TREE IMPROVEMENT

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Seed and Seedling Extension Topics

Diane Douglas - Editor

Summer is over so it's goodbye to days at the beach, mosquitoes and hot-lifts! Now comes fall with its chance of frost, winter lifts and wind. I hope everyone had a good summer and is prepared for the next phase.

This year's FNABC was held in Olympia, WA, USA during the first week of August. It was held jointly with the Western Forest and Conservation Nursery Association and was termed by (~175) attendees as a resounding success. There were speakers from as far away as Chile, Finland, and the state of Georgia, as well as a full house of commercial exhibits, great field trips and naturally - lots of food! There was a healthy mix of bare-root and container culture, with my personal favourite being the transplanting sessions. If you didn't make it, be sure to obtain a copy of the proceedings when they come out. I am sure Tom Landis will be happy to send you one (for a fee).

Many people are asking what is happening in the BC Forest Service, mainly wondering who is left and what they are/will be doing. Let's just say there is some flux and as the cannon rolls around the deck it is difficult to say where it will be pointing next. One thing is for sure; it's not over yet! My advice is to keep in touch with your colleagues and compatriots. Times may be a changing, but the overall goal is still the same.

Enjoy this edition of our Seed and Seedling Extension Topics…

Eric van Steenis
Guest Editor

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BRITISH COLUMBIA
Ministry of Forests

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GROWER'S NOTES

The Current Status of the Balsam Woolly Adelgid in British Columbia

The balsam woolly adelgid or BWA, *Adelges picea* (Ratz.), was introduced to North America from Europe in 1900 and has since dispersed through most of the habitat range of our native true firs. In British Columbia, it is unevenly distributed over 6000 km^2 of southern Vancouver Island and the southwestern region of the province. Following its initial discovery near Vancouver in 1958, extensive surveys in the early '60's established this general distribution. Concern for artificial spread and increased damage led to a voluntary restriction on the importation and movement of *Abies* stock, and then provincial quarantine regulations in 1966. The initial ban on growing *Abies* nursery stock or ornamentals was amended in 1977 and in 1992 to allow production of *Abies* but all material had to be grown under an annual permit. In essence, only permitted material could be moved and any material grown within the declared quarantine zone could not be moved outside of this zone (Figure 1). Permitted material grown outside the quarantine zone could be moved anywhere in the province. The only exemptions are seeds and cones; logs moved by water and cut Christmas trees moved from November to January.

Figure 1. Current BWA Quarantine Zone.
(see Figure 2 for proposed BWA regulated area)

In BC, BWA populations are most commonly concentrated in the crown causing swelling (gout), distortion and death of twigs and ultimately crown dieback. Heavy stem attacks are less common. It infests all *Abies* species and although *Abies lasiocarpa* is the most susceptible to damage, *Abies amabilis* and *Abies grandis* are most frequently infested in coastal British Columbia. Seedlings can be infested and seriously gouted. In 1987, surveys of long-term plots, the mortality of mature amabilis fir averaged 15% with individual plots ranging from 5% up to 95%. With the discovery in 1983 of surviving populations and damage on alpine fir and grand fir at higher elevations in Idaho, the risk and concern for potential spread into interior BC was re-emphasised. True firs are widely distributed in British Columbia, comprising 20% of the softwood volume and rank fourth at 13% of the annual harvest.

In 1995/96, surveys conducted by the Canadian Forest Service and the BC Forest Service of *Abies* stands in the Vancouver Forest Service Region found new BWA finds outside of the current quarantine zone. At this point, all reforestation and landscape nurseries on the southern BC coast were technically within infested areas. In 1997, the BWA technical group recognised that the level of resources available now and in the future would never be sufficient to effectively monitor and quarantine the movement of BWA. Therefore, the group focused on the best methods of insuring that the movement of potentially BWA infested stock did not mix with the most susceptible true fir species, *A. lasiocarpa,* which ranges throughout BC. The other true fir species that are primarily coastal in distribution, i.e. *A. amabilis,* and *A. grandis*, are less susceptible and infestations are often linked to off-site conditions. The technical group felt that as BWA is naturally dispersing up the BC coast that forest management strategies concerning outplant mix and viability would reduce the risk to existing stands.

Of greatest concern was the regulation of ornamental nursery stock and Christmas tree production. As there is no effective system to monitor production levels and transport, the technical group felt that the only effective method would be a comprehensive education program. Reforestation seedlings, the largest commodity group, represents less risk due to age, methods of production and closely monitored reforestation criteria.

At present, the following initiatives are the focus of the BWA technical committee:

- The Canadian Food Inspection Agency would administer the proposed BWA regulated area under an agreement with the Province of BC. The regulated area would be based on the biogeoclimatic zone distribution of *Abies amabilis* within the administrative boundaries of 1 forest region (Vancouver Region) & 2 coastal forest districts (North Coast & Kalum -Figure 2). This would be similar to the Gypsy Moth regulated area model. The Province would be responsible for the permit system, development of protocols within the regulated area and an education program. This would potentially do away with the current provincial BWA legislation.

Figure 2. Proposed BWA Regulated Area.

- BC Ministry of Agriculture, Fisheries & Food would continue to administer the permit system for all *Abies* grown and or brought into the province.

- The BC Forest Service seedling request system would be updated to block any *Abies* seedlings designated for planting outside the regulated area from being grown within the regulated area.

- All BC Forest Service regional & district forest health staff, and BC Ministry of Agriculture, Fisheries & Food regional specialists would be informed of the changes and requested to report any potential finds of BWA.

- An education package would be available for distribution to all commodity groups. There is currently a draft poster, pamphlet and leaflet.

- A distribution list would be created. The information will be amalgamated from the current BWA permit list, BC Forest Service & BC Ministry of Agriculture & Food staff lists, Forest Health Committee list, forestry consultants, nursery & Christmas tree association lists. A list of potential buyers/retailers/ wholesalers will be developed. The intent is to provide timely reminders and updates.

- Training sessions would be planned for Canadian Food Inspection Agency inspectors.

- Research trials to investigate BWA resistance in *Abies lasiocarpa*, effective control and management treatments to reduce BWA infestation in nursery stock and the criteria for a nursery certification programme are ongoing.

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Western White Pine Stratification

Western white pine [Pw] continues to be problematic in matching lab testing results with operational quantities of seed. Gains were realized in 2002 and this note will discuss the improvements incorporated. I would like to first provide a brief summary of western white pine use. In 2002 sowing, 61 Kg of seed was used to produce a total of 1.09 million seedlings in 62 sowing requests. This is significantly reduced from the 112 Kg of seed used to produce 1.88 million seedlings in 2001 over 101 sowing requests. For 2002 sowing, almost all requests were for orchard-produced seed (97%) and for 1-0 stock (92%). The majority of seedlings (78%) were destined for interior planting sites.

In 2002, stratification of Pw included:

1) Moving back to stratifying Pw in plastic bags *vs*. tray-type systems used in 2000 and 2001.
2) Increased moisture content in stratification by eliminating surface drying before stratification and monitoring moisture content (non-destructively) for all sowing requests at one month intervals during stratification.
3) Monitoring and 'mixing' (redistribution of moisture and gases) of all Pw sowing requests every Monday, Wednesday and Friday.
4) Extending stratification for up to 3 weeks (if possible) in consultation with the nursery. All nurseries agreed to 3 weeks additional stratification, but this was not always possible due to the late entry of some requests.

One of the biggest apparent problems with our tray-type stratification unit was that moisture was lost during stratification. Returning to stratification in plastic bags and eliminating surface drying before stratification resulted in all sowing requests being maintained at moisture contents between 35.1 and 38.6% with an average of 36.7%.

The falldowns in germination (lab vs. operational seed preparation) were reduced from an average of 37.5% in 2001 (n=29) to 19.4% (n=19). Seedlot again seems to be an important source of variation and when 3 seedlots (4 sowing requests) were removed from the database the falldown becomes more reasonable at 11.7%. For 17 sowing requests, germination tests were performed after 98 days stratification and with an additional 3 weeks of stratification (119 days). This extension in stratification resulted in an average increase in germination of 3.8%. We are recommending to seed owners and clients that **stratification be extended for 5 weeks in 2003**. This results in a total pretreatment duration of 147 days [14 day soak + 133 days cold stratification]. In order to be able to meet this objective it is extremely important that sowing requests are put into SPAR early. For example, for a February 18, 2003 sow date the request will have to go into soak on September 24th and therefore the request must be approved in SPAR a few days before this date to allow for seed withdrawal and administration.

An additional area we will investigate in 2003 is a reduction in the stratification unit size. In Pw we are currently only putting 1 000 grams in a bag versus 3 000 grams that is used for all other species. There is some indication that smaller units perform better and we will be reducing our stratification unit size to 750 grams for 2003 sowing of Pw. We will also be putting more emphasis on monitoring for chitting or seed coat cracking. With extended stratification this is a possibility and the chitted seed would be more susceptible to drying of internal contents or infection by fungi. Nurseries growing Pw should be careful in surface drying Pw to just remove the moisture from the seed coat prior to sowing.

Please forward any comments, concerns or questions regarding western white pine to me at the Tree Seed Centre. Good luck with your crops.

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TECH TALK

Pest Status and Control of Larch Adelgids

The larch adelgid, *Adelges lariciatus*, dwells on cones of larch, *Larix occidentalis*, and is a sporadic pest of larch seed orchards in the Interior of British Columbia. Five distinct generations of this insect exist, spanning two years and alternating between larch and spruce. On larch, they overwinter as 1st-instar nymphs on buds of 2-4 year old wood. These nymphs, which are immobile, start to grow in early spring (early March in the Okanagan Valley), and are adults by early April. They lay clusters of dusty green or purplish eggs, which start to hatch just as the larch buds are bursting, in mid to late April. The newly hatched nymphs are called crawlers because they crawl into the bursting cone buds, where they settle and don't move for the rest of their lives. As the cones grow, so do the adelgids, all the time producing a mass of white waxy wool (Fig. 1). By mid-June all the adelgids have become winged adults and flown off to spruce, the alternate host, leaving the cones empty. On lightly infested cones, it's difficult to tell by the end of June that there was ever an adelgid present.

Concern about the adelgids reducing seedset or extractibility led to a series of experiments in 1996, 1997, and 1999. The experiments were all slightly different, but the basic idea was that seedset, extractibility, and germination were compared between infested cones and non-infested cones. All experiments demonstrated that regardless of the level of infestation, adelgids had no effect on any aspect of production, as far as we could determine. The most careful study was conducted in 1997; the results are shown in Figure 2. Although it looks as though the adelgids influenced the filled seeds per cone (Fig 2A) or possibly the extractibility, there were no significant differences. Even this non-significant trend was not evident in the other experiments.

As part of these trials, I attempted to create adelgid-free cones by spraying them with pesticides from a backpack sprayer. Safer's Insecticidal Soap, Orthene, Pirimor, and finally Thiodan were tested against both the overwintered nymphs and the cone-dwelling stage, all

Figure 1. White waxy wool of *Adelges lariciatus*.

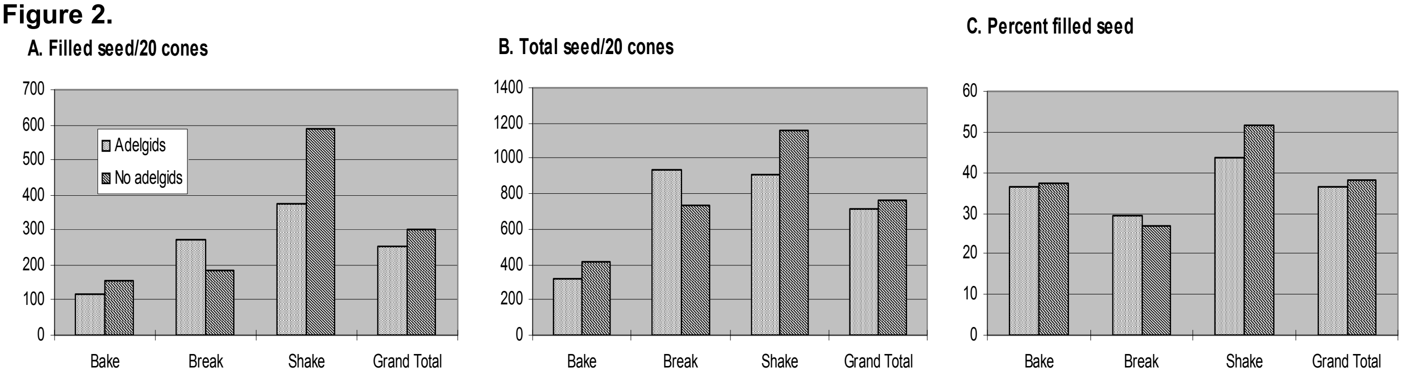
to no avail. Apparently larch adelgids on larch are extremely resistant to pesticides. I finally resorted to a rubber pencil erasor, cut at an acute angle to a feather edge, which could be slid under the cone scales to squish the adelgids. My coop student Gerad Hales spent many fine hours "erasing" adelgids!

Then, in 2001, the larch seed orchards at Kalamalka had very high densities of overwintered adelgids; something like a 10-fold increase over anything we had seen before. Concerned about the potential damage by such a heavy infestation, we obtained a special use permit for an unregistered pesticide, Admire (imidacloprid), which showed activity against other species of adelgids. We applied it on April 26, 2001, to 3 rows of trees in each of 2 orchards. These treatements killed many overwintered adults and up to 70% of nymphs in the cones. Encouraged by these results, we sprayed half of each orchard on May 17, 2001, against nymphs developing in the cones. Although the chemical is highly systemic, it looked as though it killed only those adelgids it contacted. Many cones were so heavily infested that the adelgids were stacked up like cordwood, and the bodies of the outermost layer protected the inner layers from contact with the pesticide. Consequently, we had between 30% and 50% kill, which wasn't enough to prevent damage. In the end, these two larch orchards sustained an estimated crop loss of 20% due to the adelgid infestation, as well as adding complexity to harvesting because of non-uniform ripening and cone survival.

In 2002, we decided to try Admire again, and also to try both Safer's insecticidal soap and dormant oil before bud burst. Safer's Soap, used successfully against overwintered nymphs of *Adelges cooleyi* on Spruce, was applied by airblast sprayer on March 12, when the overwintered nymphs had just started to grow. No mortality was evident, confirming my previous efforts with a backpack sprayer.

On 5 trees, Superior Oil in a 2% solution was applied March 12 by backpack sprayer to assess efficacy against overwintered nymphs, and to check phytotoxicity. No adelgid mortality was evident, so oil was reapplied to the same 5 trees on March 25. At this time most adelgids were about half grown. This application resulted in about 70% mortality. On most branches, there was nearly 100% mortality, while some branches or protected areas of branches had virtually no mortality, indicating coverage problems: where coverage was good, all adelgids were killed; otherwise many survived. No phytotoxicity was evident when buds burst on these 5 trees.

Figure 2.



Superior Oil in a 2% solution, and Admire at 234 mL/ha, were applied by airblast sprayer to portions of larch orchards at Kalamalka on April 2. Two unsprayed control areas were left in each orchard as well. At this time, many adelgids were adult size, though no eggs were apparent yet. On April 4 and 10, treatment and control areas were checked for adelgid mortality and egg laying. On April 28, conelets had come out, eggs were hatching, and crawlers were invading conelets; I assessed the number of crawlers infesting conelets in treatment and control areas. On June 11, cones from each area were rated for their infestation level on a scale of 0 to 3: 0 = no adelgids; 1 = a smattering of adelgids; 2 = half the cone infested with adelgids (usually the lower half, the upper half being more or less free of adelgids), and 3 = the entire cone harbouring adelgids. None of the cones were as densely infested as in 2001, when the cone itself was often difficult to see. Data from all these surveys are presented in Table 1.

Table 1. Measures of success of sprays applied on April 2, 2002 against larch adelgids at Kalamalka Seed Orchards. See text for explanation of infestation rating of June 11.

Date	Assessment	Mean Measure		
		Control	Admire	Oil
4-Apr-02	Mortality of overwintered adelgids	18%	52%	78%
10-Apr-02	Mortality of overwintered adelgids	40%	84%	83%
10-Apr-02	Overwintered adelgids laying eggs	46%	6%	16%
28-Apr-02	Number of crawlers in new cones	62.7	17.8	21.8
11-Jun-02	Infestation rating of cones	1.87	0.99	0.81

Both Admire and Superior Oil were effective in reducing adelgid densities, egg laying, number of crawlers in new cones, and infestation densities of cones. Oil provided a faster kill initially, though Admire was similar after 8 days. Not apparent from the mean data is that mortality from the oil was quite variable: either all adelgids on a shoot were dead, or few were, or sometimes adelgids were dead on one side of a shoot, but alive on the other side. This indicates that oil is effective but coverage is imperfect. Apparently good coverage is critical when using oil. The Admire had uniform kill on all shoots. This pattern was also apparent in the infestation ratings of cones: many trees in the Oil section had virtually no adelgids, while some trees had many. Again, the Admire sections had uniformly low adelgid densities. It seems that where good coverage was obtained, the oil provided better control than the Admire, but good coverage was hard to obtain.

In conclusion, both Superior oil at 2%, and Admire at 234 mL/ha, effectively reduced adelgid densities below unsprayed control areas, and reduced the subsequent infestation of cones. If better spray coverage was obtained, it seems that oil might provide better control than Admire. No phytotoxicity was observed with oil, probably because it was applied before the foliage had emerged. Many dormant oil products are currently registered for use on conifers, shade trees, and ornamentals, but Admire has no such registration. Registration expansion of Admire is unlikely due to the manufacturer's and regulators' concerns of soil peristence and groundwater contamination. For these reasons, Superior Oil would be a better choice when considering adelgid control in the future. I recommend using a dormant oil product at 2%, just before budburst, to control larch adelgids when densities warrant it.

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Antioxidant and Natural Biostimulant Enhancement of Seedling Growth and Stress Tolerance in Conifer Seedlings

Introduction

Trees aren't as passive as they seem. Despite their apparent simplicity, trees have awesome defences. Adapted and evolved over several hundred million years, trees are the largest and longest-lived organisms on the planet.

Subject to constant environmental change, trees have evolved an elegant defence system. This enables them to survive insect attack, disease and extremes of climate. Each attack generates a burst of oxygen radicals. Photooxidation results from the fact that not all the light energy absorbed by chloroplasts can be utilized in photosynthesis or dissipated as heat. This extra energy causes a one-electron reduction of oxygen. Photooxidation produces unpaired electrons. These form free radicals, which are highly damaging to trees.

It is the radical-scavaging activity of flavanoids and other natural compounds that maintain tree health under stress. When detoxification is inadequate, reactive oxygen species accumulate in organisms, resulting in disease, stress, and physiological aging (Miquel 1989). Free radicals react with the lipid constituents of membranes causing them to leak. Photooxidation targets the chlorophyll membrane. Chlorophyll, photosynthesis and growth all decline, in parallel with the visible colour changes that take place in the leaves of plants when they senesce.

Antioxidants defend the body against cancer, heart disease, high blood pressure and other common ailments of aging. Optimizing daily intake of anti-oxidants can increase mean life span (Harman 1978) and maximum longevity (Miquel 1989). A combination of flavanoids, phenolic acids and anthocycanins largely determines the antioxidant effect. The medicinal properties of natural antioxidants in food have been used for thousands of years. Antioxidants fall into two classes: natural antioxidants and those produced synthetically. Natural antioxidants have many potential applications. At least 14 different classes of compounds extracted from plant and animal tissues have antioxidant properties. Natural antioxidants are safer and cheaper to use than synthetic chemicals and are less environmentally damaging.

The genetic mass of conifer cells is ten times greater that of animals or humans. Up to 99% of a trees' genome consists of repetitive strands of DNA. Although previously regarded as 'junk DNA', the repeated gene sequences may be the genetic memory-bank that mobilizes tree defences so that they can respond to each types of stress. Developed over a long evolutionary history, repeated DNA sequences may provide the genetic blueprint that allows the tree to respond to a wide range of stresses.

Trees do not store defence compounds in large amounts. This would not only be energetically expensive, but it would also divert resources away from growth. When woody plants are attacked the primary pathways of metabolism shut down in favour of the phenylpropanoid pathway which produces secondary compounds that enable trees to mount a vigorous defence against any stress factor. The term 'antioxidant' refers to the ability of these compounds to interrupt oxidative stress. More precisely, antioxidants eliminate free radicals that accumulate under oxidative stress.

An array of antioxidants, antitranspirants, and antisenescence agents appear to protect trees. Colour changes signal the cessation of normal metabolism in stressed plants. The loss of chlorophyll signals the accumulation of phenylpropanoids. Pigment changes are observed in plants after they are exposed to drought, mineral nutrient deficiency, UV-B and other stresses. When the stress signal is received, phenylalanine ammonia lyase (PAL) enzyme is formed, which causes the amino acid phenylalanine to be diverted into secondary metabolites. Jack pine seedlings develop a characteristic purpling in the late fall, due to an accumulation of total phenols, proanthocyanidins and anthocycanins (Nozzolillo e*t al* 1990[1]). Irradiated with low (ambient) levels of UV-B also mobilizes flavanoid pigments, which signal the acquisition of thermotolerance in jack pine (Teklemariam 2002[2]).

Colour changes, resulting from the accumulation of flavanoids, signal the onset of hardening in stressed conifer seedlings (Teklemariam 2002).

A number of different antioxidants, antitranspirants, phenols and other antisenescence agents accelerate seed germination. They also enhance seedling growth and stress tolerance when applied to conifers.

Seed Germination

Relatively few natural compounds stimulate seed germination. Known stimulants include xyulose and strigol, which are induced by fungal infection. Anenitrile, 2-nonanone, and octylithiocyanate also stimulate germination. Synthetic compounds that stimulate germination include ethanol and several anaesthetics (chloroform and ether) (Taylorson et al., 1982[3]). The gaseous hormone ethylene also promotes germination. Although these compounds all react with cell membranes, early work on their mode of action was largely speculative.

As early as 1883 it was suggested that repeated soaking and drying may increase drought tolerance. Hydration treatments, such as spraying and dipping with water, improve seed germination in some species (Basu and Mandal 1985[4]; Basu 1990[5]), but failed to stimulate germination of conifer seed. Germination of 30-year-old jack pine seed was enhanced when seeds were soaked in a plant extract called BioProtect.

When storage conditions are cool and dry, seeds can remain viable for many years. Faster-growing seed lots decline more slowly in storage than less vigorous provenances. Seeds decline rapidly when storage conditions are warm and moist. Seed deterioration in storage can be detected by the use of embryo stains, including tetrazolium chloride, and by measuring the increase in mean germination time, electrical conductivity of seed steep water. Seed decline is often difficult to predict, which necessitates the need for seed testing.

Seeds can decline in the absence of micro-organisms, without any detectable change in seed morphology. However, seed deterioration causes membranes to become more permeable. Leakage of metabolites is most apparent in 'aged' seeds. Although supporting evidence is sparse, free radicals may cause physiological 'ageing' and loss of seed viability. Seed chemotherapy with antioxidants may help to delay or reverse the aging process in seeds.

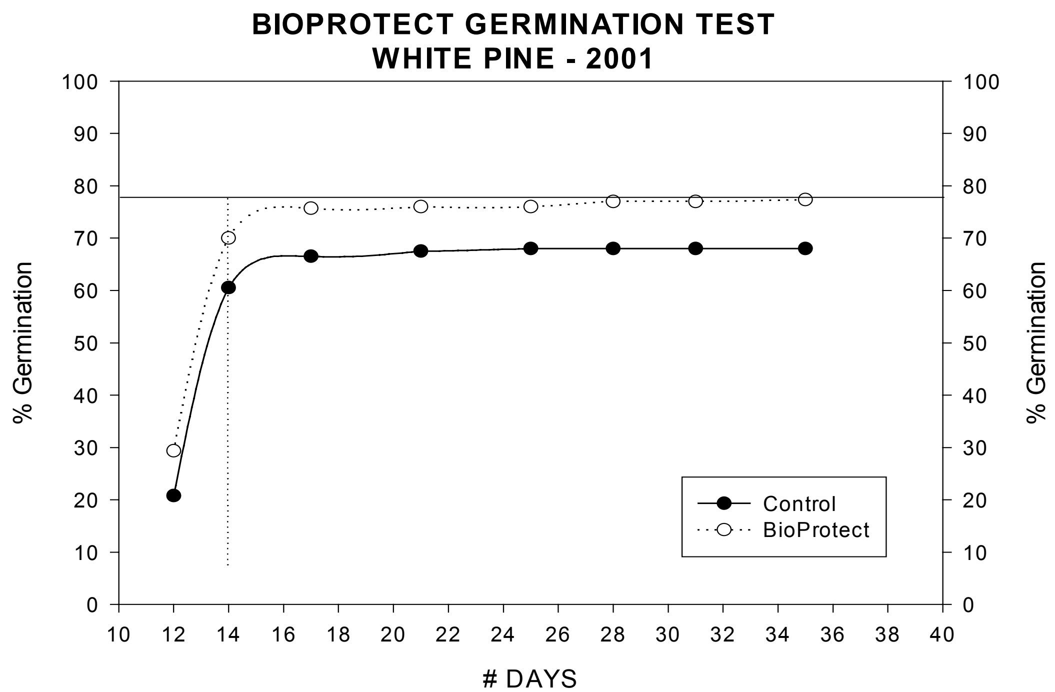
BioProtect treatment speeded germination, seedling growth and viability in greenhouse studies. BioProtect reversed part of the decline in seed viability in aged jack pine seed. Although germination was low, a 24-hour seed soak with BioProtect increased Peak Germination of 30-year-old jack pine seed by up to 50%.[6] BioProtect also accelerated the germination of young, fresh (2-year-old) white pine seed[iv] (Figure 1). A 24-hour seed-soak with BioProtect increased the overall germination rate by 10% compared to seeds soaked in water (78% vs. 68%), and increased germination energy by 30%. BioProtect also reduced the germination period by 14 days, compared to the water controls (17 days vs. 31 days). BioProtect-treated seeds produced white spruce seedlings that were more uniform in size[v] (Figure 2).

Treatment with Ambiol, a 5-hydroxybenzimidazole derivative, increased the overall germination of old, poorly-germinating seeds (Smirnov et al., 1984[7]) and speeded-up germination (Visnevetskaia et al., 1992, 1996). Ambiol also accelerated the speed of emergence of old jack pine seeds. Ambiol accelerated the germination rate and reduced the time required for emergence.

The ability of antioxidant treatment to speed-up germination may also improve seedling establishment. Treated canola (*Brassica napus*) seeds were twice as likely to have emerged 10 days after planting, compared to untreated seeds (Vishnevetskaia *et al.,* 1996). At the most effective concentration (10 mg/L), Ambiol treatment resulted in a four-fold acceleration of cucumber germination (Kritenko and Blake, unpublished data).

[iv] A. Foley, Manager, MNR Seed Plant, Angus, Ontario. (unpublished data)
[v] W. Smith and La Maison Verte, Hearst, Ont. (unpublished data)

Figure 1.


BIOPROTECT GERMINATION TEST
WHITE PINE - 2001
% Germination
# DAYS
Control
BioProtect

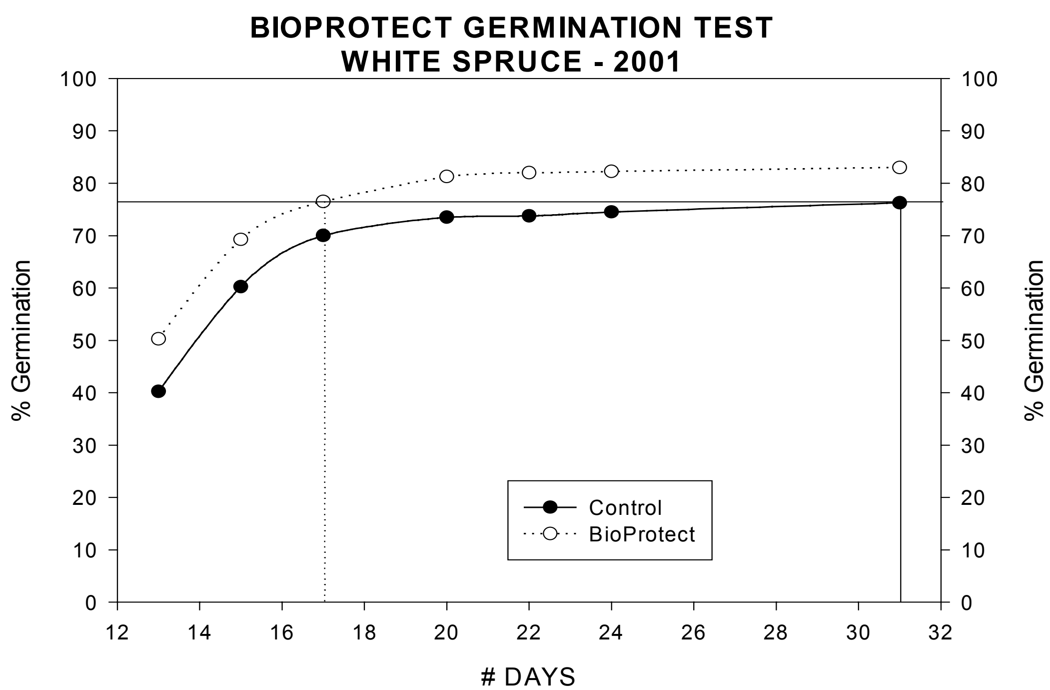

BIOPROTECT GERMINATION TEST
WHITE SPRUCE - 2001
% Germination
# DAYS
Control
BioProtect

Figure 2.

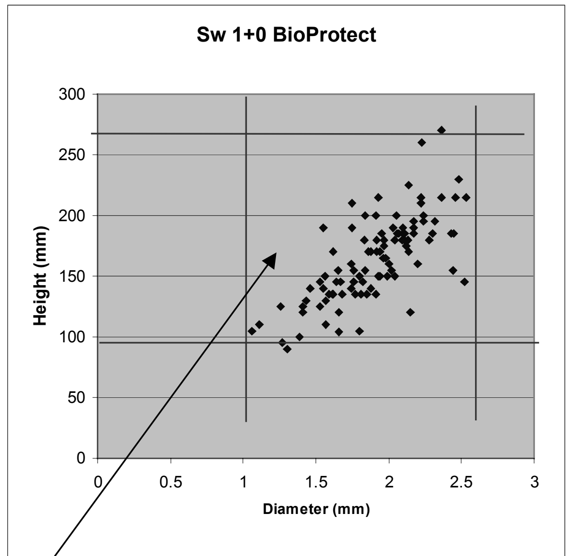
CROP GROWTH PROGRESSION / INVENTORY REPORT

CROP NAME:	**Sw 1+0**	# SEEDS / CONTAINER:	
SOWING DATE:		EXPECTED GERMINATION:	
DELIVERY DATE:	**SPRING 03**	ACTUAL GERMINATION:	

CONTAINER:	**JIFFY 96**
# CAVITIES / TRAY	**192**
# TRAYS SOWN:	
# TRAYS ON HAND:	**6000**

CROP PROFILES:

		BioProtect	Control
	% Green Count	**1,152,000**	**1,152,000**
Height (mm)	<50	0	4
	50 - 74	0	4
	75 - 99	3	9
	100 - 139	25	28
	140 - 199	60	46
	200 - 249	10	9
	250+	2	0
	Mean Ht.	165	145
	Std. Dev.	36	45
	% Target	**97**	**83**
	Total Inventory	**1,117,440**	**956,160**
	(based on minimum height = 100 mm)		
Diameter (mm)			
	<1.0	0	6
	1.0 - 1.5	10	16
	1.6 - 2.0	51	47
	2.1 - 2.5	36	29
	2.6 - 3.0	2	2
	>3.0	1	0
	Mean Dia.	1.9	1.8
	Std. Dev.	0.34	0.41



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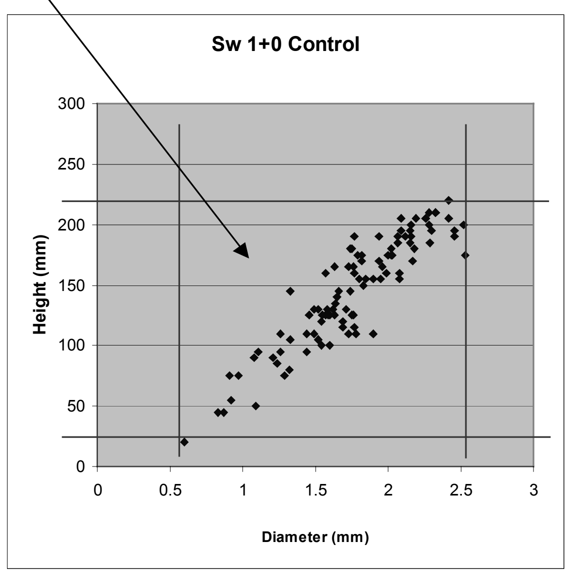

Seedling Growth
Ambiol-treated seeds reversed many of the damaging effects of radiation released during the Chernobyl incident. Scots pine (*Pinus sylvestris* L.) growing within a 30-km zone of the Chernobyl Atomic Energy Plant were not only faster-growing but were also more radiation-resistant (Vischnevetskaia and Roy 1999[8]). Seed treatment with Ambiol, spermine and aminovinylglycine (AVG is an antiethylene agent) increased osmotic adjustment and turgor maintenance in drought-stressed white pine seedlings (Islam 1999).

Ambiol also increased heat tolerance in black spruce and jack pine (Columbo 1995[9]; Beall *et al*., 1997[10]). Ambiol treatment of cucumber seed increased dry matter production of 3-week-old seedlings by 8-10% at room temperature, and also increased thermotolerance. The acceleration of growth was 30% in light-grown seedlings, but increased to 80% in etiolated seedlings, when they were compared at elevated temperatures (Kritenko and Blake, unpublished data).

Ambiol 'switches on' dormant genes in plants. The thermotolerance and antitranspirant activity induced by Ambiol was reversed by application of the protein synthesis inhibitor cyclohexamide[11]. More heterozygous and faster-growing seed provenances showed the greatest response to Ambiol (up to 130% for heterozygous jack pine). Slower-growing, homozygous families were the least responsive (Vichnevetskaia and Roy 1999).

Ambiol treatment increased the temperature tolerance of black spruce, as shown by the reduction in membrane leakage in response to high and low temperatures (Borsos-Matovina and Blake 2001).

Seedling Quality
Frost, heat, drought and a lack of stress tolerance take a great toll on transplanted conifer seedlings. In an independent forest audit (Hearnden et al., 1992)[12], fewer than 10-50% of boreal conifer stands in Ontario regenerated to the original conifer species. Logging may thus be converting prime conifer stands to lower-quality hardwood forest. The lack of regeneration success suggests the need to use more competitive seedlings for reforestation.

To increase growth and stress tolerance, black spruce, white spruce and jack pine seeds and seedlings were treated with natural and synthetic plant growth regulators. Homobrassinolide, HBR, the polyamine spermine, Ambiol, BioProtect and several combinations of these PGRs increased growth and stress tolerance in experiments conducted over the past decade. Greenhouse and field studies were conducted in 1998-2000[i], and 2000-2001[ii] to confirm the growth promoting action of anti-stress compounds.

Ambiol increased the growth of drought-stressed canola by 25-40% compared to that of untreated controls. Treated plants under drought were of a size comparable to the untreated, hydrated controls (Darlington et al., 1996). The growth acceleration of Ambiol-treated plants under stress was associated with a number of physiological changes. Ambiol increased water use efficiency (WUE) in both woody and non-woody species, indicating that carbon gain was increased per unit of water loss.[13] The response to Ambiol varied depending on the species and concentration used.

Stress Tolerance
Ambiol treatment reduces waters stress in seedlings (Islam 1999[14]). Ambiol is a patented antitranspirant, which increases water use efficiency under drought (Darlington *et al*., 1996). Ambiol's stimulation of growth is unique for an antitranspirant. Other antiotranspirants either slow growth or increase mortality (Kozlowski 1979[15]). Summaries are provided of the main treatment effects on growth (Table 1) and physiology (Table 2).

Physiological Action
The structure of Ambiol suggests it is an antioxidant. The decline in membrane leakage suggests that Ambiol

[i] The first study involved a collaboration between Ambiol Inc, La Maison Verte, Hearst Forest Management, and Agrium Inc. Both natural and synthetic plant growth regulators and a growth-promoting bacterium (Agrium[FTG]) were tested in a greenhouse and field study that involved outplanting 27,500 treated seedlings.
[ii] The second study (2000-2001) involved a collaboration between Cook Lake Nursery, Boreal Nursery, Hills Nursery) and LUSTR CO-OP. Growth was monitored on four field sites, two near Thunder Bay and two near Dryden, Ontario.

Table 1. Summary of the main effects of plant extracts (BioProtect) and other plant growth regulators (PGRs) on growth-RDW-root dry weight; SDW-shoot dry weight, TDW-total dry weight, Dia- diameter growth; Sw-white spruce, Sb-black spruce, Pj- jack pine. Amb (Ambiol), Bio (BioProtect), HBR (homobrassinolide). Combination treatment (Bio + HBR + Ambiol)[1].

Parameter	Study #1 (1998-99)	Study #2 (2000-01)	Comment
RDW	<123%	17-60%	BioProtect, alone and in combination, enhanced root growth in the greenhouse.
Root volume	<100%	88-100%	BioProtect enhanced root volume in the greenhouse
SDW	<99%	22-27%	Enhanced SDW in greenhouse & field
TDW	45-70%		BioProetct enhanced TDW of all 3 conifer species in the greenhouse
Height	<33%	<40%	BioProtect enhanced height growth in Study #2- Pj-42%; Sw-44%, Sb-18%.
Diameter	<31%		BioProtect alone, and in combination, enhanced greenhouse and field growth.

may inhibit lipid peroxidation of membranes under stress. Ambiol may donate a H^+ from the second position to oxygen radicals, which interrupts free radical chain reactions that occur during oxidation (Vishnevetskaia and Roy 1999). Ambiol has a variety of other effects. It is an effective antitranspirant, reduced water stress under drought, and inhibits stress ethylene production in drought-stressed jack pine (Rajasekaran and Blake 1999[16]).
Stress alters the balance of PGRs in favour of inhibitors such as abscisic acid (Blake and Atkinson 1986[17]), and ethylene (Blake and Reid 1981[18]) at the expense of growth promoters.

Ambiol has the structural properties of an antioxidant since it is a 5-hydroxybenzimidazole derivative. Ambiol is the only synthetic antioxidant that is known to increase both stress tolerance and growth. An Ambiol pretreatment not only stimulated growth of soybean and rapeseed (Darlington et al., 1996[19]), but also retarded senescence. Ambiol reduced membrane leakage when plants were exposed to extreme temperatures extreme (Borsos-Matovina and Blake 2001[20]) and drought (Rajasekaran and Blake 2002[21]). In each case Ambiol was found to have a membrane-sparing action.

In a three-year trial, seed pre-treatment with Ambiol increased yields of corn and growth of Scots pine (Kuznetzov *et al.,* 1986[22]). Foliar application of Ambiol increased water use efficiency and inhibited the transpiration rate of jack pine (Vichnevetskaia *et al.*, 1996). Under low humidity, Ambiol reduced midday transpiration rate and total daily water use by 25% in soybean (Darlington *et al.*, 1996). Growth of drought-stressed soybean (*Glycine m*ax) and rapeseed was increased by 25-45% by Ambiol treated plants were similar in size under drought to the fully-irrigated controls (Darlington *et al.*, 1996).

Under stress, resources are directed towards repair, which normally slows growth. Abscisic acid, ABA, Ambiol and Triacontonol reduced membrane leakage in drought-stressed jack pine (Rajasekaran and Blake 1999[23]). At 10:g per liter, ABA, Ambiol spermidine and spermine stimulated elongation growth under drought. Plants treated with Ambiol and spermidine maintained higher photosynthetic rates and a greater water use efficiency under drought, relative to the control plants (Rajasekaran and Blake 1999).

[1] Details are provided ("From Seed" Vol 6, No. 1; Vol 7, No. 1 and Vol 8, No. 1 [Blake (1999[1]), Blake (2000) and Blake and Challen (2002)]

Table 2. A summary of the physiological mechanisms induced by natural and synthetic antioxidants, BioProtect and Ambiol. (M.L. membrane leakage; Lp-root hydraulic conductivity; Q_x xylem potential).

Root Hydraulic conductivity	BioPRotect increased Lp by 100% or more. The increase, was also detected when calculated per unit RDW
Membrane Leakage	BioProtect reduced M.L. (by 23-41%), depending on the species and the type of stress. M.L. was less in plants subjected to drought and extreme temperatures.
Water stress	Plants treated with BioPro and Ambiol suffered less water stress (i.e., Θ_x did not decline under drought).
Net Photosynthesis	Carbon gain under drought enhanced by BioPro & Ambiol
Water Use efficiency	Antitranspirant effects increase carbon gain per unit water loss (water use efficiency) in Ambiol-treated plants.

Conventional wisdom suggests that compounds (e.g., gibberellins) that promote growth reduce stress tolerance. The combination of increased growth and stress tolerance in plants treated with antioxidants is unique for a PGR. Antioxidants increased root growth and hydraulic conductivity and lowered water stress. This combination of features allowed PGR-treated plants to continue growth when water became limiting (Rajasekaran and Blake 1999[24]).

Conclusions

Stress targets plant membranes. Oxidative stress causes lipid peroxidation, which destroys the polyunsaturated fatty acids in plant membranes[25]. PGRs were shown to reduce membrane leakage. Antioxidants inhibit the chain reactions initiated by free-radicals. A membrane-sparing action would provide the most likely explanation for the increase in stress resistance and growth promotion observed in plants treated with antioxidants. The ability of the these new PGRs to delay membrane breakdown under drought was associated with increases in: a) relative root development, b) hydraulic conductivity and, c) Q_x under drought.

Ambiol and BioProtect enhanced seedling height, diameter, root dry weight (RDW), and shoot dry weight (SDW). Both were effective when tested in a series of two-year trials. Ambiol increased carbon gain, reduced water loss, which together increased water use efficiency. Despite a higher transpiration rate, plants treated with BioProtect showed little or no increase in water stress under drought. Since plant diameter and root dry weight were stimulated more than height, treated plants became stockier, faster-growing, and more drought tolerant. All of these features are desirable in a transplant.

A seed soak with antioxidants (BioProtect or Ambiol) is a relatively cheap and effective method of preconditioning seedlings. Antioxidants can be applied using a single 24-hour seed treatment before seeding. Seedlings can then be grown using normal greenhouse fertilization and irrigation regimes. Natural and synthetic antioxidants are now being tested by the following organizations: the Forestry Research Institute of Sweden, the Ontario Tree Seed Plant, Hills and Boreal Nurseries (Thunder Bay.), Cook Lake Nursery (Dryden.), J.D. Irving Tree Nursery (Juniper, N.B.), La Maison Verte (Hearst.), Nipissing Forest (Webb's greenhouses) and Woodmere Nursery (B.C.). Further details on BioProtect can be viewed at www.ambiolinc.com.

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Should Seed be Sold to Nurseries?

Yes, because it will increase seed-use efficiency! No, because it will increase seedling production price! Perhaps… let's look at some container seedling production concepts first…

1 - Seedlings are grown on a contract basis. Production costs (excluding seed) largely reflect the amount of physical growing space and time a seedling requires in the nursery in order to meet morphological specification requirements. This is because a plant's light capturing ability, which ultimately governs potential productivity, is limited by time and the amount of space available into which to expand its "solar panel". All energy/dry matter incorporated into a seedling is produced through photosynthesis. Input costs such as land, growing facility capitalization, depreciation, heating, lighting, benching, etc. can be calculated on a per unit area basis, allowing for accurate determination of their contribution to individual seedling production cost. Effectively, the seedling buyer "rents" a given amount of nursery space for a set time. Larger seedlings require more space and/ or time.

2 - The empty cavity. Empty cavities are generated by pests, poor cultural management, etc. but, regardless of initial cause, cannot economically be excluded from receiving stated inputs. Production costs of empties are real, and have to be carried by producing cavities such that overall gross revenue required per unit of growing space allows a nursery to remain viable. Seed quality comes to mind, not with respect to its genetic worth, but rather its germination capacity and speed… or the potential to not generate empties and "culls". "Culls" are seedlings that do not meet required morphological and/or physiological specifications as set out in the seedling-growing contract. Within a seedlot, empties and culls resulting from the sowing of non-viable seed have to be carried by seedlings produced from viable seed. Hence seedling production price increases as seedlot germination % and speed decreases.

Given the current range of container sizes and specifications utilized, initial crop uniformity and cavity fill are extremely important in determining the success of further cultural techniques and the ability to maximize recoverable seedlings in the end. To keep up with rising expectations, costs, and competition, the level of technology employed in the BC Forest Nursery Industry has been rising steadily since its inception. As part of this, seed germination characteristics play a major role in determining overall system efficiency and the final outcome with respect to product "fitness for purpose".

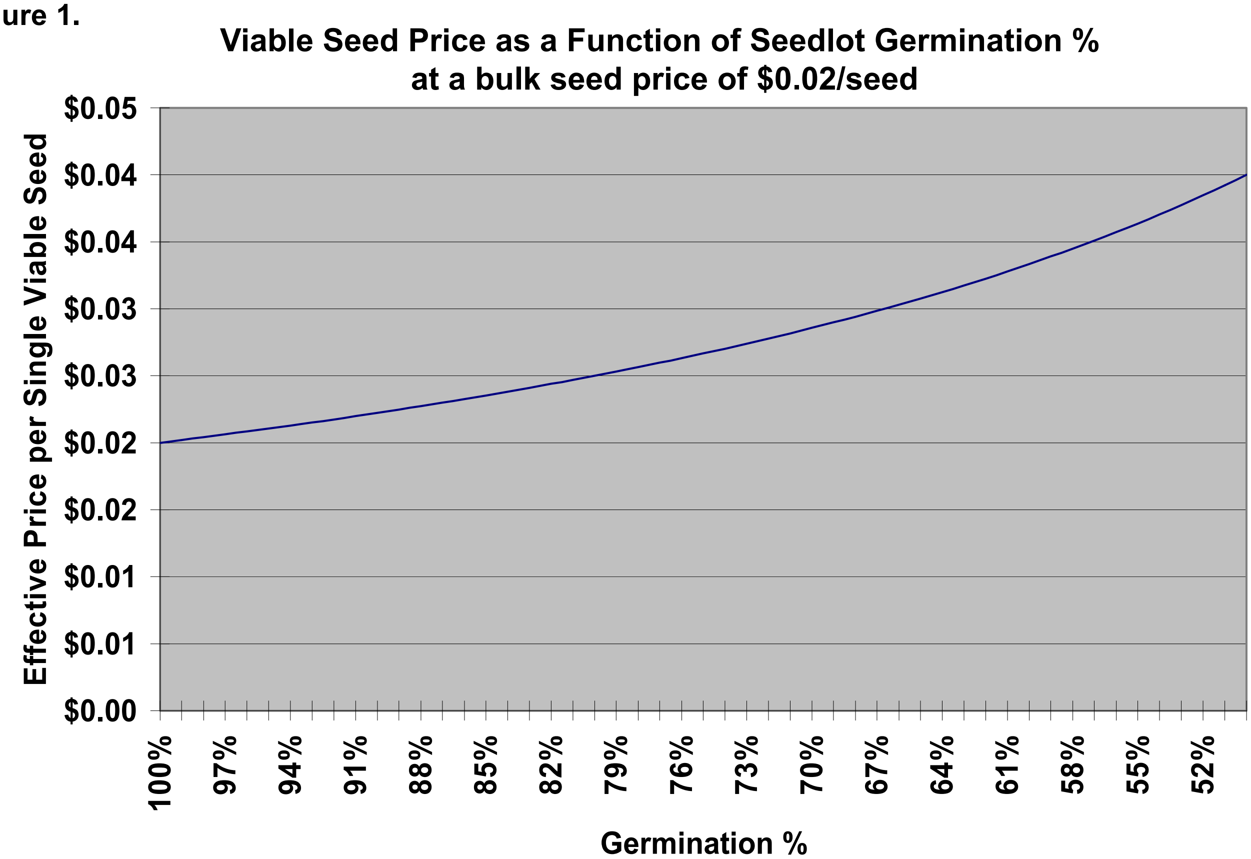
To lessen the impact of poor germination characteristics on seedling production price, seeds are multiple sown (per cavity) and thinned to single seedlings per cavity later. The cost of extra sowing (excluding seed) ranges from almost nil if employing vacuum drum seeders to up to $0.50 per styroblock ™ as slower machinery and more labor is required. Most nurseries are well automated with respect to seed sowing. The cost of thinning ranges from $0.35 to $1.25 per block depending on cavity size, materials handling system efficiency and labor rates, and is easily weighed against the cost of carrying empties to determine a break-even point. For example, in a 45-cavity block converting 1 empty cavity to a saleable seedling can pay for the cost of thinning the whole block. Labor rates, which vary from a low of $8.00 per hour to well over $20.00 per hour, induce their own trends. High labor rates reduce the break-even point for purchasing equipment, hence the level of automation is generally higher at high labor rate facilities. On the other hand, high labor rates raise the cost of thinning, thereby making single sowing and carrying some empties more attractive.

3 - Seed has a price. Wild seed prices are based on monitoring, collection, processing and storage costs. Orchard seed prices reflect the same costs in addition to orchard purchase/operating and breeding costs. To date, seed acquisition costs have been absorbed prior to entering the nursery, effectively separating seed production from seedling production costs. Generally speaking, seed owners and forest companies needing seedlings for reforestation are one and the same, hence they supply seed with which to produce their seedlings, "free" of charge. However, with the advent of more expensive and scarce (in the case of Lodgepole Pine and Western Larch) A-Class seed it has been suggested that seed be sold to nurseries to increase seed use efficiency.

Lets examine how a nursery might incorporate the purchase of seed into seedling production price and how various concepts might affect the process… Will there be savings and increases in efficiency?

Nurseries will purchase the minimum amount of seed required to accomplish the job. However, if nurseries purchase seed with which to grow seedlings they must and will, at the very least, **add** the price per single viable seed to the production cost of each seedling delivered. Viable seeds are those which contain a live embryo capable of germinating under favorable conditions. Viable seed price increases as germination % and speed (vigor) of a seedlot decreases. For example if a batch of (bulk) seed is sold for $1000/Kg but has a germination % of 80, then the viable seed price is $1250/Kg.

Figure 1.



In reality it is virtually impossible to produce a plantable seedling from every viable seed. There is always a nursery-handling factor to account for minimum equipment requirements, spillage, etc. Also, seedling culls are created regardless of seedlot genetic worth. In fact, most culls are the result of factors other than genetics. Density dependent competition effects within a crop are major contributing factors, as are pest pressures, cultural dilemmas, exaggerated morphological specifications, etc. So whether one multiple sows some or all cavities to make up for <100% germination capacity seed, or sows extra cavities to make up for culls/empties generated for other reasons, the end result is the utilization of more than 1 viable seed per plantable seedling produced.

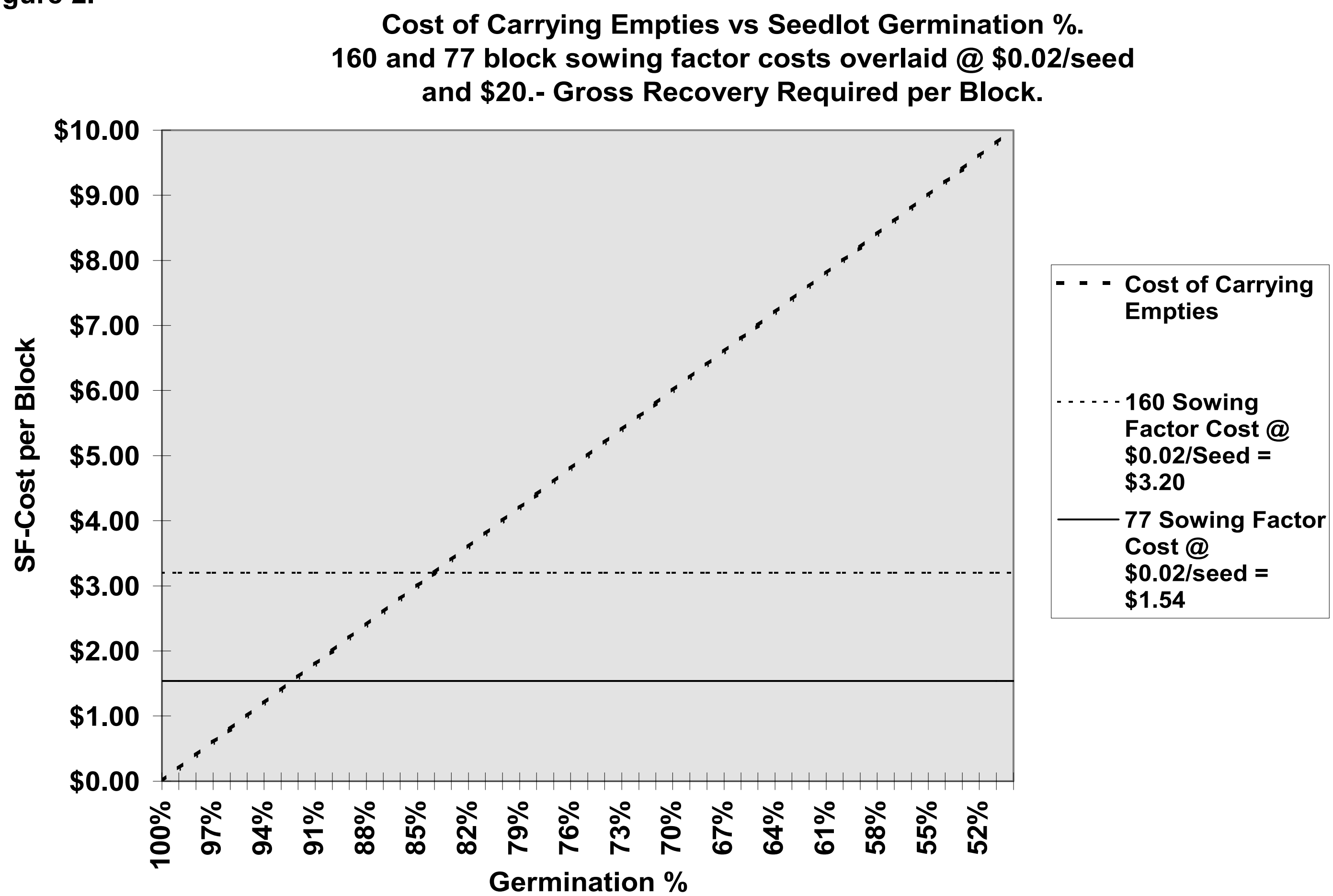
The seed Owner/forest Company may sell seed to the nursery, but will subsequently buy it back again as part of the final seedling price.

If seed cost is charged to the nursery and incorporated into seedling production price, the cost of multiple sowing can be weighed on a per unit growing area or block basis. The number of empties or culls converted to specification seedlings has to pay for:

a) Operational cost of sowing extra seed (machine and personnel time, seed)
b) Entering the (whole) crop for the purpose of thinning
c) Thinning those cavities with multiple germinants back to one seedling/cavity

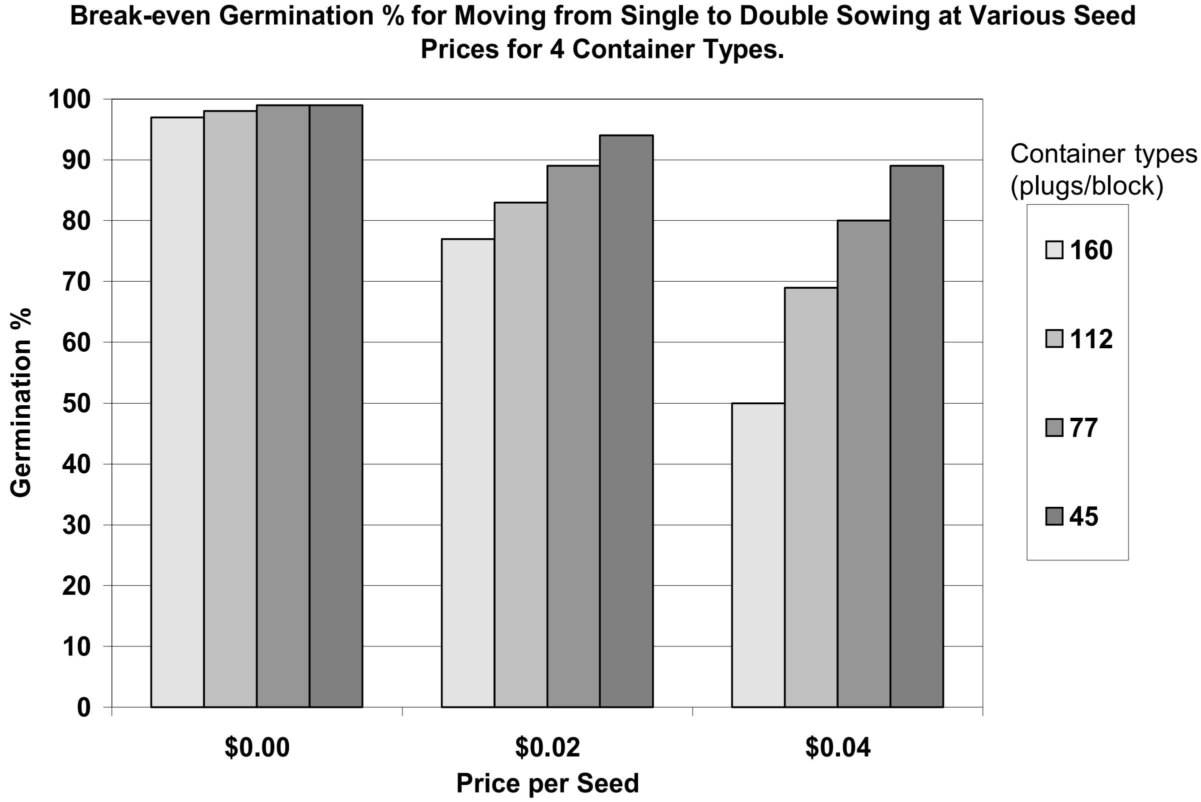
For ease of comparison lets assume a nursery sows 1, 2 or 3 seeds in every cavity, i.e. does not practice fractional sowing. One can work with the cost of one whole sowing factor worth of seed. Conceptually speaking this would be 77 seeds for a 77-cavity block, 160 seeds for a 160-cavity block.

Figure 2.



*** Note that the cost of carrying empties is independent of cavity size, since unoccupied growing space is the issue. For example, given 1000 Styroblocks™, it does not matter whether they are 160 or 77 cavity blocks, an 80% germination seedlot single sown will leave 20% of the cavities (growing space) empty in each one. However, do note the difference in break even points (germination %) for 77 and 160 blocks. This is where the cost of carrying empties equals the cost of one sowing factor worth of seed, implying that moving to double sow (with available seed) becomes an economic option at the stated seed cost and gross revenue requirement. Thinning costs are not included here but would raise the break-even cost, thereby lowering the break-even germination %.

Figure 3.



To carry empties (as seed price increases) is economically justified sooner in high-density blocks than in low-density blocks.

Thus there are a number of criteria that influence where a nursery finds its break-even point for moving from single to double sow, or double to triple sow. Mentioned so far:

- price per viable seed
- seedlot germination % and speed
- cavity type (number per block)
- sowing and thinning costs
- risk of generating culls for a given stocktype/ specification combination
- gross revenue requirements for the particular growing facility

4 – Seed Scarcity. If seed is supplied free of charge so as to maximize cavity fill, the industry makes production decisions based on a viable seed cost of zero, minimizing seedling production cost accordingly. If the forest nursery industry is to produce seedlings as efficiently as possible while incorporating seed costs > zero, then they will need to be able to purchase as much seed as desired at the stated price. This is a necessity so that they can choose the most economic combination of sowing factor, empty cavity count, and oversow factor for the particular seed price/quality and stocktype combination.

To artificially impose a low sowing factor based on seed scarcity or genetic worth may save or "stretch" seed resources but not allow economics to govern production process analysis, resulting in inflated seedling production costs. If scarcity or genetic value of seed needs to be incorporated into seedling production cost, then the seed should be sold to the highest bidder on the open market. Once genetic worth is translated into $/seed, economics will prevail to supply the lowest cost seedling. A seed owner/seedling customer may be unpleasantly surprised by the increase in effective seed cost he/she is paying through imposition of a low sowing factor. For example, imposing a sowing factor of one (1) on an 80% germination seedlot being grown in a 60-cavity block requiring $20. - Gross revenue, translates into an increase in effective seed "value" of (20% of $20)/60 = 6.7 cents. In other words, one is forcing 2 cent seed to be grown as if it is worth 8.7 cents? Would its genetic worth or scarcity bring this price on the open market?

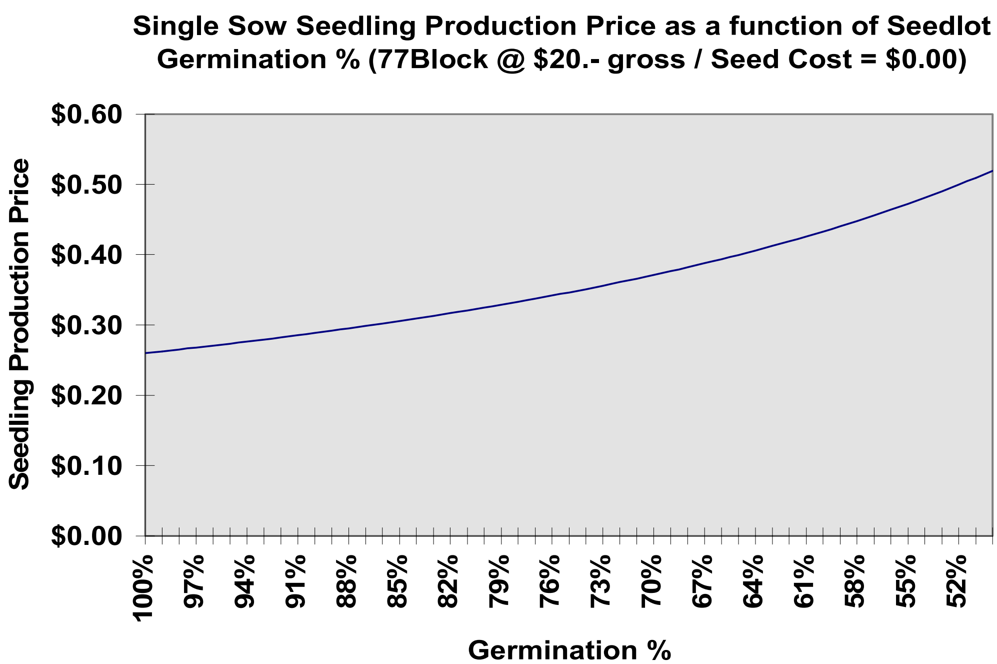
It all comes down to… What do you want and are you willing to pay the price. One can save seed by insisting on single sowing but not necessarily save money…

As mentioned previously, a seedling customer "rents" greenhouse or compound production space and chooses through imposition of seedling morphological specifications and cavity size (seedling growing density) how many seedlings he/she obtains from that amount of growing space. If a client further reduces seedling-growing density in the nursery by requiring single sowing of <100% germination capacity seed, it will mean an increase in the production price per individual seedling.

What will be difficult during the transition from growing with "free" versus purchased seed, is the fact that seedling customers have in mind "seedling production prices" for the stocktypes they currently utilize - prices from which they are generally not willing to budge. It is interesting to note that these seedling production prices are based on recovering viable seedlings from well over 90% of occupied growing space in the nursery and **do not include** seed (production) costs.

Figure 4 emphasizes how seedling production price must increase as a function of seedlot germination % if single sowing. The relationship implies that if every cavity yields a plantable seedling the price/seedling is $0.26. If however, only 50% of the cavities yield a plantable seedling then the price/seedling has to rise to $0.52 in order to keep the nursery in business (seed price is extra).

Figure 4.

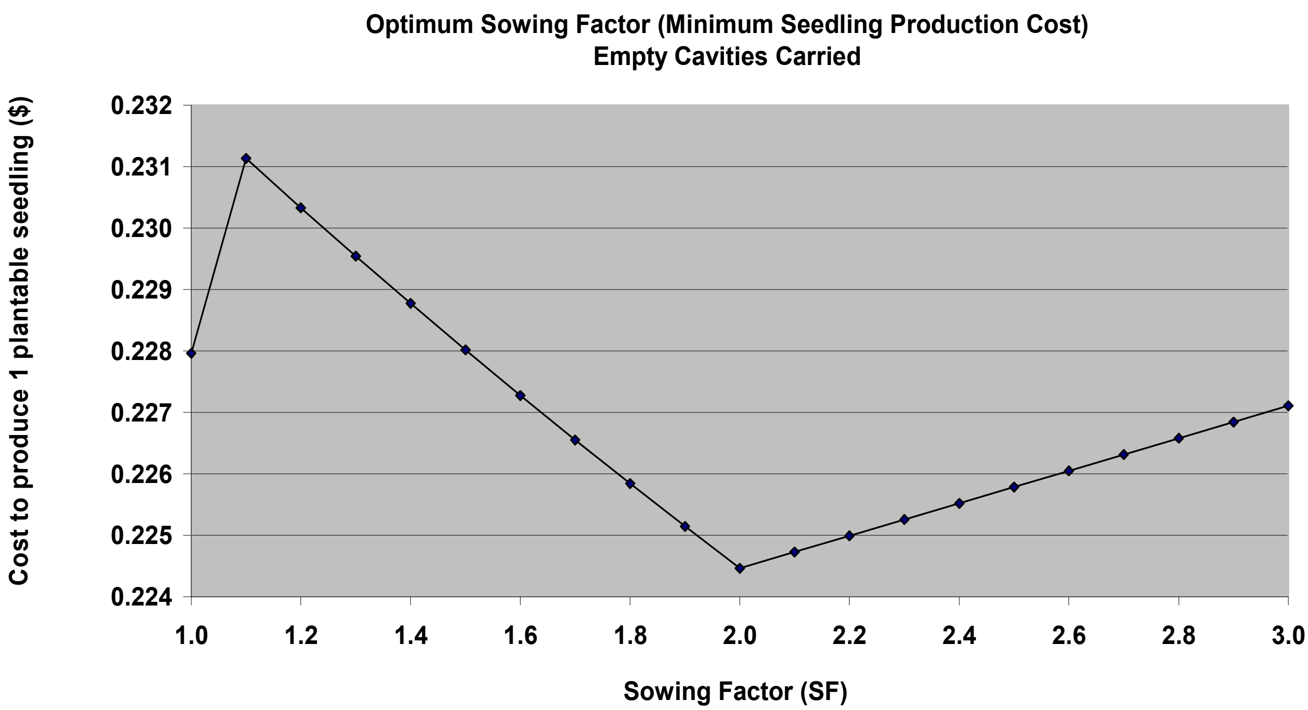


A spreadsheet can be constructed to help determine the optimum (lowest seedling production cost) for various combinations of input costs. In particular, it can be used to determine the most economic seed use pattern over a range sowing factors, seed prices, seedlot germination capacities, thinning costs, etc. Three examples are depicted in Tables 1, 2, 3 and Figures 5, 6, 7 respectively.

Table 1. (Example 1: Seedling Production Parameters)

Seedlot Germination Capacity	94	%
Cost per seed	0.000	$
Cavities per block	112	
Cost to grow/produce a block	20.00	$
Cost to enter crop for thinning purposes	0.35	$/block
Cost to thin first germinant (1>SF≤2)	0.005	$/germinant
Cost to thin extra germinants (SF>2)	0.003	$/germinant
Desired/Required Green Stems	1.20	
Desired/Required Nursery Handling Factor	0.15	seeds/cavity sown

Figure 5. (Example 1)

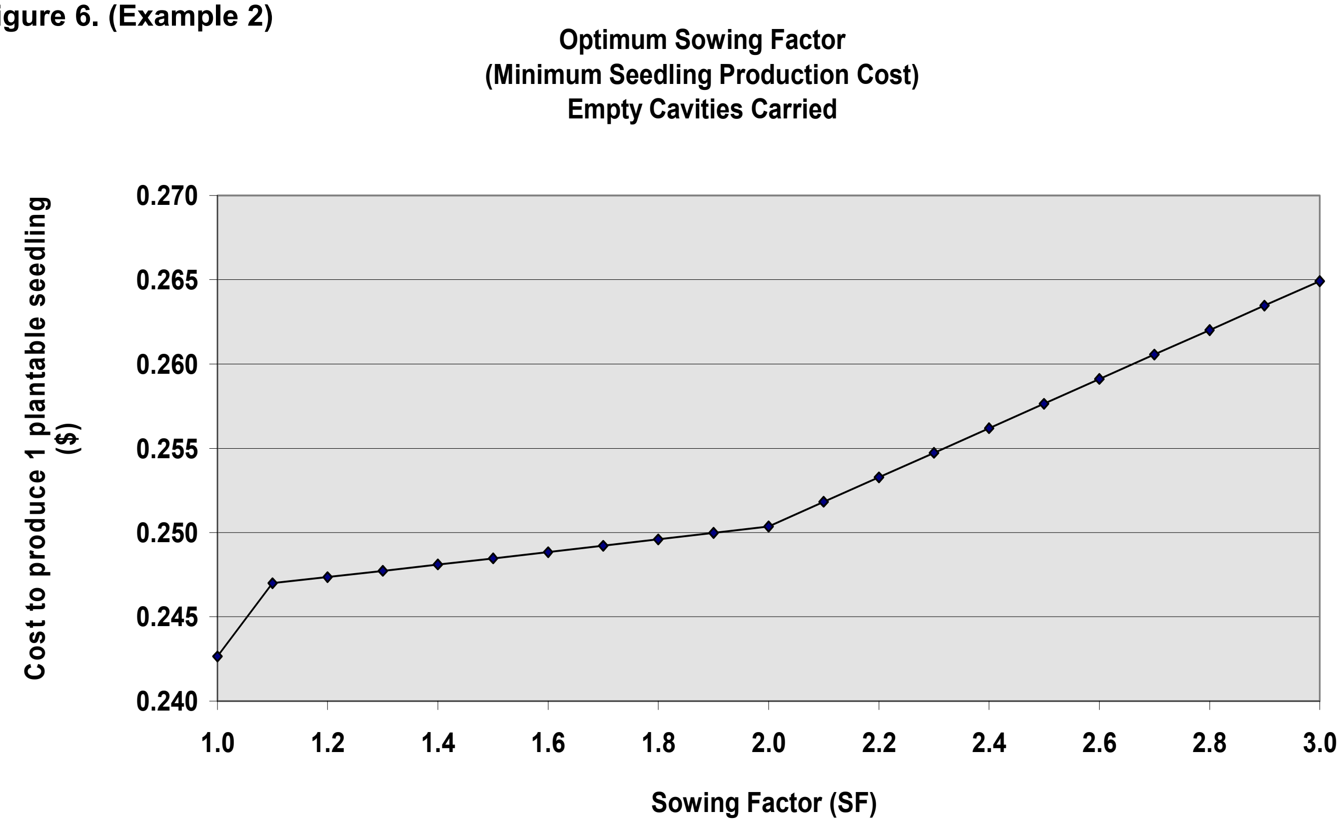


Note: (Table 1 & Fig. 5) Given the nursery parameter values in table 1 with a seed cost of $0.00, the minimum seedling production cost is achieved at a sowing factor of 2. Due to crop entry and thinning costs, it does not pay to single sow or multiple sow at a rate less than 1.5 seeds/cavity. Between 1 and 2 seeds per cavity the largest gains are made in reducing empty cavities, hence the quick drop in seedling production cost. Over 2 seeds/cavity, seedling production cost increases gradually since the increased cost of thinning outweighs the savings from reduced empty cavities (for a 94% GC seedlot).

Table 2. (Example 2)

Seedlot Germination apacity	94	%
Cost per seed	0.010	$
Cavities per block	112	
Cost to grow/produce a block	20.00	$
Cost to enter crop for thinning purposes	0.35	$/block
Cost to thin first germinant (1>SF≤2)	0.005	$/germinant
Cost to thin extra germinants (SF>2)	0.003	$/germinant
Desired/Required Green Stems	1.20	
Desired/Required Nursery Handling Factor	0.15	seeds/cavity sown

Figure 6. (Example 2)

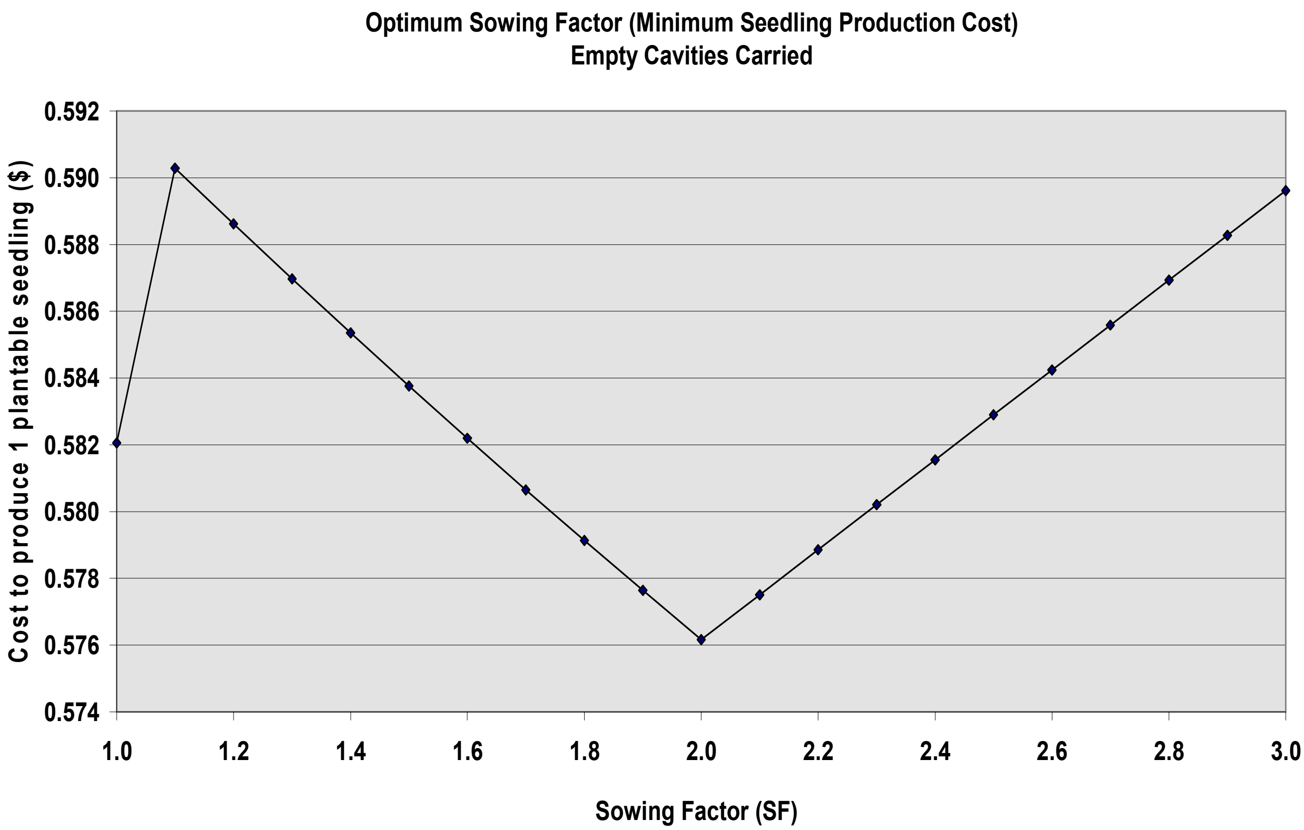


Note: (Table 2 & Fig. 6) Given the same parameter values except that seeds are now worth 1 cent each, the most economic scenario for the nursery is to single sow, not thin, and carry the 6% empty cavities. In a 112 block the cost of additional seed (and thinning) does not economically justify the associated reduction in empty cavities.

Table 3. (Example 3)

Seedlot Germination Capacity	94	%
Cost per seed	0.010	$
Cavities per block	45	
Cost to grow/produce a block	20.00	$
Cost to enter crop for thinning purposes	0.35	$/block
Cost to thin first germinant (1>SF≤2)	0.005	$/germinant
Cost to thin extra germinants (SF>2)	0.003	$/germinant
Desired/Required Green Stems	1.20	
Desired/Required Nursery Handling Factor	0.15	Seeds/cavity sown

Figure 7. (Example 3)



Note: (Table 3 & Fig. 7) In a 45-cavity block, with the same parameters and 1 cent/seed, it does pay to multiple-sow. Minimum seedling production cost is achieved at 2 seeds/cavity. This is because of the increased seedling value relative to seed value. The cost of carrying a large empty cavity is greater than a small one; hence buying 2 seeds to eliminate it is cost-effective. Again, beyond 2 seeds/cavity the rate at which empty cavities are eliminated slows down substantially, and seed consumption begins to drive up overall seedling production cost.

This spreadsheet is available on request by contacting Eric.vanSteenis@gems5.gov.bc.ca.

Some Thoughts…

Nurseries have the ability to incorporate seed cost into seedling production cost, and determine the least cost or most efficient production scenario for a particular seed price and stocktype combination.

There are situations where multiple sowing of high value seed will be the most efficient (cost-effective) way to produce a particular seedling order. Under such circumstances nurseries will need to be able to purchase extra seed in order to achieve these efficiencies.

Seed purity, germination capacity, vigor, as well as genetic worth need to be reflected in seed pricing schedules. To be fair, these should be determined on the open market.

If seed genetic worth and availability is deemed to be such that no viable seeds can be sacrificed under any circumstances then single sowing may be warranted. However, the seed owner/seedling customer must then be willing to absorb a subsequent increase in seedling production price.

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ADDRESS ______________________________
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CITY ______________________ PROV/STATE ______________
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