comparative account of the trees indigenous in much of northeastern North America* which is currently only available in hardcover.

Lengthy titles, but I know Graham would appreciate not having them abbreviated. You made great contributions to tree biology -Thank you.

Dave Kolotelo

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National Tree Seed Centre Update

I'd like to take the opportunity to introduce myself to the Tree Seed Working Group. My name is Darren Derbowka and I am the new Coordinator of the Canada's National Tree Seed Centre (NTSC). I started with NTSC in June of 2024 and had the great honour of being Donnie McPhee's understudy until his retirement this past December. Prior to moving to Fredericton, I worked for the Ontario Ministry of Natural Resources and Forestry (OMNRF) for the past 25 years with the Forest Research and Monitoring Section (FRMS) located at the Ontario Forest Research Institute (OFRI) in Sault Ste Marie, Ontario. I fulfilled several roles during this time, most recently acting as Science Coordinator and Science Operations Supervisor for the FRMS management team. Prior to this I was the Growth Facilities Leader, providing leadership and operational support to the research greenhouses, Arboretum, and the Ontario Tree Seed Archive program. I also worked as the Genetics Project Forester for the Forest Genetic Diversity program, providing operational and science support for the white pine blister rust breeding program and running the somatic embryogenesis laboratory.



The NTSC is part of the Canadian Forest Service within Natural Resources Canada. Our primary operational mandate as Canada's forest genetics genebank is obtaining a representative collection of seed from every tree and shrub species across the country. We are targeting 15 to 20 individual tree collections from each ecodistrict of species' occurrence, with a minimum of 5000 seed per seedlot. Although we are interested in all tree and shrub species, we attempt to prioritize collections from threatened species with eastern hemlock (*Tsuga canadensis*) and black ash (*Fraxinus nigra*) being our targets in 2025. In addition, we support Canada's 2 Billion Tree (2BT) program through knowledge mobilization activities that assist proponents, professionals, and the general public alike with education and training to ensure that genetically appropriate and high quality seed are utilized in tree planting projects. Through additional support from 2BT, NTSC also launched the Indigenous Seed Collection Program (ISCP) during the spring equinox of 2022. The aim of the ISCP is to assist Indigenous communities by supporting their seed collection and conservation objectives for species of commercial, cultural, and medicinal importance. Since the program started, NTSC has travelled across the country and met with more than 75 communities and 230 participants with plans to meet with even more communities in 2025. For more information about the NTSC and the ISCP, please visit our [website](#).

I am very excited to be part of the NTSC team and in this new role. Sadly however, nothing could have prepared me for the tragedy last August. I met Melissa several years prior, through my work at OFRI and earlier by way of her reputation. Her vibrant passion for seed collection and conservation was an immediate and palpable energy right from our very first conversation. She was the first person at NTSC to graciously tour me around Fredericton, regaling me with her stories of wine, food, garlic farming, greenhouses, and (mostly) seed. We shared more than one single malt beverage as we talked about her ideas and aspirations, particularly with regards to her Master's thesis. She was quickly becoming a good friend to both my wife and me.

The shocking news struck me during the first leg of the Indigenous Seed Collection Program trip across the country. Standing in a truck stop parking lot east of Ottawa over come by grief, confusion, and helplessness, the journey home was long and emotional. The emotional

impact to the entire NTSC family was immense and devastating, as Melissa had been an integral part of our team for almost 5 years. Her absence has been sorely missed every day since on both a personal and professional level, as she brought a passion and energy to the NTSC that was second only to her depth of knowledge and connection to the seed conservation world.

It was initially hard to imagine the NTSC going on without her influence, as many of us wrestled with confusion, guilt, and sorrow at the thought of carrying on with our daily work. Tragedy has a sad way of pulling people together though. To truly keep Melissa's spirit alive, we knew in our hearts that every seed we collected connected us to her memory. To say that "Melissa would have wanted it this way" may seem colloquial, but we all know what her work meant to her and the importance she attributed to forest conservation. So, we persevere.

The Tree Seed Working Group was something that Melissa was deeply passionate about and many (if not all) of you had the privilege of knowing, learning, and/or working with her, which stands testament to the impact she has made to the seed collection world. She was an inspiration to all of us and a measure of what could be attained when your heart is in the right place. She will be forever missed.

Darren Derbowka

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BC Tree Seed Centre Update

In the last News Bulletin I provided an update on our freezer and cooler expansion project and promised an update. We are about 98% complete with a few minor outstanding items. The new and older exterior of the brick framework for the freezers and coolers was covered in a vapor barrier, rock wool insulation and then covered with wood cladding (Figure 1) which amounted to an insulation value increase of R12.

We also have a new backup generator on site able to run our facility for a minimum of four days before needing to be refueled. The new (and updated for older coolers and

freezer) HVAC systems have redundancy built in with two systems alternating in a lead/lag configuration. Modernization also included a conversion from open drive belt driven compressors to scroll type compressors which are on our rooftop (Figure2).

These are considerably quieter and far more energy efficient than our previous compressors. Another major update is found with our environmental monitoring systems that provide much more detailed information for monitoring and identifying problems. Figure 3a shows our full system overview and Figure 3b is a more detailed look at information provided for one of our six freezer vaults.

We are currently using our new cooler space and it has been far more efficient to have dedicated cooler (2-5 °C)

spaces for seedlots at various stages of processing (Figure 4a) and seed stratification (Figure 4b). As freezer completion occurred after we started this production season we are waiting until spring/summer to move the seed inventory into our new freezer vaults.

Dave Kolotelo

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Figure 1: a & b. The new and older exterior of the brick framework for the freezers and coolers was covered in a vapor barrier, rock wool insulation and then covered with wood cladding.



Figure 2: Enclosed scroll type compressors on the roof.

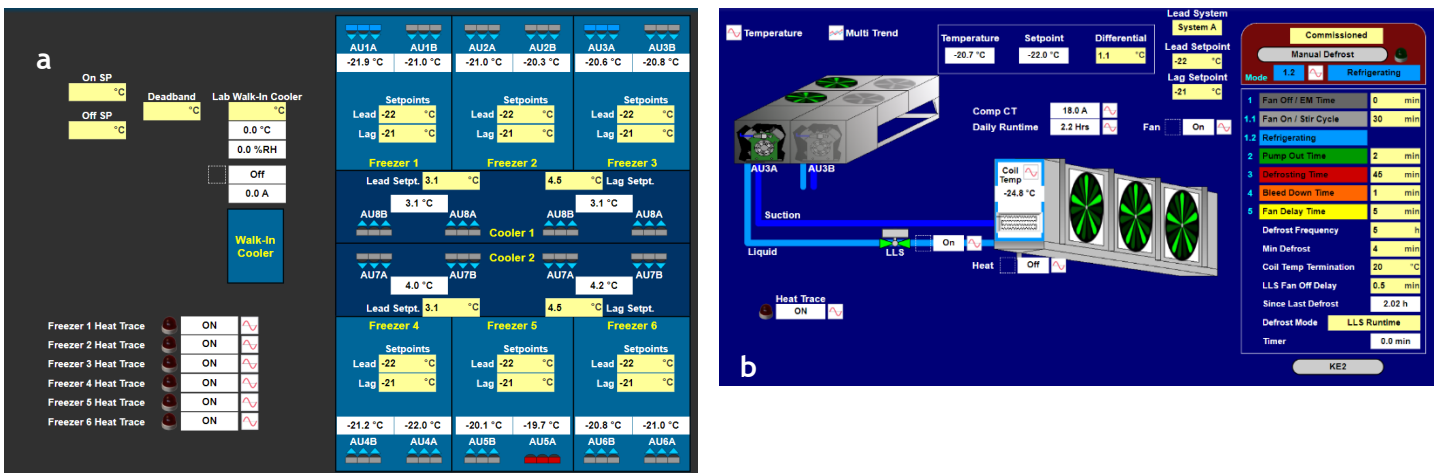


Figure 3: a) the environmental monitoring system a) for the entire freezer and cooler system ; b) a detailed view of information provided for one of the freezers



Figure 4. New cooler space a) dedicated to seedlots at various processing stages and b) our cooler dedicated to seed in stratification.



BC Tree Seed Centre Staff, March 2025

Pines and People: Human Impacts on Five-Needle Pines.

Whitebark pine Science and Management Conference Summary. October 11-14 2023, Revelstoke BC

Around 150 five-needle pine enthusiasts convened in Revelstoke, BC to attend a conference co-hosted by the Whitebark Pine Ecosystem Foundation of Canada and Parks Canada. A field trip to the Kalamalka Research Station in Vernon to see practical grafting demonstrations, and a field tour of whitebark pine at Kicking Horse Mountain resort in Golden bookended the conference with two days of talks in between. Twenty-six talks and 14 posters addressed the key theme of human impacts on five-needle pines. There was plenty of time for participants to share ideas and connect with at a social at Begbie Brewery, an evening of live music provided by the Tulsa Naturals and various breakout sessions during the day.

Participants left with novel ideas, new connections, and a renewed sense of energy to move forward on key conservation and restoration themes. Many thanks to all the organizers and sponsors that made it possible to host the event. More information as well as Abstracts and Presentation from the conference can be found [here](#).



Field demonstration at Kalamalka. Photo: Natalie Staffl/Parks Canada



Conference venue in Revelstoke. Photo: Teodora Rautu/WPEF



Field Trip to Kicking Horse Mountain Resort. Photo: Kevin Lavelle

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An X-ray Scanner Darkly: Estimating Germination in Whitebark Pine

The BC Tree Seed Centre (TSC) stores seed of the endangered whitebark pine, *Pinus albicaulis* Engelm., (henceforth PA) for operational planting, blister rust screening and genetic conservation purposes. The TSC provides viable seed estimates based on x-ray image analysis for white bark pine seed due to its high value and scarcity. We x-ray our PA seed for 12 seconds at 20 kilovolts. A random sample of 100 seeds from a seedlot is split into two replicates of 50 and each replicate is imaged separately. Images are assessed to categorize every seed into viable or non-viable. The total viable seed from both images comprise the viable seed percent estimate. With the appropriate dormancy breaking treatment, the viable seed estimate should be a good approximation of germination. This process relies heavily on subjective evaluation of seed viability. To improve accuracy of x-ray germination estimates a trial was initiated tracking individual seeds from x-ray, through stratification to germination test conditions.

Single Seed Trial

Seed with questionable viability was selected from a variety of standard x-rays on PA samples destined for the conservation seedbank storage in 2022 and 2023. Every seed was individually x-rayed and assigned a number for

tracking through the trial. To break dormancy seed was soaked in running water for 72 hours and then spent 56 days in warm stratification (20 °C) followed by 112 days in cold stratification (2-5 °C). The seed was then transferred to a germinator on a cycle of 30° C with light for 8 hours and, 20° C & dark for 16 hours and monitored for 28 days. Germinants were counted and removed when the radicle was greater than 4x the seed coat. Abnormal germinants, seed that germinated but wouldn't result in a viable seedling, were categorized, and counted as having not germinated. The traits examined in this trial are some of the factors we consider while assessing viability and include:

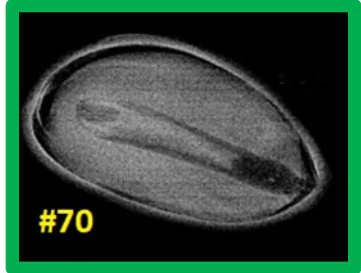
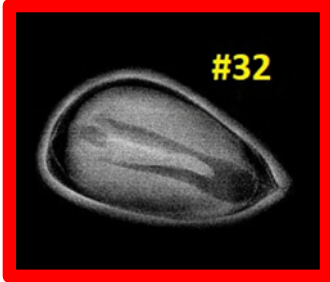
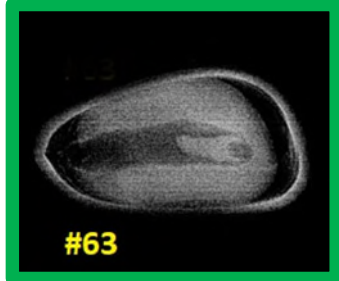


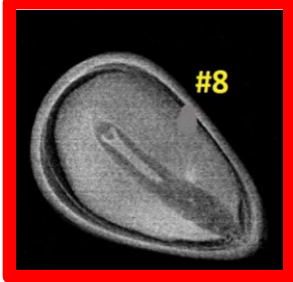
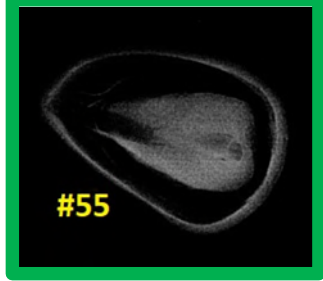
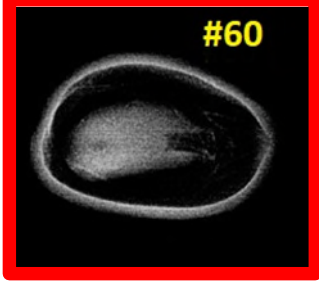
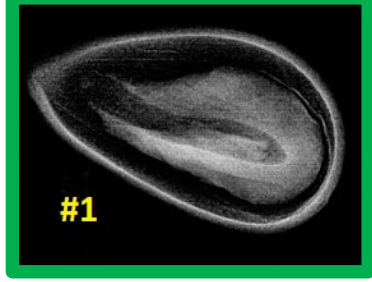
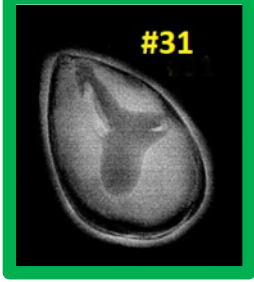
- embryo length relative to corrosion cavity (>50% has been the criteria we have used to help separate viable and non-viable seed).
- embryo width (some seed have uncharacteristically thin embryos, an example of embryos classified as skinny can be seen in Table 1.)
- Megagametophyte health categorized based on visual estimate of the volume within the seed coat that consists of empty space: “bad” megagametophyte status (>50% volume empty), “concerning” (30-50% volume empty), “Potentially Viable” (0-30% volume empty).

Data was analyzed using a chi-square test under a null hypothesis that there would be no differences in germination capacity between categories for any of the traits observed and an alternative hypothesis that there would be differences between the categories for a trait.

Results

The trial followed 112 seeds. Seven seeds germinated during warm stratification, 44 seeds during the germination test, and 10 seeds germinated after the trial concluded. All seeds that germinated normally, no matter which temporal category, were counted as germinants. Seven seeds germinated abnormally and are analyzed as having not germinated. X-ray images of germinated and non-germinated seed for each trait can be seen in Table 1. Seed #70 is an example of what we would consider a viable seed with the typical immature embryo. Germination outcomes from the trial are presented in Figure 1.

Table 1: X-rays from some of the categories of the questionable seed across the traits we observed in the trial. A green border indicates a seed that germinated and a red border one that did not germinate.

<p>Potentially Viable Seed</p> <p>#70: Viable looking embryo and megagametophyte.</p> <p>#32: Viable looking embryo and megagametophyte.</p>		
<p>Embryo <50% Corrosion Cavity</p> <p>#63: Embryo only 1/3 corrosion cavity</p> <p>#29: Embryo just short of 50% corrosion cavity</p>		
<p>Skinny Embryo</p> <p>#19: Skinny embryo but otherwise viable appearing seed.</p> <p>#8: Skinny embryo with embryo length only about 50% corrosion cavity.</p>		
<p>Megagametophyte Abnormal</p> <p>#55: Significant space between megagametophyte and seed coat. Some irregularity in shape.</p> <p>#60: Significant space between megagametophyte and seed coat.</p>		
<p>Multiple Issues</p> <p>#1: Poor Megagametophyte health and an embryo less than 50% corrosion cavity.</p> <p>#31: Small embryos and a misshaped corrosion cavity. Seed germinated after trial concluded.</p>		

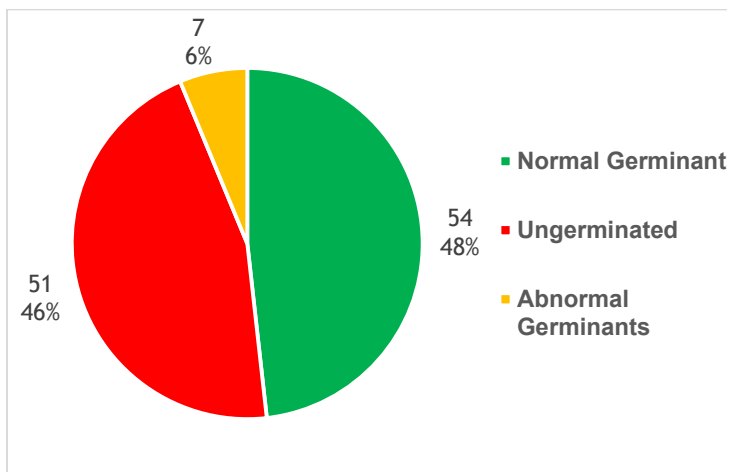


Figure 1. Pie chart showing the germination outcome of all questionable seed included in the trial.

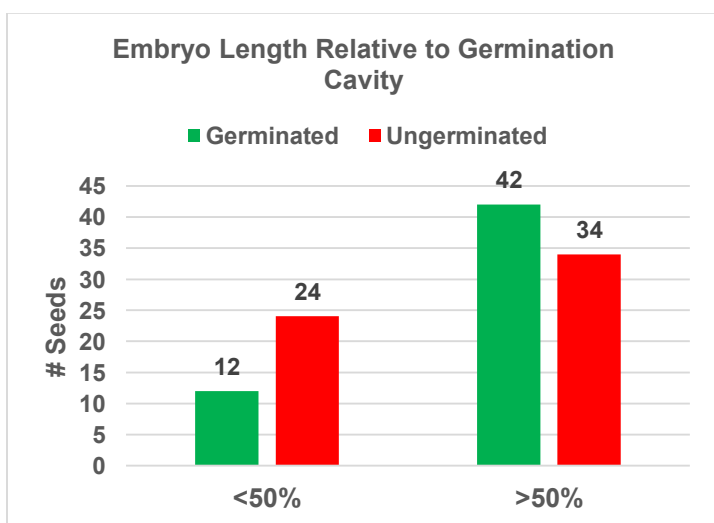


Figure 2. Bar plot showing seed germination outcomes for those meeting and not meeting the embryo >50% the length of the corrosion cavity criterion. P-value of 0.030 from a chi-square test comparing the categories.

Figure 2 illustrates the difference in germination capacity between seed meeting and failing the 50% embryo length relative to corrosion cavity that our lab uses to classify if a seed is viable. Of the 112 seeds involved in the trial, 36 (32%) have an embryo length less than 50% of the corrosion cavity and 12 of these germinated. Slightly over half of the seed with embryos >50% germinated while only a third of those with embryos <50% germinated, but seed in this category had other characteristics causing them to be considered questionable. A chi-square test yielded a P-value of 0.030 indicating that there is a

significant difference between the germination capacities for seed with embryos greater than or less than 50% the length of the corrosion cavity.

There were 32 seeds identified as skinny during the trial. Seed with skinny embryos achieved a germination capacity of 50% compared to the 40% achieved by the normal width embryos. A chi-square test yielded no significant differences between the skinny and normal embryo and a P-value of 0.81. Figure 3 shows the data for both skinny and normal embryos.

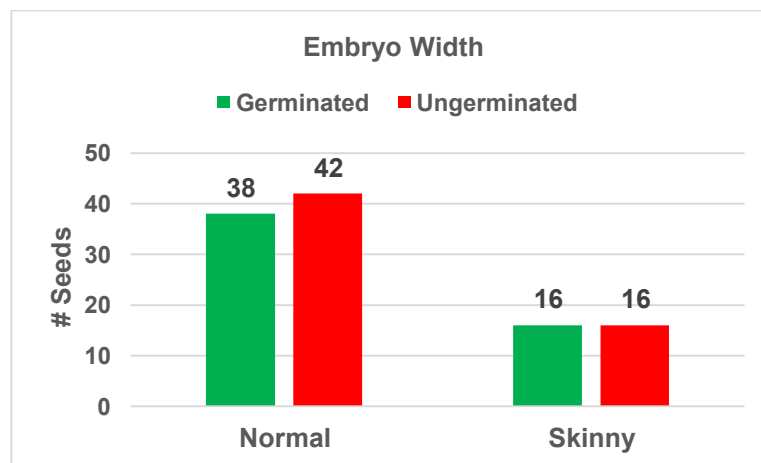


Figure 3. Bar plot showing the germination outcomes for seed with normal and skinny embryos. P-value of 0.81 from a chi-square test comparing the categories.

There were 18 seeds in both the “Bad” (>50% volume empty) and “Concerning” (30-50% volume empty) megagametophyte categories and 76 in the Potentially Viable (0-30% volume empty) category. No significant difference was found between the categories (P-value of 0.13), perhaps due to the low sample size in some categories. Figure 4 does show an observed reduction in germination for both the “Bad” and “Concerning” categories. This reduction is large enough in the “Bad” category to be operationally significant even if lacking statistical significance. We also performed a chi square test grouping the “Concerning” and “Normal” categories together which had a P-value of 0.058.

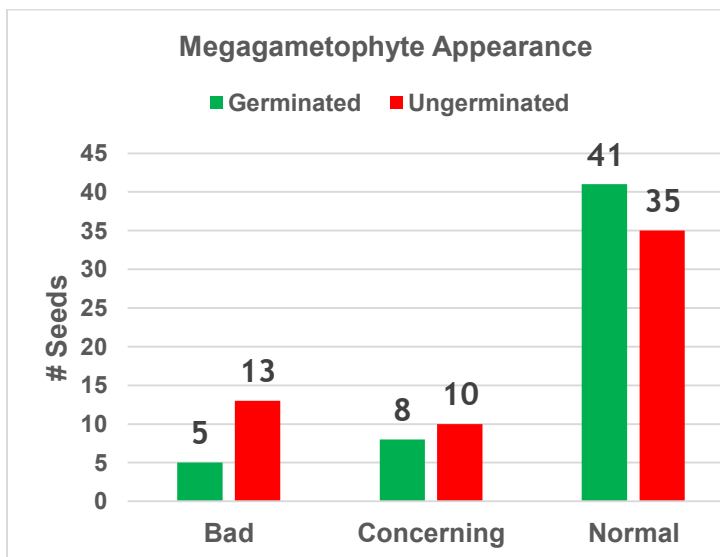


Figure 4. Bar plot showing germination outcomes for seed from the megagametophyte health categories. P-value of 0.13 from a chi-square test comparing the categories.

Discussion

Most seeds are relatively easy to classify as viable or non-viable. Only seed with questionable viability were included in the trial and as such germination capacities are not representative of typical seedlots. Some seed included for one trait may have confounded results for other traits as all seed was included in the analysis of every trait. This is the first attempt we are aware of to establish visually distinguishable X-ray criterion to assess PA seed viability, and we will continue to build our database of seed images to improve accuracy going forward.

The trial will inform lab policy on viability assessment for germination estimates. Embryo length being less than 50% of the corrosion cavity was found to be a reasonable metric for excluding seed from being considered viable. Seed with an embryo length >50% corrosion cavity achieved a total germination percent of 55.2%, the highest of any category we observed. We found that having a skinny embryo is not a valid reason to classify a seed as non-viable. Irregular shape of the megagametophyte and volume of space between the megagametophyte and seedcoat are the most visually captivating factors when examining x-rays. Megagametophyte health had the greatest variability and some of the most surprising germinants (seeds #1 and #55 in Table 1). While no significant differences were found there is an observed reduction in the germination capacity for seed exhibiting

megagametophyte health concerns (~27-44% germination versus ~54% in 'Normal' appearing seed). Too much emphasis on megagametophyte health can potentially lead to underestimation of viable seed as although there is an observed reduction in germination it is not large drop between the 'Normal' and 'Concerning' groups.

PA seed considered non-viable based on embryo length or megagametophyte health for the purposes of germination estimates may still be capable of germination but at reduced frequency. It is therefore important not to screen this seed out during seed processing. We had several surprising germinants during our trial such as numbers 1, 31, 55, and 63 which can be seen in Table 1. At the end of the trial seed was retained for cut tests that occurred 6 months after the germination test concluded. During this time several germinants were observed, this is in line with observations from nurseries in which seed may be retained an additional year for a second flush of germination due in part to the deep dormancy occurring in this species (D. Kolotelo, personal communication, February 17, 2024). This deep dormancy may account for some of the variance we saw with early and late germinants. Some seed exhibiting significant megagametophyte health or embryo size concerns in the x-ray appeared very healthy during cuts. It is likely that the issues observed with embryo size and megagametophyte health are related to maturity and dehydration and thus salvageable through proper imbibition with warm and cold stratification protocols. It is difficult to visually distinguish between immature/dehydrated seed and deteriorating seed on an x-ray, so determining which are salvageable is a problem.

Germination estimates are of benefit to growers in approximating seed need to meet seedling requirements. The accuracy of the estimate derived from x-ray analysis for PA is less accurate and precise compared to commercial conifers due to the subjective evaluation. Some seedlots have very little questionable seed and others have many leading to varying amounts of subjective judgement required. Overestimation of the germination is likely since even viable seed may not germinate.

The trial spanned nearly two years and a great deal of care was put into selecting seed since it takes nearly 200 days from soaking the seed to collect results. In the future more trials will be conducted further examining these traits

quantitatively and other traits such as cotyledon length or degree of development. If readers here have ideas for what traits observable in x-rays may impact germination capacity, please reach out! We would also welcome any germination results from nurseries which have sown registered PA seedlots.

Bendix Hollmann

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2024 BCSOA Conference

The BCSOA (British Columbia Seed Orchard Association) hosted its biannual meeting on June 18th and 19th in Sidney, BC. There were 60 people in attendance from BC, Alberta, the USA and even a few farther afield from England and France. The meeting got started with an evening icebreaker on June 17th at Small God's Brewing Company which was well attended and a fun time had by all. The meeting was then split into an indoor conference day and evening banquet on June 18th and a tour day on the 19th.

The conference day was divided into three main sessions:

1. Climate Change and Adaptation
2. New Technologies for our Practices
3. Program Updates and Genetics



The conference booklet which contains more details, presentation pdf's and photos can be viewed at this

[weblink](#). The evening entertainment featured a wonderful meal and live music provided by the band The Smokestacks. We were also able to offer game 5 of the Stanley Cup finals on the big screen for those that still had hope.

The tour day started at the Pacific Forestry Centre to view some of the ongoing research projects directly related to drought and disease resistance. Many of these are collaborations with geneticists. We also visited [Applied Bio-nomics](#) which conducts research and produces biological control agents for industrial use. Their webpage has a great deal of additional resources, so if interested in this topic it is worth checking out. We visited the Western Forest Products Saanich Forestry Centre facility that includes a production nursery and several seed orchards.



The final stop was The Ministry of Forests orchard site in Saanich where Mark Vendrig of [Precision Crop Tech](#) provided a discussion of drone use and a demonstration of their drone spraying technology.



The meeting went very smoothly, and I'd like to acknowledge our organizing committee: Rut Serra, Corey



Mathieson; Chris Halldorson, Brian Barber, Dave Kolotelo, Abbi Vernier and Sanya Nar. The next meeting of the BCSOA will occur in Northern BC in 2026.

Dave Kolotelo

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2024 Forest Nursery Association of British Columbia Annual Conference

On September 11th-13th, 2024, the Forest Nursery Association of British Columbia (FNABC) held its 42nd Annual Conference in Vernon, BC. Over 100 attendees from BC and Alberta forest nurseries, suppliers, Canadian and US agency representatives, gathered in-person for the three day event. The meeting focused on a number of major themes and presentations: seedling demand and the Canadian Tree Nursery Association; Protected Agriculture Stewardship Program; workplace safety; panel sessions on container alternatives and loose pack/wrapped stored seedlings; pesticide trials & regulations; tree seed dormancy release; seed orchard seed supply; IPM & biocontrol alternatives.

Two awards were presented and two scholarship recipients announced at the conference banquet. The first is the Green Timbers Award by which the forest nursery community recognizes the history and major contributions of an individual or organization to reforestation in British Columbia. The 2024 recipient is Don Pigott for his unwavering contributions to all manner of seed management in BC. The Chief Forester's Award is a competition award presented by the Chief Forester or designate to the nursery that succeeds in producing the best specification seedlings of a BC forest species and stocktype chosen by the AGM Organizing Committee. The 2024 winner was Landing Nursery Ltd. located in Vernon, BC. The two 2024 scholarships were awarded to Emma Lloyd and Teagan Findlay. Congratulation to all award winners for their past, present and future contributions to the forest nursery sector.

The conference rounded out with post-conference tours of the PRT Vernon nursery and the Ministry of Forests Kalamalka Seed Orchard and Forestry Centre. The agenda is available from the FNABC website: [Final Agenda](#)

This conference would not have been possible without the generous support from our industry and agency sponsors, the Organizing Committee, and several volunteers. The FNABC would like to thank all for their dedication and contributions. Thank you to all the speakers for their submitted presentations.

The next FNABC Conference and AGM will be held from September 23rd-25th, 2025 at the Mary Winspear Centre in Sidney, BC. Please consult the FNABC website for future updates in the Spring.

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Goodbye Old Friend

At [Yellow Point Propagation Ltd](#) we have recently sold our old S47D (Super) (Figure 1) to Mitchell Farms in Victoria.



This was the first seed cleaner the BC Forest Service bought in 1957 and is made out of solid oak. It was the main seed cleaner used until the provincial Tree Seed Centre moved to Surrey in 1986. I bought it on BC Bid in 1987. The S47D purred like a kitten. It was a little harder to clean out but was fantastic for large seedlots. It was also not easy to remove from our processing building as illustrated in Figure 2. I also bought an M2B (also a 1957 model) which is still used in seed processing.



Mitchell Farm's grows and malts barley for many of the breweries in Victoria. (They won 2 silver's and one bronze in the malt Olympics last year!). They already

have the sister of our seed cleaner and going to a good place to be used operationally! After almost 40 years in the seed cleaning business I'm not closing shop, just trimming the equipment space. My reflections on this industry resulted in a presentation to the BC History Society titled "[Seedy Business](#)" which was posted on Youtube

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Conservation Challenges for Seaside Juniper, *Juniperus maritima*, a Dioecious Conifer Endemic to the Salish Sea

Seaside juniper, *Juniperus maritima*, is one of the few dioecious conifer species in Canada. Trees grow on sand or on granite. Seaside juniper's range is also restricted to that region of the Salish Sea basin characterized by a Mediterranean climate, which includes islands and adjacent shorelines (Fig. 1) found along the waters of Georgia Strait, Puget Sound and some of the Strait of Juan de Fuca (Gleaves, 2018). Trees tolerate salt spray and drought. Because of some unique morphological features, an ability to reproduce under natural conditions, and reproductive isolation from other taxa in this group of juniper species, *J. maritima* was recently recognized as a species (Adams, 2007). In addition to sea level populations, the species can be found up to 1700 m on the Olympic Peninsula. These trees are thought to be descendants of refuge populations during the Vachon Glaciation that covered the Salish Sea during the Pleistocene (Adams et al., 2010).



Figure 1. Nearly pure forest of seaside juniper (*Juniperus maritima*) on Shoal Island, Vancouver Island BC

Seaside juniper was and is still culturally important to local First Nations (pers. comm., Nancy Turner). It was planted as a medicinal plant (Pojar et al., 1994). Branches were used to safeguard houses from illness, e.g. they were hung in the house, or placed under mattresses (Turner and Hebda, 2012). Branches have a strong pleasant aroma and continue to be used in ritual ceremonies connected with sweat lodges. (Charlie and Turner, 2021). Indeed, the type specimen for the species is to be found on Tsartlip First Nation’s land along Brentwood Bay (Adams, 2007).

Colonists were attracted by the aromatic quality of the wood, as well as the heartwood’s pink colouring. Seaside juniper has been extensively logged, which has resulted in a drop in numbers over recent decades (pers. comm. Don Piggot).

The species is considered to be reproductively limited (Adams et al., 2010; Gleaves, 2018), not unlike other some juniper species in which problems associated with seed biology, such as long stratification requirements, high insect predation and low yield have been identified (Scianna, 2001; McCartan and Gosling, 2013; Wilkins and Duckworth, 2011).

Like its close relative, *Juniperus scopulorum*, *J. maritima* has berry-like glabuli that house their ovules, which are wind-pollinated. Seeds mature in the following year. Adams (2007) noted that seed were exserted (Fig. 2), meaning they appear to push out of the mature berry. He used this as a taxonomic character, but as you will learn in this note, exserted seed has little to do with taxonomy and more to do with entomology. Mature seed cones may remain on the tree for up to three years.

We will consider seaside juniper seed from three different points of view: 1. the proportions of gender in this dioecious species, 2. exserted seed and what it really means, and 3. what estimates of filled seed per cone can tell us about population differences.



Figure 2. Photo of seed cones of seaside juniper, one of which has exserted seed (e).

Materials and Methods

Trees and Seed

We studied trees in and around southeastern Vancouver Island from the following locations: various parks in Greater Victoria (Copley, Gyro, Nymph Point, Summit), locations along the Vancouver Island seashore (Brentwood Bay, Deep Cove, Shoal Island, Yellow Point) as well as by Mesachie Lake and Gordon Bay, Lake Cowichan. In addition, we looked at trees in the Gulf Islands, including Winter Cove, Saturna Island; Montague Harbour, Galiano Island; Roesland Gulf Islands National Parks Reserve, Pender Island (Fig. 3). The gender of each tree was recorded.

We collected seed from a subset of these locations: Copley Park, Deep Cove, Mesachie Lake, Shoal Island, and Yellow Point. Cut tests were done to measure filled seed per cone. Empty or aborted seed was assessed for the type of damage.

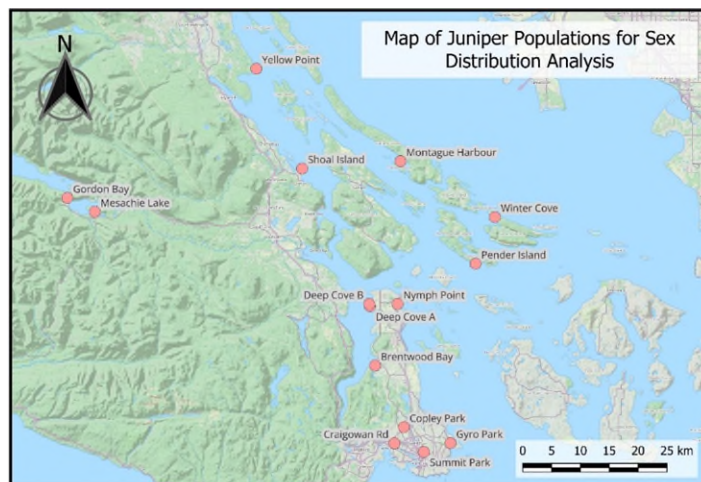


Figure 3. Map of seaside juniper populations analyzed for sex distribution. Study area included Central and Southern Vancouver Island, as well as Galiano Island, Saturna Island, Shoal Island and Pender Island.

Results and Discussion

Dioecious trees and gender

Trees were either classified as trees having cones that produced pollen, trees having cones that produced seed, or trees that had neither pollen nor seed cones and were classified as undetermined (Table 1). Dioeciousness was generally well-maintained. One out of 405 that we assessed had a branch of seed cones on an otherwise pollen cone-bearing tree. It was not included in the table. A large percentage (44%) were undetermined, meaning the functional gender could not be determined: these trees lacked pollen cones and seed cones. Some of these were young and likely sexually immature. However, most were older trees. As this was a single-year survey, there was no way to know whether these trees were just non-reproductive in our survey year. Given that junipers hang on to their cones for more than a year, these trees were likely to be unproductive for more than just this year. A longer-term study is required to reveal whether these trees are capable of reproduction.

Overall, the ratio of functionally female trees (seed cone-bearing) to functional male trees (pollen-cone bearing) favoured the former (60:40). This figure should be treated with caution. There are so many undetermined trees that one year's worth of sampling may provide only limited information. A five or ten-year study would be in order. We know little to nothing about masting or other related phenomena in this species. By comparison, other dioecious gymnosperms typically have a high male to female tree ratio (Walas et al., 2018). Studies of sex bias in angiosperms have found that it may be influenced by clonal reproduction (Timerman and Barrett, 2019). In seaside juniper natural layering occurred in some populations of older seaside juniper trees, i.e. rooting of branches and subsequent establishment of ramets. This possible contribution to sex bias merits closer study. Among the undetermined trees, few were juvenile.

Southern Vancouver Island populations generally had few trees. Most locations had just one individual. The biggest populations were on the southern end of the island at Deep Cove and Brentwood Bay. These had up to 18 individuals.

These larger populations at these two locations had a female to male percentage ratio of 70:30. The Gulf Islands juniper population numbers were highly variable. It was observed that almost all of the trees to be found were in reserves, such as parks.

We found naturally occurring nurseries of small trees in the Mesachie Lake populations. These were limited to a few dozen small saplings. Elsewhere juvenile plants were rare. Taken together, the lack of juvenile trees implies problems of recruitment in the populations that we studied. A more detailed survey should be undertaken of reproductively mature trees, which would allow us to compare central and northern Salish Sea populations with those studied by Gleaves (2018) in the southern Salish Sea. He estimated that seaside junipers were slow to mature: trees only had their optimal reproduction when they were between 50 and 200 years of age.

Table 1. Number of pollen-bearing and seed-bearing individual trees, as well as undetermined individuals. Juveniles and trees lacking pollen and seed cones were classified as undetermined.

Location	Type of Cones			Population Size
	Pollen	Seed	Undetermined	
Vancouver Island				
Gyro Park Victoria	0	0	1	1
Summit Park, Victoria	0	1	0	1
Craigowan Rd, Victoria	1	0	0	1
Nymph Point, Victoria	1	0	0	1
Deep Cove Beach Area 60	2	5	1	8
Brentwood Bay	5	4	0	9
Copley Park, Victoria	0	1	0	1
Deep Cove Beach Area 59-58	3	15	0	18
Mesachie Lake	4	6	19	29
Mesachie Mountain	12	20	114	146
Gordon Bay	2	1	2	5
Shoal Island	20	33	9	62
Yellow Point	27	37	3	67
Gulf Islands				
Winter Cove, Saturna	10	7	23	40
Montague Harbour, Galiano	1	2	0	3
Roesland Park, Pender Island	3	4	5	12
Total	91	136	177	404

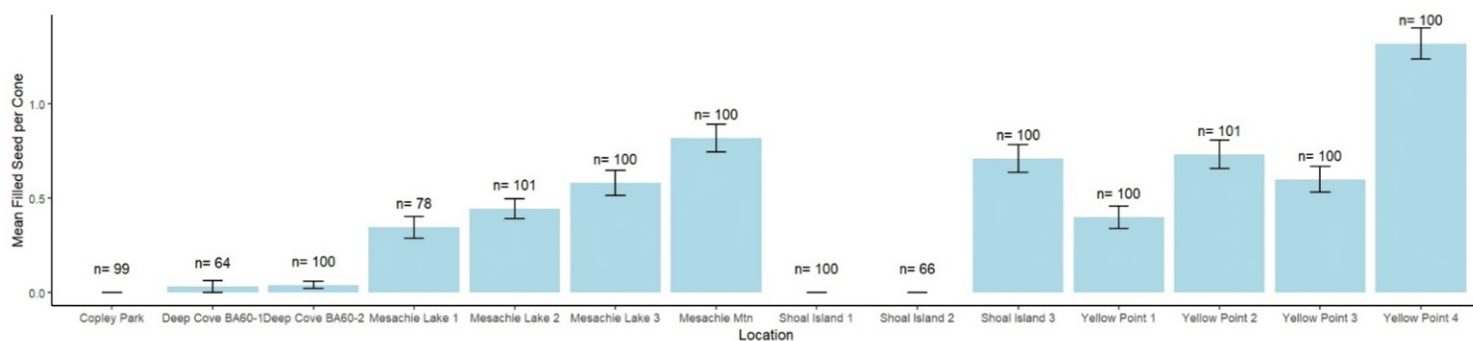


Figure 4. Filled seed per cone (FSPC) at 14 Vancouver Island locations. FSPC was determined through the cut-test performed on each cone. Locations are ordered from south to north. Mean \pm s.e.

Exserted seed

As seed cones aged, their colour changed. Initially, mature cones with viable seed were yellowish-green to green; by the second year they had turned purple. Exserted seed were occasionally seen on green seed cones, but mostly were found on purple cones. Of the nearly 1300 seeds, two-thirds had round cones, and only a third had cones with exserted seed morphology. Cut tests revealed that of 476 cones of the exserted seed cone phenotype, only one had filled seed. The remaining 475 were filled with dead seed. The seeds of these cones were consistently insect-infested. Such a high percentage of exserted seed present in seaside juniper cones, inevitably raises question of the long-term health and recruitment of this species.

Adams (2019) considered exserted seed to be a key characteristic when he wrote his species description in which he differentiated seaside juniper from the closely related Rocky Mountain juniper. Our results show that exserted seed is a phenotype entirely associated with insect damage. Exserted seeds appear to be due to larvae of at least two different insects. One is orange larva—as yet unidentified, and the other is a chalcid wasp of the genus *Megastigmus*. There are likely to be others.

Filled Seed Per Cone (FSPC)

The mean FSPC for the 14 locations for this part of the study was 0.45 ± 0.02 FSPC. At most locations the average was less than one filled seed per cone (Fig. 4). The majority of seed cones in the samples only contained empty seed, meaning seed with a coat, but the insides of which had either aborted, or been infested by an insect, or were nonviable for other reasons. It was a surprise just how few filled seed there were. In particular, samples from Copley Park and Shoal Islands had not one living seed in any of the 300 cones assessed. A Kruskal-Wallis Rank Sum test determined there were significant differences between populations for FSPC ($H = 421.16$, $df = 13$, p -value of $<2.2 \times 10^{-16}$). A subsequent post-hoc Dunn test showed that all locations differed from one another. Furthermore, southern locations were more likely to have very low FSPC compared to northern locations.

Possible explanations for why so few seeds are filled must include lack of pollination, failure to fertilize and insect

predation. A study by Gruwez et al. (2012) looked at phases critical to seed development in *Juniperus communis*. They found that up to 50% of the ovules were unpollinated. Similar high rates of failed seed have been recorded in other dioecious trees (Owens, 1995; Anderson and Owens, 2000). Insect predation as a cause has already been mentioned above. Juniper mites are known to cause substantial damage. In *J. occidentalis*, chalcid wasps may destroy up to 25 % of seed (Dimitri et al., 2018).

The link between insect damage and exserted seed implies that there is a major physiological response by the berry to insect infestation that results in deformation of the berry. In the end exserted seed with no FSPC are so common as to have misled taxonomists.

Conclusions and Perspectives

Factors such as low numbers of filled seed per cone due to poor pollination biology and insect predation, and the general rarity of healthy populations suggest seaside juniper is in decline and in need of conservation measures to ensure its future. In addition, more attention needs to be paid to its cultural status. Since there are First Nations' oral traditions associating this tree both in the past and present with spiritual and medicinal usages, as well as its use today in rituals, cultural conservation is warranted. Lastly, stories of its population decline since colonial settlement imply a steady neglect of this species that needs to be reversed if we are to protect this valued and beautiful tree.

We recommend that a number of different approaches should be undertaken. Closer study of reproduction is needed. Seed storage and germination trials should be done. Dioecious trees have their unique conservation challenges. Efforts need to be made to start to collect seed and, where that is not possible, i.e. male trees, cuttings from different populations need to be rooted in a common garden. Ecophysiological study of its habitat preferences need to be carried out to better understand where it can be successfully replanted. Thus far, only populations in the southern Gulf Islands of Canada and neighbouring Orca Islands in the United States have received attention: northern Gulf Islands' populations have only been cursorily investigated to date and require revisiting. If we extend our attention to this species in the same way that we have with other non-economic tree species in the province, such as arbutus and bigleaf maple, we can, with

proper conservation and management, give this species the protection it deserves.

Currently, seaside juniper is considered a top priority tree species for conservation in British Columbia (Wang et al 2020).

Acknowledgments

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Winter damage and fall-down of 2024 Douglas-fir (*Pseudotsuga mensezii*) in the interior of British Columbia: A Tale of two Months

The Lead-up to the 2024 crop

The summer of 2023 continued the hot and dry conditions pattern seen for several years in the interior of British Columbia. Above-average temperatures and very low precipitation (<25% seasonal averages) in June and July during the period of bud initiation and differentiation for interior Douglas-fir (Fdi) resulted in conditions ideal for a very large Fdi crop for the 2024 season.

The above-average temperatures and low precipitation carried through the fall and early winter of 2023 and through to the start of 2024. Temperatures were well above seasonal normals for this period and orchardists in Okanagan noted continued development of reproductive buds in Fdi during this time. Reproductive structures were observed to be larger than normal for that time of year and further-on in development than normal and easily identifiable. Early crop estimates at Fdi orchards in the North Okanagan at this time predicted very large crops across region.

Winter arrives: January 2024

Following the very warm November, December and early January in which temperatures in the region were consistently above freezing, a sudden and brutal cold snap hit the region, bringing a rapid drop in temperature. In a 24 hour period spanning January 11-12 2024, the Environment Canada weather station in Vernon BC

Table 1 : List of Orchards surveyed

recorded the temperature dropping from -1°C to -25°C reaching as low as -28°C on January 13th (Figure 1).

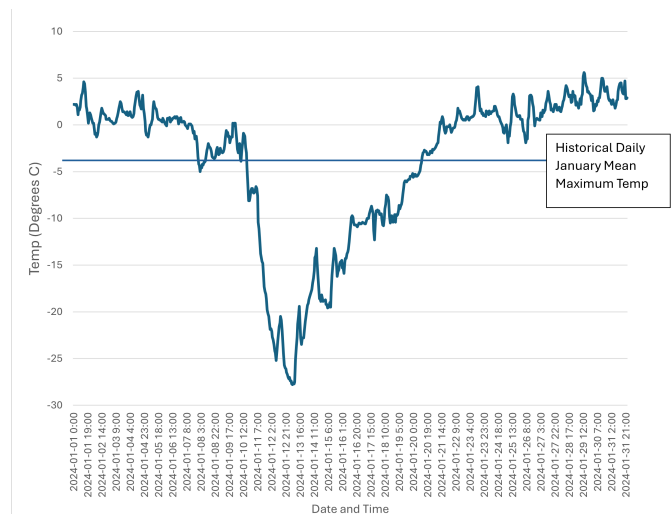


Figure 1: January 2024 Temperature recorded at the Environment Canada Vernon Weather station

This cold snap had a major impact on crop producers across the region, wiping out the crop for most wine grape and stone fruit producers (Delury, 2024), and as would be later noted it also had a severe impact on Douglas-fir production in the area.

Spring 2024: Dude, where's my cones?

Early in seasonal phenology monitoring in Fdi orchard staff noted that the development of cones seemed to be delayed. It was soon apparent that in Fdi orchards across the region a large portion of the expected cone crop was not developing.

To determine the extent of crop loss in the orchards a survey plan was implemented in five Fdi orchards at four different orchard sites. (see Table 1). The goals of this survey were:

1. Determine the scope of damage in each orchard
2. Identify potential clonal relationship or relationship with origin of parent material
3. Identify potential for relationship between aspect or slope and damage
4. Identify potential relationship between location on the tree and damage severity

Orchard	BEC Var	SPU	Location
233	SBSdh1	Prince George Low Elevation	Vernon Seed Orchard Company,
321	ICHdw1	Nelson Low Elevation	PRT Armstrong
324	ICHmw2	Nelson High Elevation	Kalamalka-Bailey Rd Seed Orchard
354	ICHmw3	Thompson Okanagan High Elevation	PRT Armstrong
355	IDFmw1	Thompson Okanagan Low Elevation	Kalamalka-Reservoir Rd Seed Orchard

In each orchard, every 4th row was surveyed and within the row every-other tree was examined. On each tree 2 branches on each side of the tree corresponding with the major aspect of the site (eg. upslope vs downslope or north vs south) were selected. On each side of the tree one branch from the top half of the tree and one from the bottom half were surveyed, for 4 branches per tree total. For each selected branch the main stem and each side-branch within the 30 cm proximal to the first internode were examined and staff recorded the number of expanded reproductive structures (male and female cones) and number of unexpanded buds

The survey was conducted when vegetative buds were visibly flushing or enlarged sufficiently to easily identify them and exclude them from the survey. Figure 2 shows examples of surveyed structures.

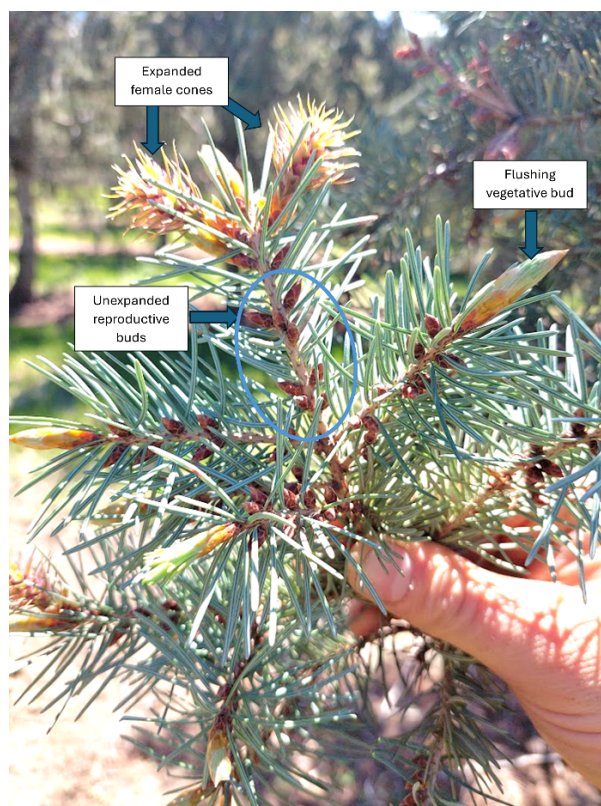


Figure 2: Example of surveyed structures

Results and discussion.

The collected survey results showed that crop loss had occurred in all orchards across the region, however the severity of this loss varied substantially between orchards and ranged from 24.6% to 98.2% of buds showing damage (Figure 3).

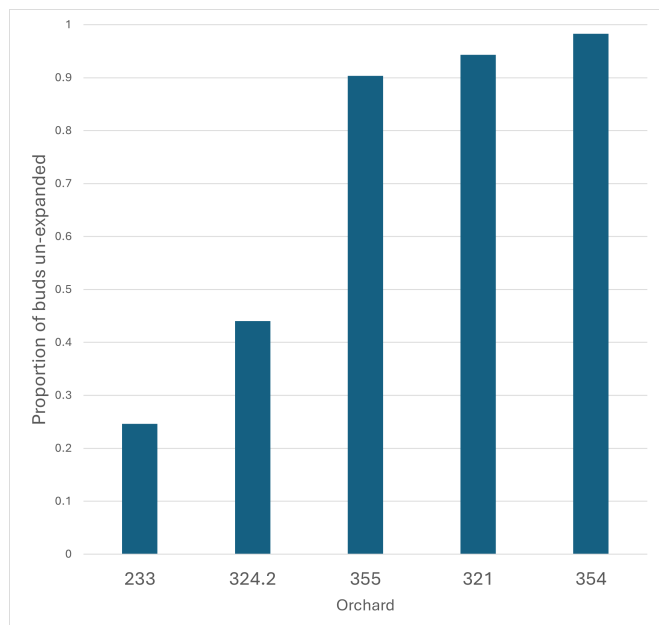


Figure 3: Proportion of Un-expanded reproductive buds in surveyed orchards

There was no noted effect of branch height or aspect within orchards across all damage levels (Figure 4 and 5).

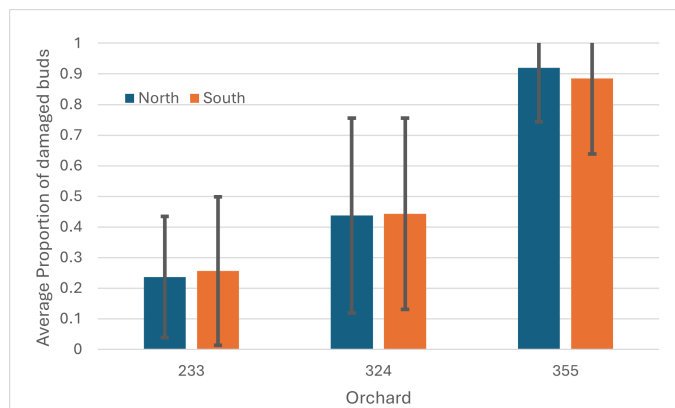


Figure 4: Effect of aspect on bud damage in orchards with low (233) moderate (324) and severe (355) frost damage. Error bars indicate standard deviations.

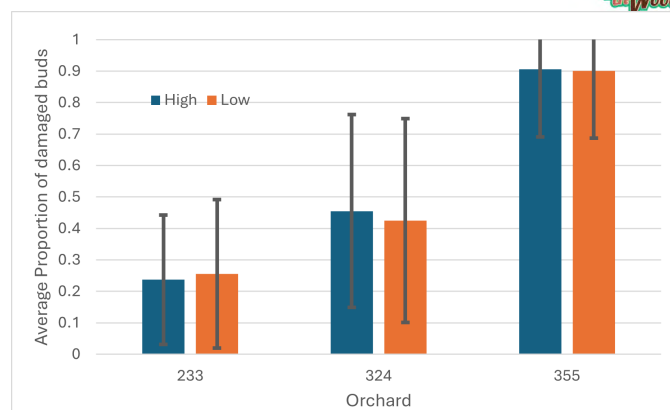


Figure 5: Effect of branch height on bud damage in orchards with low (233) moderate (324) and severe (355) frost damage. Error bars indicate standard deviations

Parental origin/climatic deployment zone of the orchards does appear to be correlated with the extent of freeze damage seen in the orchard. Orchards with more southern and low elevation parental origin showed the highest level of damage. Orchard 233, with a parental Biogeoclimatic Environmental Classification (BEC) variant of SBSdh1 (formerly for the more northerly Prince George Seed Planning Unit(SPU)) had only 24% damage, orchard 324 (ICHmw2, formerly Nelson High Elevation SPU) had 44% damage, while all of the other surveyed orchards had over 90% bud damage (Table 3) implying that there is likely variation in adaptation for cold tolerance between the parents in these orchard populations. The presence of variation based on parental origin is supported by a body of work on this species (Bansal et al., 2015, Bigras & Colombo, 2001).

Previous work has shown that seedlings of *P. menziesii* are shown to be experimentally cold tolerant without damage to -32°C but requires exposure to short days and sufficient chilling temperatures prior to exposure to this temperature (Bigras & Columbo, 2001). Female cones of coastal Douglas-fir have been shown to tolerate -22°C with appropriate cold exposures. Female reproductive structures are less tolerant of cold than male reproductive structures and vegetative buds by up to 3° C (Timmis, 1977). For the purposes of this survey, we did not attempt to determine the percentage of unexpanded buds which were male or female and so it is difficult to say whether there was differential survival of the different bud types, but anecdotally pollen cones seemed to have greater apparent survival.



The rate of freezing plays an important role in the severity of damage. With rapid chilling, physiological processes may not be able to keep up to prevent ice nucleation especially when no acclimation period has occurred (Bigras & Colombo, 2001). Due to the higher than normal temperatures in the early winter, cold hardiness during dormancy was either not fully gained, or was lost as temperatures warmed, putting the material at risk for freeze damage. The greatest risk of freezing damage in this species is most commonly from hard frosts early in the fall or late in the spring (Timmis *et al.*, 1994), however severe damage from extreme cold mid-winter has been previously reported. A similar freezing event in the BC Interior was reported by van der Kamp & Worrall, 1990 in which weather in the winter of 1988-89 followed very similar patterns as seen in the winter of 2023-24 and resulted in widespread damage to vegetative and reproductive buds in a range of conifer species. Interestingly, in the case of frost injury which occurred in January 2024, little to no damage was observed in vegetative buds in any orchard surveyed.

Ultimately, orchards 233 and 324 had sufficient crop with adequate effective population for a harvest in 2024, however no crop was harvested from any of the other surveyed orchards, a disappointing result given the 2023 estimates of extremely large Fdi crops across the region and deficits of seed in inventory.

The abnormal, though not totally unprecedented weather pattern in BC's interior during the winter of 2023-24 impacted agricultural production and as we have observed, also production of Douglas-fir seed. This unusual damage to seed crops highlights a potential risk that less predictable and abnormal weather patterns may play in the future of seed production. Unfortunately, there is little that seed orchard producers can easily do to manage extreme weather events such as these but this situation demonstrates the importance of ensuring that we include preparing for uncertainty into our orchard management planning.

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Berthier Nursery's latest improvements in sugar maple seedling production

In a previous article (Colas et al. 2022), we presented the evolution of our strategy for sugar maple (ERS) seedling production at the Berthier nursery.

The main reason for initiating the work was the significant labour needs at a very critical time in the nursery, as sowing is done manually with pre-germinated seeds during the busy month of May. Several significant improvements have been made, allowing us to increase the proportion of seedlings produced in this scenario (+20% of the target of 627 k seedlings in 2024). Here are the details.

Difficult to predict germination

ERS seeds are deeply dormant and require long stratification if used soon after harvest. Initial germination

remains stable for 3-4 years, with some lots being very good and others much less so. International Seed Testing Rules recommend 2 months cold stratification, then germination at 20°C for 21 days (ISTA 2024).

The germination test is carried out every year to ensure that sufficient seeds are allocated. For years, the nursery's seedling production method is based on the use of pre-germinated seeds. The seeds are soaked for two weeks at 3°C, then placed in big trays for stratification protected from air and light (Janerette 1978, 1979). After 15 days of stratification, germination is repeatedly estimated. When it reaches 25 to 30 %, the manual (hand) sorting begins, radicles must not exceed 10 mm long. Our germination test was adapted to fit, with these conditions. Since 2022, the germination test was slightly changed: still soaking for 2 weeks, but stratification / germination for 42 days, with germination determined at the end to obtain the maximum germination of the seedlots.

Mechanized production (20% of our production)

Seedlot selection and Stratification Method

The seedlots used for mechanical transplanting must have a minimum of 70% germination to ensure good cavity fill. After soaking, seeds are lightly dried and mechanically sown in the trays (Jiffy Preforma® 288-10, Figure 1), then kept in cold storage (3°C) for 42 days. After this cold stratification / germination period, the trays are transferred to an outdoor tunnel for seedling development. The transplantable stage is reached in about 15 days and only the best seedlings are transplanted into containers (Styroblock® 28-340) with a 100% occupancy rate (see details in Colas et al. 2022). Seedling growth is more uniform (Figure 2), making it easier to monitor the crop and saving 12% of production space.



Figure 1: Tray with seeds. A thin layer of vermiculite will be added.



Figure 2: seedling production 2 months after transplanting.

Seedling malformation and irrigation

During the first trials in 2021, we observed that some radicles emerged from the mini cells during germination, resulting in malformed seedlings that were unsuitable for transplanting (Figure 3; see Colas et al. 2022 for details). At first, we thought this was due to the initial orientation of the seed in the cavity. However, this is not important and mechanical seeding can be used.



Figure 3: Malformed seedlings

To overcome this problem, we tested different coverings to maintain pressure on the seedlings without compromising the ease of watering required midway through the cold treatment. The best results were obtained by covering the trays with clean, disinfected Styrofoam sheets 5 cm thick, held firmly to the surface to prevent germination outside the cavity. This occupies up a lot of space in our cold room (Figure 4) and requires complicated watering. A small amount of non-pathogenic mold was observed on the vermiculite covering the trays.



Figure 4: Trays covered with Styrofoam sheet and weight in the cold room

To reduce this space, in a final trial conducted this winter, we stacked 2 x 5 mini-cell trays, separated them by a thin plastic sheet, and then wrapped the stack in plastic wrap. The result was positive as the mini cells did not dry out and the stacking created enough pressure to prevent germinants from growing outside the cavities. A small amount of mold was observed on the seeds, but this did not affect the seedlings. This new method saves a lot of space in the cold room and eliminates the need for watering. This method will be used for the 2025 sowing.

Continuous improvement of the « traditional » method (80% of our production)

Although the mechanical sowing / transplanting is very promising, we reserve it for the best quality seedlots. In cases where we have to use seedlots with less than 70% germination, we continue with the traditional method: pregermination and manual (hand) sorting.

Stratification in bucket

However, the stratification is now done in a bucket, which is much simpler than the previous box method (see Colas et al. 2022), easier to handle and requires less equipment.

After soaking for 14 days, the seeds are placed in a perforated bucket, a water-saturated Kimpak® is placed in the bottom of the bucket and on top of the seeds. The lid is then placed on top. This container is not completely airtight but keeps seed moist and minimizes contact with the air, preventing the seeds from drying out. The containers are kept cold (3-5°C; Figure 5).



Figure 5. Stratification in a bucket

Integration of optical sorting to facilitate manual sorting

To speed up the sorting of pre-germinated seeds, we have integrated the use of an optical sorter before manual sorting. When the germination % reaches 15% in the bucket, the seeds are passed through the sorter. Prior to this, they are subjected to light surface drying, which does not affect the subsequent quality of the seed and radicle.

The sorting recipe was difficult to establish since the colors between the integument and the germinant are quite similar (light green and brown are colors very difficult to sort, Figure 6).





Figure 6: a-Germinated vs non germinated ERS seed, b-ERS germinated seeds.

Recent trials with a thicker chute on the optical sorter greatly improved the sorting efficiency. This allows us to double the percentage of germinated seeds in the batch to be manually sorted, thus increasing the hand-sorting yield. Ungerminated seeds are returned to the bucket and then to 3°C. The process is repeated 2-3 times to achieve the required number of seeds. We have observed a time saving in sorting with enrichment after optical sorting.

Conclusion and future developments

The implementation of mechanized ERS seedling production allows a better distribution of labor during the season, as 20% of the production is sown in March, a month with little activity in the nursery. The addition of optical seed sorting also speeds up the manual sorting process, reducing the labour devoted to ERS seeding.

There are still several trials to increase our efficiency: introduce optical sorting prior to seeding mini cells to reduce the time in the cold room and reduce the surface area needed ; validate the effect of drying excess seed after priming to see if these seeds can be returned to the bank without affecting their future germination ; to optimize the production line, characterize each seedlot for its optimal cold germination time (35 to 48 days).

We invite you to follow the next step in our journey to optimize ERS seedling production!

Acknowledgement

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Western Larch Germination Test Updates

In News Bulletin 71 the results of our western larch (LW) germination testing experiment and operational test adjustments made were discussed (Kolotelo 2021). This is the promised update. Starting in August 2021 all new LW germination tests used 25 instead of the standard 37.5 ml of water in the germination dish for the 21-day stratification period and the 21-day count duration. For all new seedlots and seedlots with a history of fungal problems the running water soak (RWS) was used in place of a standing water soak. Although it would be preferable to use the RWS with all seedlots it does add an additional 15 minutes per test and was not adopted in seedlots without a history of fungal problems.

Since August 2021 we have performed 293 germination tests using 25 ml of water; 103 with a RWS [G10new]; 133 with the traditional standing water soak [G10sws] and 57 tests with an added seed sanitation step (GH1). There was no difference in germination capacity (GC) between the updated test types, but the G10sws test had fastest germination represented by the higher Peak Value (PV) (Czabator 1962) (Table 1). One of our operational testing quality assurance (QA) checks is the use of the International Seed Testing Association (ISTA 2024; Table 5.1) tolerances for the maximum tolerated range between the 4 replicates of 100 seeds in a germination test. If a germination test exceeds these tolerances then the test is repeated. You will likely not find anyone using these tolerances in scientific publications, but they are a key component in most seed testing facilities. To provide some perspective, when LW germination problems arose

with family testing in 2016 the out-of-tolerance (OOT) rate was 70% (21/30). There has been a large decrease in number of OOT germination tests needing repeating, but the levels are still higher than other species and a mean GC of 86% is still disappointing. The G10sws actually had the lowest OOT rate of the three tests. The explanation is related to the seedlot origin as only 3% of these tests are derived from seed orchards. Fungal problems have been a larger testing issue with seed orchard seedlots even though orchard seedlots have a lower average *Fusarium* contamination level of 0.9% (n=66) compared to 1.6% (n=199) with wild stand seedlots. This is an additional reason for species identification as total *Fusarium* presence, for example, may not be reflective of pathogen levels.

Table 1. Operational seed testing results for G10new, G10sws and GH1 germination tests.

	# Tests	% Tests from seed orchard seedlots	Mean GC	Mean PV	%OOT
G10new	103	82%	85.5	7.3	11.6
G10sws	133	3%	85.6	8.3	6.8
GH1	57	56%	85.3	6.1	14.0
TOTAL	293				

The News Bulletin #71 article discussed treatment G (operationally a GH1 at our facility) that incorporates a RWS, 25 ml of water in dishes and a 2.5 hour 3% hydrogen peroxide soak following stratification. The sanitized seed was placed on fresh filter paper, kimpack in a cleaned germination dish. In addition to the 2.5 hour time the seed is soaking in hydrogen peroxide, performing the sanitation step and respreading the seeds in germination tests adds an additional 20 minutes to a G10new test type. There were 57 GH1 tests performed with 8 OOT tests. The remaining 49 tests averaged 85.3% germination with the tests on seed orchard seed averaging 90% and wild stand seedlots averaging 80%. This is encouraging as 90% of western larch seed used annually is produced in seed orchards.

The results in Table 1 provide an overview of effort in trying to tackle the LW germination problem and to provide the best information possible for seed use efficiency and nursery sowing decisions, but they do not provide a clear quantification of the gains resulting from changing our testing procedures. There are many reasons for this including: different seedlots getting different test

types, seed pathology and out of tolerance tests, the dynamic nature of the inventory with new seedlots not getting tested with old test types and seedlots expiring before new tests can be performed. In the transition period when we were evaluating the new test type there is a dataset of 20 seed orchard seedlots which received i) G10old our old standing water soak with 37.5 ml of moisture in the dishes ii) our G10new and iii) our GH1 treatment. Due to OOT tests there are a few G10old tests that are older than the other two tests, but I am still confident in the average gain estimates from the testing adjustments. Results are presented in Table 2 showing **an average 5.4% gain by incorporation of the running water soak and 25 ml of germination test moisture and an additional 4.2% gain through the incorporation of the hydrogen peroxide sanitation step.**

Table 2. Results of germination testing on 20 seed orchard western larch seedlots. GC=germination Capacity; AB%= percentage of abnormal germinants; ROT%= percentage of Rotten, diseased germinants with proportion of total abnormal germinants in brackets; STDEV= average standard deviation of 4 replicates in a germination test and PV=Peak Value.

	G10 old	G10new	GH1
GC	80.5	85.9	90.1
AB%	11.8	6.8	3.4
ROT%	8.7 (75%)	5.8 (82%)	1.1 (31%)
STDEV	4.234	3.787	2.646
PV	6.3	7.5	6.6

A few seedlots showed much higher gains (11, 16, 19 and 25%) through use of the running water soak and reduced moisture levels. Some seedlots also showed higher gains (13,16, 25%) with the addition of the hydrogen peroxide soak. Average gains are a great tool, but not all seedlots respond equally to the various treatments and Figure 1 is provided to illustrate that different seedlots respond differently to the various treatments.

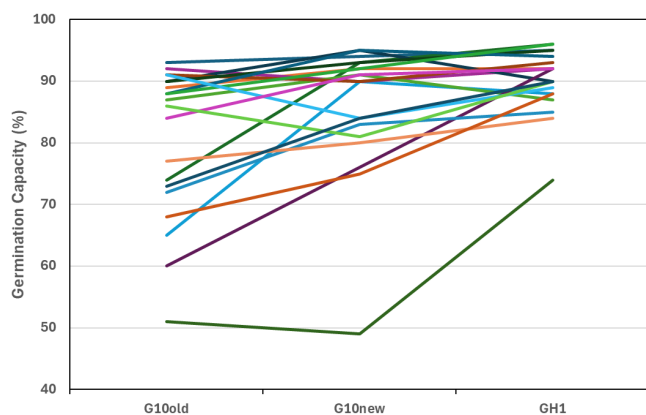


Figure 1. The performance of 20 western larch seed orchard seedlots across three different test types.

The amount of abnormal germinants, rotten germinants and replicate variability decreased as one moves from the G10old to G10 new and GH1 test results. The PV showed a increased rate of germination with the G10new treatment, but a decrease when the hydrogen peroxide was added and this agrees with the overview results presented in Table 1.

DISCUSSION

There has been progress in western larch germination testing, but it has been a slow and labour intensive process. Testing problems are unquestionably tied to seed pathology, but we still are lacking confirmation of the pathogenic organism(s), although *Fusarium* and possibly *Alternaria* spp. are still our primary suspects. Variability within seedlots is problematic as well as the seedlot * treatment interactions found in 2021 and illustrated in Figure 1. In general, the amount of test variation decreases from the G10s to the G10 new and the GH1 Tests, but not all seedlots show this simplistic pattern. It was surprising that the GH1 treatment showed a reduction in germination speed quantified using the Peak Value as hydrogen peroxide has been shown to be a germination accelerator in Douglas-fir (Ching 1962).

We are confident that improvements made in our germination testing are more reflective of what nurseries are able to achieve. This is partly due to the incorporation of a hydrogen peroxide treatment for LW at most nurseries. Currently the G10 new or G10s results are used to calculate amount of grams used and the GH1 result is possibly used to reduce grams required by each nursery. Hopefully the illustration of seedlot specific germination

gains through seed sanitation will cause all nurseries to adopt the practice and the GH1 may be used to calculate the required grams in the future. Our facility in BC is unique as we do about 95% of the stratification and we try and maintain the same exact treatments for testing and stratification practices, but it is not possible to incorporate seed sanitation into our operational seedling request activities.

The testing problem may simply be due to the highly repeatable 100% humidity conditions in a germination dish being more conducive to disease expression than in an operational nursery. We try to update germination results for all seedlots in inventory, but with 90% of the seedlings produced from 24 seedlots how much attention do we pay to the 122 wild stand western larch seedlots? There are still 15 of these seedlots being used this year, but those may vary annually based on cut block location and seed ownership. Our greatest testing efficiencies would be gained by the designation of some seedlots as “backup” material and focus testing on seedlots that have a high probability of use.

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Western Larch Seed Pathology Update

In News Bulletin #71 (October 2021) there was an article on western larch (LW) germination test updates. The primary problem related to seed pathology, specifically in seed testing. This article provides an overview of the background and current efforts in testing for seed borne pathogens in western larch.

Traditional Fungal Assay Plating

Western larch has been a high priority for *Fusarium* testing in our fungal assay program. Testing is performed on dry seed to determine base levels and keep testing consistent, but it has been shown that *Fusarium* can bulk up unpredictably during cold stratification (Neumann 1977). We have conducted 265 *Fusarium* tests for LW which resulted in an overall average of 1.4% contamination. The probability of a seedlot being contaminated (result >0.0) is 62% or 165 of 265 seedlots showed some level of *Fusarium*. The maximum level found was 43.2%. Some of these tested seedlots have been totally used, but the above results on the remaining 143 seedlots are almost exactly the same and fortunately the seedlot contaminated at 43.2% has been expired. For current seedlots in inventory there is a difference between seed orchard and wild stand seedlots. The probability of contamination for current seed orchard seedlots is only 45% (vs. 66% for wild stand seedlots), the overall average is 1.8% (vs. 1.3%). Although the probability of contamination is less for seed orchard seedlots in inventory, the contamination level if *Fusarium* is present is 4.0% vs. only 1.9% for wild stand seedlots. The higher problems with seed orchard seedlots have also been observed in our germination testing, but these averages also reflect use of better quality seedlots - meaning lower germination and higher contaminated seed orchard seedlots are not being used. We fortunately have adequate inventories of western larch seed orchard seedlots and in 2024, 91% of LW seed used was from seed orchards for the 10.1 M seedlings of this species grown.

The germination problem with western larch is clearly tied to seed pathogens resulting in lower and highly variable germination. Initial results on fungi present on diseased germinants, based on traditional plating techniques, in two laboratories were not consistent, but fungi of the *Fusarium* and *Alternaria* genera were the most common fungi found on diseased germinants.

Molecular Methods

I'd like to thank Dr. Nicolas Feau of the Pacific Forestry Centre for editing and in some cases writing the details of these molecular methods that I have only a cursory familiarity with. To try and determine the casual organism we sampled diseased and healthy germinants from six different western larch seedlots. These samples were forwarded to the University of British Columbia (UBC) for DNA extraction and amplified by Polymerase Chain Reaction (PCR) using the primer pair i., ITS1/ITS4 to target the ITS1 fungal DNA barcode, and ii. the RPS19 primers specific to the oomycetes (water molds, e.g., *Phytophthora* and *Pythium* spp.). The PCR products (or "DNA-metabarcodes") obtained were then sent to the Centre d'Expertise et de Services Genome Quebec for high throughput (next-generation) sequencing. This technique allows for the simultaneous identification of several species and is appropriate for this fishing expedition into the causal fungal organism. This is a very brief synopsis of this investigation (Hamelin et al. 2022) with literature cited provided here to allow readers to dig deeper.

No amplification of the RPS19 gene occurred indicating that oomycete pathogens were not involved in the damage to the radicles. Amplification and sequencing of the ITS1 gene was very successful resulting in over 2 million fungal sequence reads for both healthy and diseased samples. A total of 104 amplicon sequence variants (ASV's i.e., a consensus of sequencing reads based on sequence similarity) representing potentially different taxa belonging to 35 different fungal genera were identified. The diseased samples had fewer (mean=14) ASV's than the healthy samples (mean=22) and this difference was statistically significant ($p=0.047$).

Most healthy and diseased samples indicated the presence of *Alternaria* species with no statistical difference between these sample groups. This genus represented 88% of the reads in diseased samples and 83% of the reads in the healthy samples. *Alternaria* is generally considered to be a pathogen/saprophyte (Pölme et al. 2020) and shown to have the capability to act as a pathogen at high concentrations with very young seedlings of Douglas-fir and Engelmann spruce (James and Woo 1987).

The second most common fungal genus found was *Fusarium* which is generally acknowledged as our most destructive seed pathogen causing pre- and post-emergence damping off as well as root rots later in the seedling production phase. *Fusarium* is a taxonomically complex genus, making species identification challenging



and pathogenicity can vary greatly between individual species. *Fusarium* ASVs were found on both healthy and diseased samples and although more diseased samples had been associated with *Fusarium* there was no significant difference in occurrence between these sample types. *Fusarium* was also less common than *Alternaria* with only two samples having more than 50,000 sequencing reads while almost all samples had *Alternaria* reads above this threshold. For me, one of the most significant points of the discussion section of the report were the comments on the disease cycle. *Alternaria* could be the causal organism. However, it could also be that another organism such as *Fusarium* killed the seed and *Alternaria* is now acting as a saprophyte on the dead germinant. This is stated as a common pathology problem in diagnosing the cause of death in plants, but an important point to consider in seed pathology. Timing can be just as important as location.

Several different ASVs identified as *Fusarium* were found in the samples. Phylogenetic analysis suggests up to five different *Fusarium* species complexes were found: *Fusarium fujikuroi*; *F. oxysporum*; *F. tricinctum*; *F. sambucinum* and *F. solani*. A species from the *F. fujikuroi* complex was found on 67% of the diseased samples and only 39% of healthy samples. In comparison to an available CBS (Centraalbureau voor Schimmelcultures) isolate the best species match is *F. proliferatum* which is a common species found in the Pacific Northwest (Stewart et al 2020) and shows high virulence on Douglas-fir seedlings (James et al 1997). Another sequence variant (ASV_4) matched *F. acuminatum*, but it was more frequent on healthy than diseased germinants. This could be related to organism presence prior to symptoms appearing, differences in pathogenicity or differences in symptom expression.

The study had a few hiccups worth reporting: 1) At the Tree Seed Centre two people verified the classification in distinguishing healthy from disease germinants, but upon arrival at UBC the “Healthy” sample for one of the seedlots contained diseased seedlings and these were removed at UBC. To me it emphasizes the importance of timing and I was the one doing the sampling which was then verified by another staff member; 2) One of the blank samples was contaminated with several of the fungi found on the germinants. DNA-metabarcoding is a very sensitive approach based on the PCR technology that is prone to cross-contaminations. The authors caution that

results should be used with caution and preliminary as the contamination could skew the results or draw false conclusions. It was also pointed out to me that not all published studies that use this approach include blank samples to eliminate the possibility of contamination in results obtained.

This study did not conclusively determine the smoking gun for the disease symptoms on western larch germinants, but there are still some learnings to be gained.

- 1) There is no evidence to suggest the oomycetes are part of the problem and for practical purposes can be eliminated as a suspect.
- 2) For species identification the ITS1 gene is insufficient to identify *Fusarium* and *Alternaria* to the species level. For *Fusarium* species identification even the ITS1 and ITS2 may be insufficient.
- 3) Preliminary results indicate that *F. proliferatum* and *F. acuminatum* are good candidates to explain the observed disease and deserve further investigation.

There was some additional work on obtaining isolates from contaminated seeds and this work was sponsored by the Tree Seed Centre and performed by Michael Peterson at Applied Forest Sciences Ltd. It was primarily focused on Douglas-fir, but three western larch seedlots were also included. Seedlot selection was simply based on new seedlots that were targeted for traditional fungal assays. The *Fusarium* cultures sent by Michael were identified using the Translation elongation factor gene TEF1. DNA was extracted directly from each fungal culture using the Qiagen Plant Pro DNA extraction kit following the manufacturer’s instructions. DNA was amplified by PCR with *Fusarium* specific primers that target the TEF1 gene, and the PCR products obtained were sequenced at the CHU de Québec-Université Laval Research Center SANGER Sequencing platform. Identifications at the species level were performed by submitting the sequences obtained to a similarity search against the NCBI nucleotide collection using the BLASTn algorithm.

In Table 1, the *Fusarium* species identification for the isolates obtained from three western larch seedlots is provided. These isolates will be a valuable tool for further work on seed pathology and some of that work has already

been initiated at the Pacific Forestry Centre with Douglas-fir. In that work *Fusarium proliferatum* was one of the most aggressive species causing damping-off.

Table 1. *Fusarium* species found in three western larch seedlots. Numbers indicate the number of *Fusarium* cultures obtained for each species.

Seedlot	<i>F. sporotrichoides</i>	<i>F. redolens</i>	<i>F. oxysporum</i>	<i>F. proliferatum</i>	<i>F. culmorum</i>	<i>F. tricinctum</i>
63375	0	2	0	0	0	0
63941	1	2	3	3	0	0
63793	4	1	4	2	2	1

Discussion

With significant seed pathology issues evident in western larch germination testing since 2016 it is embarrassing that the causal organism(s) have not been more confidently confirmed. Progress has certainly been made in adjusting our germination testing regime to reduce amount of ‘rotten’ germinants, reduce out of tolerance tests and also introduce a germination test that incorporates a hydrogen peroxide seed sanitation step (see accompanying article). This discussion will focus on seed pathology.

The current plating techniques of tree seed have provided estimates of contamination (*Fusarium* sp.) and infection (*Sirococcus conigenus* and *Caloscypha fulgens*) levels on operational seedlots. These results have provided “red-flags” for the presence of these pathogens. These methods are not without complications as more competitive fungi (e.g., *Penicillium* and *Trichoderma* spp.) in petri dishes may mask the real pathogen. I am more comfortable with the *Fusarium* result being based on the percentage of seeds contaminated vs. molecular methods which assess a homogenate of usually far less seeds and providing an indication (Yes/No) of whether it is above a specific threshold. Mathematical models exist to determine actual levels (Geng et al 1983), but validation trials are required and would likely only be conducted on our most important species. Plating techniques can be performed on any species without calibration allowing a red flag to be provided for major and minor reforestation species.

Fusarium is generally considered the most common and problematic of the three pathogens assayed for and has the most practical dilution (running water soak) or sanitation (hydrogen peroxide soaks) tools available in addition to fungicides. The *Fusarium* problem is complicated by the fact that seed is only one of the ways *Fusarium* can be introduced into the nursery, levels can bulk up greatly during stratification, and there is a wide variability in the virulence of *Fusarium* species which are complicated to identify based on spore morphology and through molecular methods. Molecular methods continue to be reduced in cost and are becoming more accessible, but the seed virulence of different *Fusarium* species is not well known across very many tree species.

Several nurseries in BC will simply sanitize all western larch seed prior to sowing regardless of the *Fusarium* result. How will *Fusarium* species identification and virulence information reduce seedling losses? Are there more *Fusarium* species specific tools available? I am supportive of the development of molecular tools and greater understanding of seed pathology, but am still not clear on how it will impact nursery practices.

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Ottawa, Ontario

<https://sites.google.com/fgca.net/forestgenetics25/home>

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Mary Winspear Centre

Sidney, BC

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