

FIGURE 14 Relationship between filled seed per cone and respiration. *The relationship between percent filled seed per cone and respiration for 40 dehydrated western hemlock pollen lots segregated into four viability classes (poor=diamond, low=circle, moderate=square, and good=cross). The parameters for the logistic function are $a=58.5$, $b=-0.45$, and $c=4.35$. The coefficient of determination (r^2) is 0.697.*

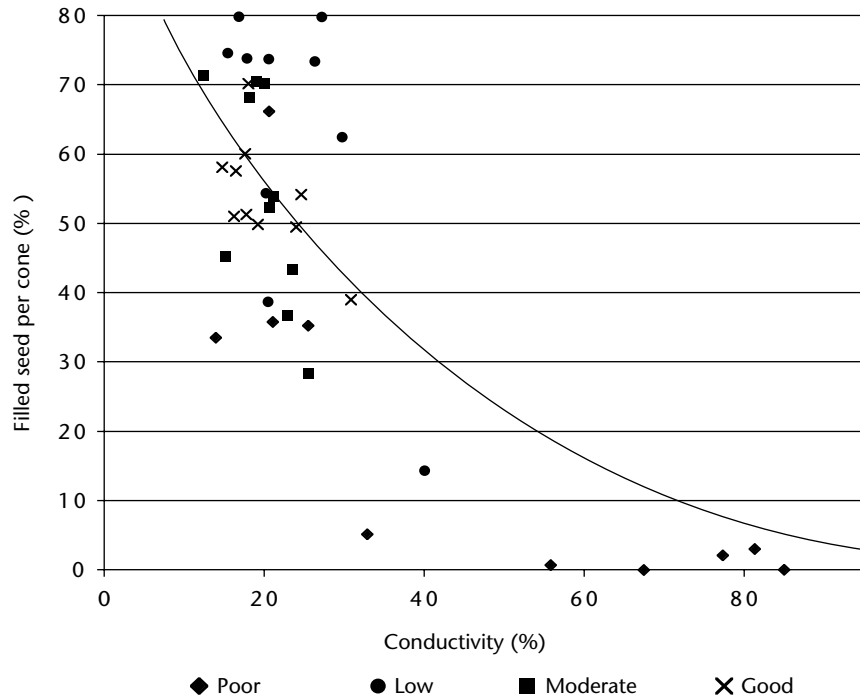


FIGURE 15 Relationship between filled seed per cone and conductivity. *The relationship between percent filled seed per cone and percent conductivity for 40 hydrated western hemlock pollen lots segregated into four viability classes (poor=diamond, low=circle, moderate=square, and good=cross). The parameters for the hyperbolic function are $a=91.7$ and $b=-0.02$. The coefficient of determination (r^2) is 0.645.*

For percent conductivity, however, the r^2 are 0.410 and 0.645 respectively. The improved relationship between percent conductivity and seed yields attributed to hydrating pollen prior to assay is the effect on mending “leaky” pollen membranes.

If we use the 50% potential filled seed per cone as an operational limit for seeds yields in western hemlock, then the corresponding assay response for percent germination, respiration, and percent conductivity are about 45% germination, 15 $\mu\text{L O}_2/\text{min/gdw}$ respiration, and 35% conductivity. Values considerably lower than this could still be used for controlled crossing using a single lot pollen parent, but under open pollinated conditions it would be advisable to use only the highest viability lots. We do not know what effect lower viability lots would have on paternal contribution, especially when used in open pollinated conditions. When specific paternity analyses using DNA fingerprint techniques are available, then pollen competition and SP efficacy studies can be completed.

When formulating pollen poly-mixes, two techniques can be considered. First use equal volumes of all pollen lots but use many lots, not less than 12–15. If fewer lots are required then it is possible to use the techniques described by Stoehr et al. (1999), where lot volume is adjusted to viability. Lower viability lots have higher volumes and higher viability lots have lower volumes. Again, when specific molecular markers (DNA fingerprints) are available, these questions can be answered more directly.

4.3.4 Field pollination techniques On average, seed yields for western hemlock are about 20 filled seed per cone (Edwards 1976). Western hemlock has about 20–30 scales per cone (Owens and Molder 1984) suggesting that the seed potential is about 40–60 filled seed per cone (e.g., two ovules are borne on each scale). This potential is seldom realized because about one-third of the scales are not fertile. Other factors affecting seed losses in western hemlock include ovule abortion, insufficient pollination, no fertilization, and embryo degeneration (Colangeli and Owens 1990). From our experience using good pollination technique this should result in about 25 filled seeds per cone. However, considerable variation can occur between clones and within clones, ramets, and cones. This variation results from variability in the timing of pollination, the amount of pollen applied, and from male–female interactions.

To study the optimal time to pollinate western hemlock seed cones, we define six stages of seed cone development. Seed cone buds are initiated in the spring and differentiated by mid-summer. The newly formed dormant bud that overwinters is our first stage of seed cone development (see Plate 1A). Both seed and pollen cones are rather small at this time and they are difficult to distinguish. Identification by dissection is possible but the technique is technically demanding and requires considerable experience.

Starting in February of the second year the seed cone buds begin to enlarge (stage 2) as the result of the onset of meiosis. The cone tips begin to push through the bud scales (stage 3) about 3 weeks before pollination begins (towards the end of March or early April).

The seed cone bracts separate, exposing the ovules (stage 4) at about the same time that the microsporangia dry and split, releasing the pollen. Throughout the pollination period the seed cones are usually purple and remain horizontal or pendant. The ovuliferous scales then enlarge and seal the cone (stage 5). Visually, the seed cone changes from red or purple during receptivity to green at the pendant stage and then brown at maturity. From early bud burst through receptivity and cone closure, the scales and

seed cone axes continue to enlarge and elongate. About 3 weeks after cone closure, the cones become pendant and continue to mature until mid-August when the scales start turning brown and the entire cone begins to loosen (Stage 6). The mature cone is ready for harvest. A more complete summary of seed cone development can be found in Appendix 1.

Western hemlock has a relatively long receptivity period, which is principally regulated by temperature. Optimal receptivity occurs between 2 and 8 days after bud burst (defined to occur when 40–50% of the bud scales are visible) but can still be receptive to pollen up to 12–14 days after bud burst. Figure 16 shows the seed yields for various pollination times beginning at bud burst (day 0) and for 2, 4, 8, and 12 days after bud burst.

While late pollination (e.g., 12 days after bud burst) does produce good seed set, it is likely that earlier pollination may be more effective for enhancing genetic worth. In Douglas-fir (see Webber and Yeh 1987), we have demonstrated that early pollination is preferentially favoured over later pollination (first-on, first-in). This is a result of the unique pollination mechanism of Douglas-fir, which traps early-arriving pollen in the stigmatic hairs surrounding the micropyle.

In western hemlock, the pollination mechanism is quite different. Unlike most other conifer species where the pollen sifts through the space between the seed cone scales and bracts, pollen seldom reaches the micropyle or integument (see Plate 6A; Colangeli and Owens 1989, Appendix 1) but rather adheres to the outer edges of the bracts and (to a lesser extent) scales. The surface of western hemlock pollen is roughly sculptured with spines that adhere to the waxy hair-like surface of the bracts very much analogous to the fastening system of Velcro (see Plate 6B). About 8–9 weeks after bud burst, pollen grains begin to germinate, forming very long pollen tubes that

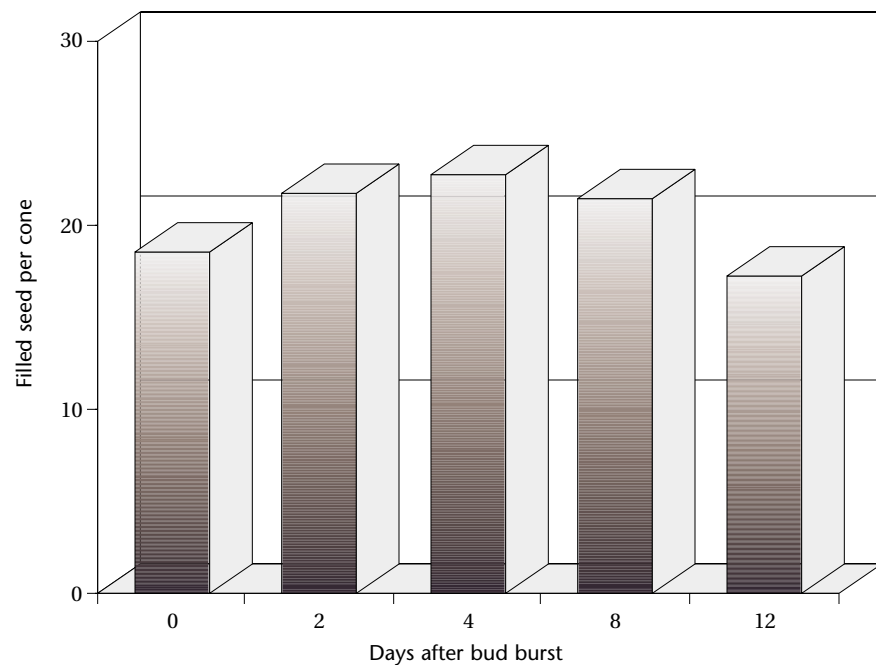


FIGURE 16 Seed yields and pollination timing. *The effect of pollinating western hemlock seed cone buds at bud burst (0 days) and at 2, 4, 8, and 12 days beyond bud burst on seed yields (filled seed per cone).*

Photographic Plate 4
Seed cone development; stages 2, 3, and 4

A. Stage 2 *Meiotic seed bud; seed cone is beginning to develop. (See Plate 1A for stage 1 dormant seed cones in terminal positions.)*



B. Stage 3 *Seed cone bud burst with 50% of the scales exposed.*



C. Stage 3 *Seed cone bud burst with scales beginning to reflex.*



D. Early Stage 4 *Receptive seed cone bud with more than 50% of the cone bud scales exposed due to cone elongation.*



Photographic Plate 5.
Seed cone development; stages 4, 5 and 6

A. Late Stage 4 *Receptive seed cone bud with all scales fully exposed and reflexing.*



B. Stage 5 *Cone closure. Scale swelling closes the gap between the scale and bract, eliminating further pollen access.*



C. Stage 6 *Mature seed cone bud.*



D. Seed cone development sequence
From very early stage 3 bud burst through receptive seed cone bud at stage 4 to cone closure in stage 5.



grow towards the micropyle and about 1 week later, penetrate the nucellus (see Plate 6C). The important point here is the distance that the pollen tube must cover to reach the nucellus. Because of this particular mechanism, pollen viability and vigour must be important to fertilization success. This suggests that some pollen grains will out-compete others in the race towards fertilization.

We are not certain how to use this pollen mechanism to our advantage when we pollinate western hemlock. Will pollen adhering to the bracts first have an advantage over other pollen grains arriving later? Will high viability pollen out-compete low viability pollen? We have attempted to answer the first question and expect the answer to the second will be yes.

Figure 17 shows the seed yields from a time of pollination experiment in which known good and poor viability pollen lots were applied to receptive seed cone buds. Both poor and good pollen lots were applied first at BB+2 days and then each was followed by either the good or poor lot at 24, 48, and 96 hours. For comparison, seed yields for only the good and poor lots are shown as well as a 50% mixture of good and poor lots.

The data in Figure 17 show indeed that the poor lot resulted in poor seed yields and the good lot produced good seed. The yields from the good lot were not diminished by mixing with 50% poor pollen but, because the poor lot was virtually dead, we cannot make any comments on whether pollen competition could occur. When the good lot was applied first, followed by the poor lot at 24, 48, and 96 hours, there was no effect on seed yield by the later-arriving poor lot. When the poor lot was applied first, followed by the

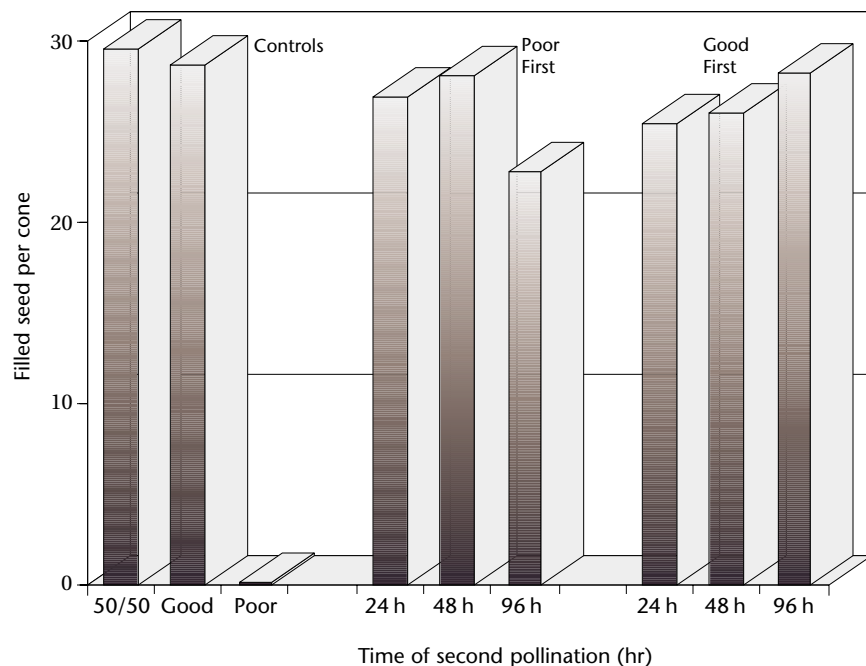


FIGURE 17 Seed yields and time of arrival for good and poor pollen lots. *The effect on seed yields of good and poor viability pollen lots applied at BB+2 in western hemlock and then followed with poor and good lots, respectively, applied at 24, 48, and 96 hours after the initial pollination. For comparison, seed yield values for the good and poor lots only are shown as well as the seed yields from a 50% mixture of good and poor lots.*

good lot at 24, 48, and 96 hours, there may have been a slight reduction in seed yields when the good lot was applied at 96 hours but not at 24 or 48 hours. Apparently the time of arrival of pollen in western hemlock does not affect its ability to complete the events leading to fertilization, as we have noticed for Douglas-fir.

4.3.5 Supplemental pollination Supplemental pollination (SP) is the broadcast application of pollen to female receptive seed cone buds that are not isolated from airborne pollen (Bridgwater et al. 1993). It has been successfully used to improve seed yields, to control paternal composition of orchard seed, to introduce specific genotypes into the seed orchard, and to reduce self-fertilization and contamination. Since contamination is not a serious issue in western hemlock orchards, the principal use of SP in hemlock will be the enhancement of seed yields and the improvement of genetic worth and paternal balance.

For western hemlock orchards in British Columbia the species plan does not specify the type of delivery system for genetic gain, and the available options are few. Currently, controlled crossing with bulking up using rooted cuttings is our only other strategy for producing high genetic worth seed lots. However, these bulking up techniques have not been fully implemented operationally, so we will still require effective SP techniques to meet objectives for seed yields and doubling the gain.

Timing and concentration Success of supplemental pollination is very dependent on the quality of applied pollen (viability, vigour, or fertility potential), and how and when the pollen is applied. Most practitioners experienced with supplemental pollination agree that the technique should be more successful than actual studies indicate. Bridgwater et al. (1993) provide a good review of the advantages of supplemental pollination, including the criteria for success. Pollen quality, timing of application, frequency of application, and amount of pollen are all important factors. We have discussed techniques for maintaining pollen fertility at a high level and its application at the optimal stage of seed cone receptivity. We have also mentioned the importance of quantifying pollen cloud density since it too is a principal determinant of supplemental pollination success. Next we will show that SP can improve seed yields.

Figure 18 shows the seed yield results for an SP trial in the Mount Newton western hemlock seed orchard (1994). One ramet from each of eight clones was treated with a pollen poly-mix composed of nine pollen parents. Pollen was applied at BB+4 days, BB+6 days, and BB+8 days beyond seed cone bud burst. Each was a single application and the treated branch was either bagged or left unbagged after application. Since there was virtually no ambient pollen cloud within the orchard, virtually all seed arose from SP application. Timing of application (e.g., the number of days beyond seed cone bud receptivity) was not important and the loss of seed due to leaving the pollinated seed cones exposed (e.g., unbagged) was also not significant. For this test, pollen supply from SP was adequate and losses due to insects were minimal. These results suggest that supplemental pollination for western hemlock is effective, but we have not tested these procedures under heavy pollen cloud competition or when insect populations are more prevalent.

A question we are often asked about SP application is “what is the dilution rate of applied pollen?” We do know that pollen viability can affect seed yield, and the relationship is not linear (see Figures 13, 14, and 15).

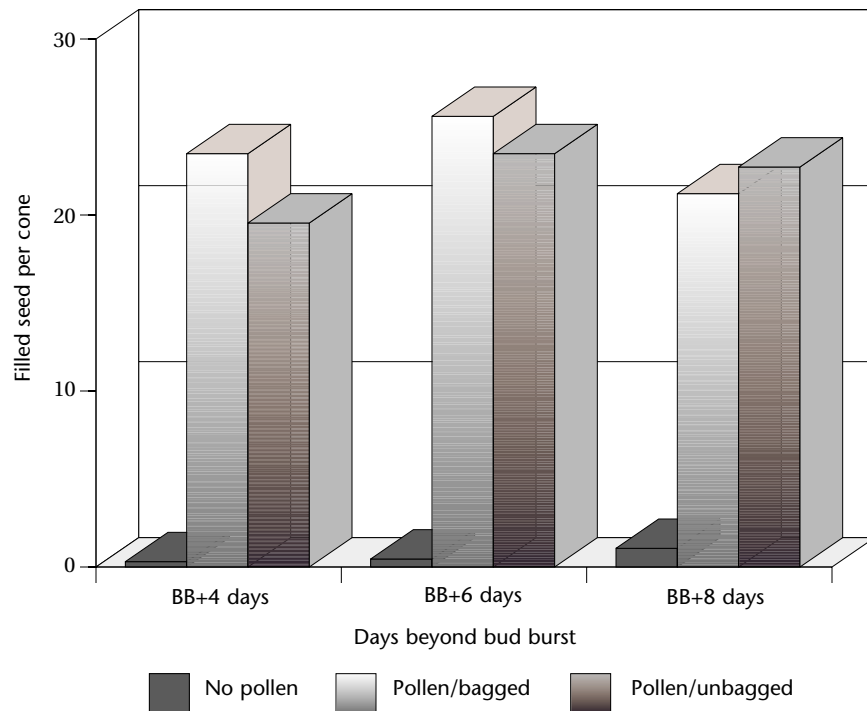


FIGURE 18 Supplemental pollination and seed cone receptivity. *The effect of supplemental pollination at three stages of western hemlock seed cone receptivity for single pollen applications using a nine-pollen poly-mix and comparing treated seed cones left bagged and unbagged.*

In general, seed yields rise in a linear fashion as viability increases. However, there is a point (the asymptote) beyond which increasing pollen viability is not associated with a further increase in seed yields. This suggests that high viability pollen lots could be diluted without jeopardizing seed yields. Figure 19 shows this to be true.

In a pollen dilution study, both dead pollen and talc were used to create a series of live/dead ratios. From 0% live, seed yields rose somewhat linearly to about 40–50% dilution rates. Beyond 50% live pollen, there was no corresponding increase in seed yield with an increasing proportion of live pollen. Up to 50% dilution, there were some differences between dead pollen or talc as a diluting agent but we do not feel that these differences were significant.

Although Figure 19 shows seed yield values to be better for talc as the diluting agent at the 50 and 60% dilution, we do not feel that this is biologically significant. For general field pollination, diluting pollen to 40 or 50% may be an effective tool for stretching limited pollen supplies, especially for controlled cross pollination. However, we have not determined the effect of diluting pollen when used under open pollinated conditions or when used in competition with high viability pollen from both known and unknown sources. Again, this pollination technique can be better assessed when we have access to DNA fingerprint analyses.

The biggest question that remains to be answered is “what is the efficacy of SP applied under various pollen cloud densities?” If we do not see an increase in seed yields (e.g., as we see in Figure 18), then we do not know if there is a corresponding increase in genetic worth. This question must wait for the development of unambiguous techniques for determining contributing pollen parents (paternity analyses). We are working on

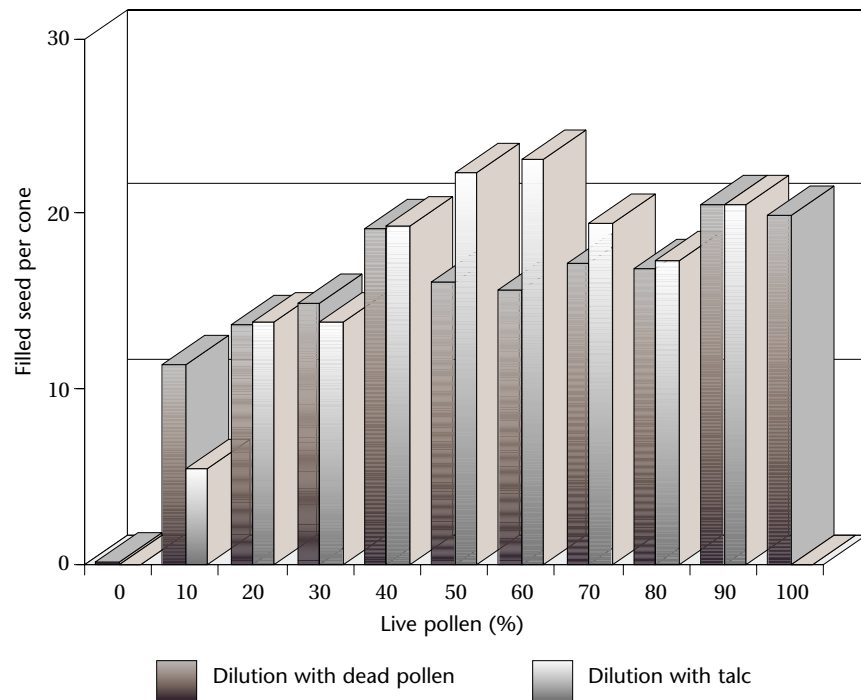


FIGURE 19 Pollen dilution and seed yield.
The effect on seed yields of diluting live western hemlock pollen to specific ratios with either dead pollen or talc powder.

developing specific DNA fingerprinting techniques, and as our diagnostic tools for paternity analyses improve so will our ability to develop SP technique under all orchard conditions improve.

Pollen monitoring We have stressed the effect of competing pollen cloud density (PCD) on optimizing seed yields and genetic worth of western hemlock production crops. Because western hemlock orchards will rely on supplemental pollination to improve yields and genetic worth (at least in the early stages of establishing high gain orchards), then the relationship between competing pollen cloud density and seed yields will be important information to know. Sorensen and Webber (1997) have described this relationship mathematically for western hemlock. Using a log-log model, seed set is seen to rise rapidly with initial increases of PCD, and reaches an asymptote at PCD values that are low compared with maximum values measured under field conditions.

The techniques for capturing airborne pollen and quantifying the captured pollen into cloud density is detailed in the *Douglas-fir Pollen Management Manual* (Webber and Painter 1996). It is strongly recommended that these procedures be used in conjunction with any open pollinated treatments. For controlled crossing and unmanaged orchards, pollen monitoring is not needed. However, for orchards attempting to maximize their genetic worth, knowing the year-to-year variation of within-orchard pollen clouds will help explain the differences observed in SP response for any particular year.

Pollen applicators This manual has not detailed the various types of pollen applicators available. Again, the *Douglas-fir Pollen Management Manual* (Webber and Painter 1996) does provide comprehensive descriptions of the applicator devices we have developed plus others we have tested. There are many types of applicators, each with its own advantages. There are, however, features that make some better than others. It is our experience that applicators that work off compressed air and direct a “blast” of pollen directly at the receptive seed cone are consistently better than those that create only a mist of pollen that is allowed to “float” over the seed cones. Regardless of the way SP pollen is applied, we encourage all users to determine paternal contribution as the best way to guide this developing technique.

5 CONCLUSIONS

The Forest Genetics Council's objectives for western hemlock seedling production requirements up to the year 2007 can be met with current operational seed production capabilities if we:

- develop seed orchard protocols based on field-tested techniques,
- continue to develop supplemental pollination technologies and DNA fingerprint analyses for their assessment, and
- vigorously integrate the new technologies into management of existing and future seed orchards.

What we have learned is summarized in this manual and includes research summaries for cone induction and crown management, and research results for a wide range of pollen management techniques. Although all of these are important, focusing on pollen management, especially supplemental pollination, will enable us to make significant gains immediately.

The most severe limitation to developing technologies for producing seed of the highest genetic worth is determining supplemental pollination efficacy. A major unknown in this process is the effect of competing pollen, and we need to complete trials under various pollen loads to develop supplemental pollination techniques that work for western hemlock.

When these pollination techniques are considered in conjunction with management techniques already developed, we will be able to design orchard systems that will help us meet our target goals for high genetic worth seed lots. In the meantime, if we vigorously utilize recently developed techniques in existing orchards, significant improvement in seed yields and gains in genetic worth are possible.

John Owens and Marje Molder have provided an excellent review of the reproductive biology of western and mountain hemlock (Owens and Molder 1984) with 40 references to additional work. The following summary relies heavily on that review, and there has been no attempt to cite the work every time it was used.

Reproductive potential Western hemlock at lower elevations is generally a good cone and seed producer. On open grown trees the best cone crops form between 25 and 30 years. Individual trees usually produce some cones every year and heavy (collectible) cone crops occur every 3 or 4 years. Cones produce about 30 or 40 seeds each, about half of them being filled.

Growth cycles A schematic of the development of reproductive buds and cones of western hemlock is shown in Figure 20. The schematic is for western hemlock in southern or low elevation regions where activity starts earlier in the spring and continues later into the fall than for trees from northern or high elevation regions.

Figure 20 illustrates the 2-year growth cycle starting from a dormant vegetative bud and ending with a mature seed cone. This schematic shows the flushing of the dormant vegetative bud that occurs in mid-May but no other vegetative development. Vegetative stages not shown in this schematic are flushing that precedes bud swell in late March, rapid shoot elongation in late spring and early summer, and slow shoot elongation through mid-summer.

Axillary bud initiation begins in April, and these axillary buds have the potential of developing into vegetative shoots, pollen cones or seed cones. The dormant vegetative bud shown as the start point of Figure 20 is the second year stage of an axillary, vegetative bud initiated the previous year. Pollen cone development starts in June and seed cone development in July, and both types of cones develop during the fall of the first year.

Pollen development with the associated meiosis in late February and March is the first activity of the second spring. This is followed by seed cone development and pollination in late March and April. Fertilization occurs in late May following female gametophyte development. Embryo development proceeds through the summer, and the seed cones are mature by the end of August.

The following seven sections describe the events outlined in the previous three paragraphs in more detail. Parenthetical references to plates are to the colour plates in the Collection/Extraction (Plates 1 and 2) and Field pollination techniques (Plates 4 and 5) sections of the main text.

Bud differentiation Differentiation of axillary buds occurs after bud scales have been initiated. This normally occurs soon after the rapid elongation stage of the current year's shoot. An axillary bud may (1) initiate leaf primordia to become a vegetative bud, (2) initiate bracts to become a seed cone bud, or (3) initiate microsporophylls to become a pollen cone bud.

Seed cones always form on the terminal buds of lateral vegetative shoots that are 1 year or older. Pollen cone buds most often form on newly initiated lateral buds. Male buds are differentiated first on shorter proximal shoots in the lower crown, followed several weeks later by seed cones typically borne on longer distal shoots in the upper crown. States of

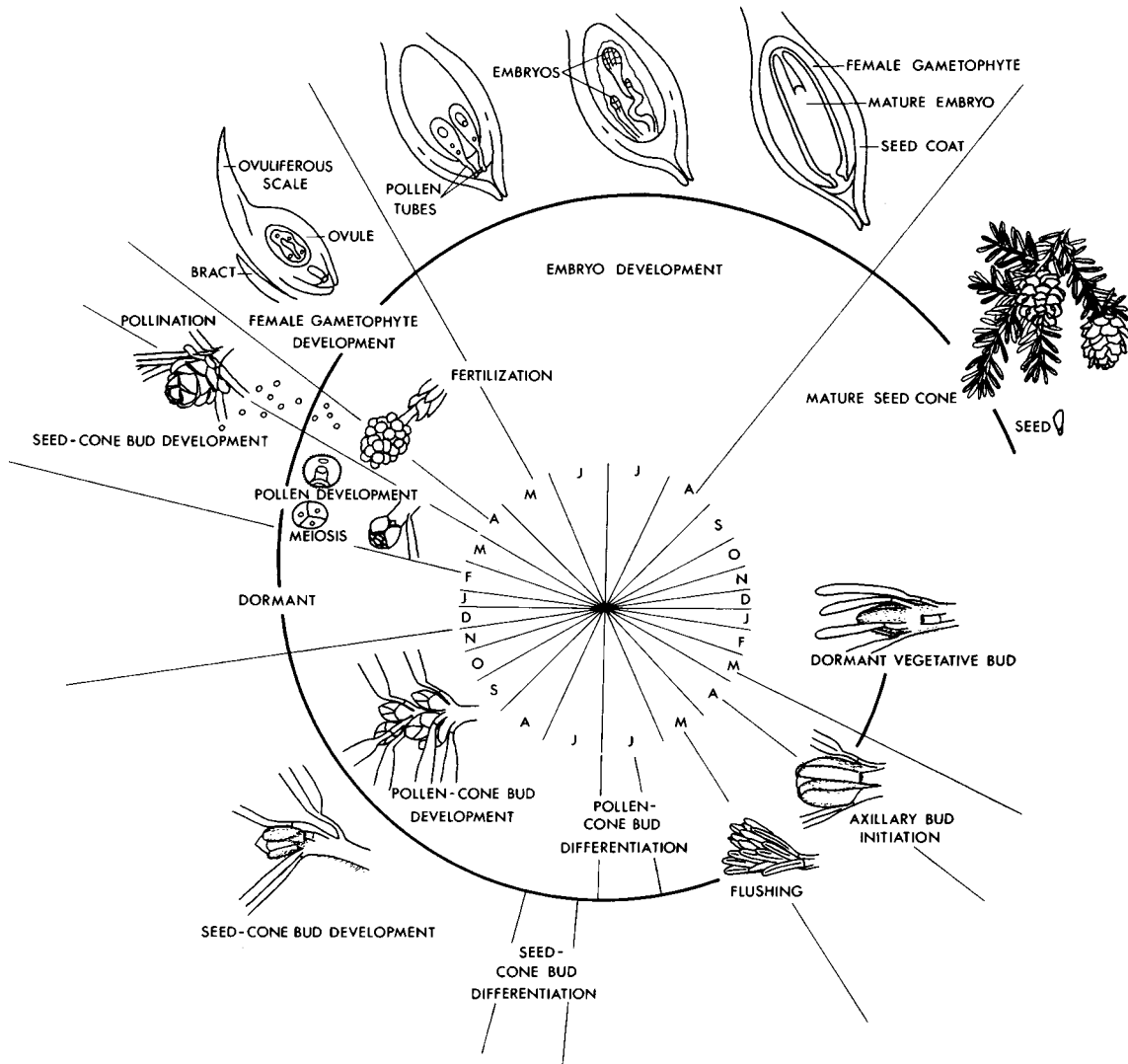


FIGURE 20 Development of reproductive buds and cones in western hemlock. Schematic of the 2-year cycle from dormant vegetative bud to spent seed cone of western hemlock. (From Owens and Molder 1984, Figure 5.)

differentiation occur during a 2-week period and are only visible microscopically. The methods of early identification of buds are too tedious for routine work.

By summer, buds can usually be distinguished on the basis of external characteristics. Vegetative buds are more pointed than cone buds. Seed cone buds are broader than vegetative buds and the shoot at the base of the seed cone bud bends at a distinct angle from the subtending shoot. Pollen cone buds tend to be more rounded than seed cones but are most easily distinguished by their distribution. Several buds are usually clustered around the base of a short shoot (Plate 1A).

Pollen cone and pollen development After differentiation in the first year, pollen buds overwinter at the pollen mother cell stage (see Ho and Owens 1974). Development resumes the following spring with the onset of **meiosis**, the process by which the **chromosome** number is halved (from 24 to 12). This halving is accomplished by the division of the pollen mother cell into four microspores. This is the first identifiable stage of spring

development (Plate 1B). The pollen sacs (microsporangia) borne on bract-like structures (microsporophylls) begin to swell and break their bud scales from February into early March. The first appearance of the pollen cone, known as bud burst, is the purple cluster of microsporophylls as they break through the bud scales (Plates 1C and D).

The pollen cone continues to elongate and the microspores expand with the accumulation of starch and lipids (Colangeli and Owens 1988). When the microsporophylls begin to separate, the cones are very near shedding (Plate 2A). Maturation of the microspore occurs during the elongation of the cone and is complete when the cone axis has completely emerged from the bud scales (Plate 2B). The microspores go through a series of divisions that produce mature, five-celled pollen grains, and these grains are generally shed a few days after being formed (Plate 2C). On the coast, pollen shed can begin towards the end of March to early April. Empty cones (Plate 2D) may remain on the trees for many months.

The early stages of meiosis and microspore development can be sensitive to severe environmental changes, particularly low temperatures (see Webber and Painter 1996 and references therein).

Anna Colangeli and John Owens (1988a) divided pollen cone development into nine phenological stages and compared these with pollen cytology in field-grown and container-grown western hemlock clones. Phenology proved to be an accurate indicator of cytology. The following table is a simplified summary of their work.

In the main text we use only six pollen cone development stages; our stage 4 correlates with stages 4 through 7 in the table below. For field management purposes the pollen is either mature or not, and we do not need be concerned with the cytological stages being considered by Colangeli and Owens.

TABLE A1.1 Western hemlock pollen cone development

Approximate dates of phenological and cytological events for two groups of western hemlock. (Modified from Colangeli and Owens 1988, Table 1.)

Stage	Date	Phenology	Cytology
1	mid-Jan. to late Feb.	Quiescent bud	PMC to end of meiosis
2	early Feb. to early Mar.	Swollen bud	Tetrads of microspores, Separation of tetrads
3	early Feb. to early Mar.	Bud burst	One-celled pollen
4	late Feb. to mid-Mar.	One-half emerged cone	One-celled pollen, Reserve accumulation
5	late Feb. to mid-Mar.	Three-quarters emerged cone	Two-celled pollen, Reserve accumulation
6	early Mar. to late Mar.	Fully emerged cone	Three- and four-celled pollen
7	late Mar. to early Apr.	Stalk elongation	Four- and five-celled pollen
8	late Mar. to mid Apr.	Pollen shed	Five-celled pollen
9	late Mar. to early Apr.	Empty pollen cone	

Seed cone and seed development The number of vegetative lateral buds initiated varies little from year to year, but their subsequent development varies greatly, with dense crops of both seed cone and pollen cone occurring occasionally. The underlying physiology of this is poorly understood, but much information on the effects of external factors on cone bud differentiation has been collected. For cone production, the important stages of development are the later stages of rapid shoot elongation (Owens and Colangeli 1989; Ross 1989).

Environmental factors that enhance cone bud formation include abundant sunshine, warm temperature, and low rainfall preceding and during the time of cone bud differentiation. Of the many artificial methods of enhancing cone bud formation that have been tried, the most successful for western hemlock involves combined use of gibberellins, nitrate fertilizers, and water stress. Generally, however, gibberellin A4 and A7 combined with naturally occurring summer drought seem to be sufficient to induce flowering in hemlock.

After being initiated in the spring, seed cone buds are differentiated by mid-summer. Differentiation consists of the initiation of bracts followed by the formation of ovuliferous scales at the base of the bracts. Ovule primordia are initiated on the larger ovuliferous scales before the end of the first year, making it possible to distinguish the buds visually by the end of the first fall (Plate 1A).

Starting in February of the second year the **nucellus** develops on each ovule. Integument tissue then forms on the end of each ovule; this develops into a short funnel with a wide opening known as the integument tip (Plate 4A). What one sees as the result of these cellular developments is that the seed cone buds enlarge. Bud burst, the emergence of the cone tips through the bud scales, occurs about 3 weeks before pollination begins at the end of March (Plates 4B and C).

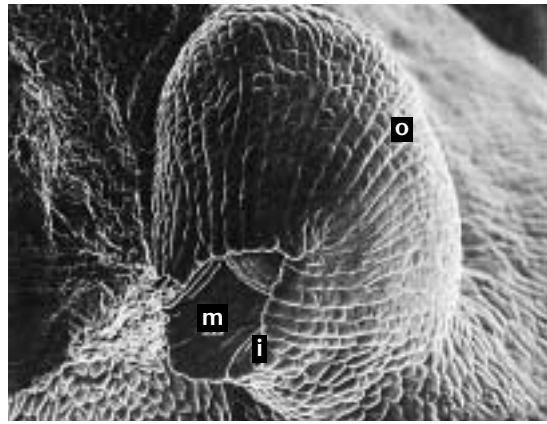
Pollination Pollen cone and seed cone development varies markedly with temperature, so pollination can vary significantly in different locations within a given year and in the same location in different years. Pollen and seed cone development can also be non-synchronous. However, in a specific time and place the pollen cone axis elongates and the seed cone emerges from the bud scales in unison. The seed cone bracts separate, exposing the ovules, and the microsporangia dry and split, releasing the pollen. Throughout the pollination period the seed cones are usually purple and remain horizontal or pendant (Plates 4D and 5A).

After pollination in the early spring the pollen adheres to the bract, ovuliferous scale, ovule, or integument tip of the seed cone (Plate 5B). After 5–6 weeks the pollen will germinate on almost any surface within the cone. After germination each pollen grain forms a pollen tube that grows down to each nucellus. The ovuliferous scales then enlarge and seal the cone (Plate 5C).

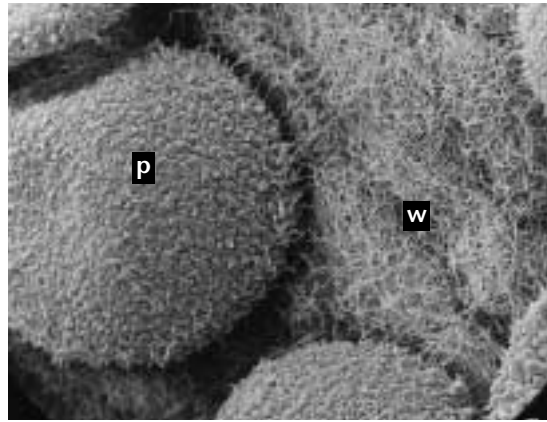
Ovule development and fertilization Meiosis starts after winter dormancy (i.e., concurrent with pollination) and the cell divisions that are part of this process result in the formation of the female **gamete**. In the next 6-week to 2-month period specialized cells form around the gamete, resulting in the filled seed. The seeds will mature (i.e., the gametes, ovules, and cones will develop fully) without pollen. Visually, the best indicator of seed maturity

Photographic Plate 6
Seed cone development and pollination

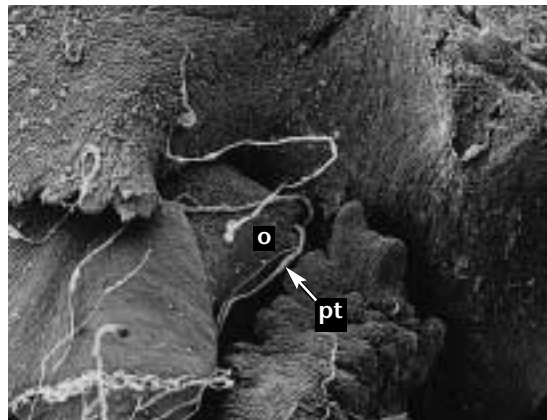
- A. Scanning electron micrograph (SEM) of a fully enlarged ovule (o) in which the integument (i) has elongated, forming a short microphyle (m) (Colangeli and Owens 1989, Figure 14).



- B. SEM of pollen (p) entangled in the epicuticular waxes (w) on the bract (Colangeli and Owens 1989, Figure 19).



- C. SEM of a seed cone with an ovuliferous scale removed, showing pollen tubes (pt) growing into the microphyle of an ovule (o) (Colangeli and Owens 1989, Figure 28).



is the integument and scales.

There are multiple eggs within an ovule. Several pollen tubes may grow into the nucellus, but no more than one will penetrate each **archegonium**. The body cell that is carried through the pollen tube divides to form two unequal-sized male gametes. The contents of the pollen tube are released into the egg cytoplasm, and the larger male gamete moves through the egg cytoplasm and fuses with the egg nucleus. This constitutes fertilization. If no eggs are fertilized within a female gametophyte, it will degenerate, leaving a normal-appearing but empty seed.

Seed development, cone maturation, seed release, and regeneration The embryo undergoes a short development stage within the archegonium that produces 16 cells and a longer phase that takes place in the female gametophyte tissue. While a seed is maturing, as many as 16 young embryos may be formed by two different cell division processes, but normally only one mature embryo develops. Once this embryo begins development within the female gametophyte, the embryo enlarges rapidly and specialized cells form. By fall, mature seeds are formed.

The seed coat begins to differentiate from the integument before fertilization. At pollination a seed wing starts to develop from the ovuliferous scale opposite the micropyle, and the seed with its wing is completely separated from the scale before the embryo is mature. Cones begin to shed seed in September as soon as the seed is mature. The time from pollination to seed release ranges from 120 to 160 days. Shorter maturity periods are associated with drier, warmer weather. Visually, the cones change from red or purple during development to brown at maturity.

Most seeds are shed in the fall when cones first open. Subsequent cycles in wet and dry weather can result in seed release into the next spring. Although cones may remain on the tree throughout the next year, they contain few viable seeds. Western hemlock cones have 20–30 scales. Those in the centre of the cone typically produce about 40 full-sized seeds with less than half of them filled.

Forecasting cone crops Estimating the potential for pollen and seed cone crops is best done in the fall a year previous to harvest. Production of pollen and seed cones on a single tree is generally related, and it is easier to estimate crops based on the more easily recognized pollen cone buds.

The presence or absence of clusters of pollen cone buds at the base of short shoots or from branches collected from the middle portion of the crown gives the most reliable results. Samples must be collected from several trees in an area because individual trees appear to have their own cycle of cone production. Sampling gives an accurate account of the absence of cones, but, because of cone abortion, poor pollination, insects, and disease, the presence of cones does not necessarily mean a good seed production.

This appendix presents the technical details for pollen preconditioning and the three laboratory methods of determining pollen viability. We also provide technical details for controlled pollination, which we use to verify the assay response for the three laboratory tests.

Pollen preconditioning The response for all three viability assays improves somewhat by hydrating pollen just prior to the assay. However, the relationship (regression coefficient, r) between assay response and field fertility (filled seeds per cone) improves substantially only for conductivity (percent conductivity) when the pollen is hydrated (Figures 12, 13, and 14). All pollen lots can be hydrated prior to assay, but we recommend that they always be hydrated before doing the conductivity assay.

The technique we use is to evenly spread about 100 mg of pollen (the dehydrated weight of pollen to be used for the specific viability test). The dish is placed in a closed container saturated with water (100% RH at 20° C). We use a 4-L ice cream bucket with a screen suspended over the water. We do not put the petri dish lid on but we do use a suspended lid to prevent water from forming drops on the petri dish lid and dripping onto the exposed pollen. For western hemlock, we expose the pollen to these conditions for 16 hours (overnight). It is not necessary to reweigh the hydrated pollen if the weight of the dehydrated pollen is recorded before hydration, and all the hydrated pollen is used in the assay. Rehydrated pollen will nearly double in weight and must be used immediately for the test (i.e., rehydrated pollen cannot be stored for future use).

Conductivity Conductivity analysis to estimate fertility potential of western hemlock pollen is recommended for operational use. The procedure involves measurements of cold and hot conductivity and a simple calculation of the ratio of cold to hot (expressed as percentage). It is a relatively simple process that can be completed in less than 2 hours.

Cold conductivity

- 1 Hydration: hydrate pollen at 100% relative humidity at 25° C for 16 hours. If tap water is used, let it sit overnight to allow the chlorine to evaporate.
- 2 Pollen weight: weigh approximately 100 mg of dehydrated pollen, hydrate at 100% for 16 hours, and leach at 25° C for 1 hour. The actual weight of dehydrated pollen can range from 20 to 200 mg. Since pollen weight is affected by moisture content (remember that the weight increases with hydration), all weights should be expressed on a dry weight basis. To calculate the dry weight of pollen, first determine the pollen's moisture content $[(Wt_w - Wt_d)/(Wt_w)] \times 100\%$. The dry weight is then calculated as:

$$Wt_d = Wt_w - (o.MC)(Wt_w)$$

where Wt_d = oven weight of dry pollen
 Wt_w = weight of dehydrated pollen (before hydration)

Before hydration begins, weigh the sample to be treated and calculate the dry weight of pollen. Hydrate the pollen for the specified period and use the entire hydrated sample to determine cold conductivity.

- 3 Leaching volume: 30 mL de-ionized water yields values that are easily determined by commercial conductivity meters. Since variation within lots can be considerable, it is recommended that two replicates of each sample be assayed.
- 4 Containers: use a plastic container with a tight-fitting, leakproof seal. Do not use a metal container. A 50-mL centrifuge tube with a conical bottom that allows pollen to settle before measurement is recommended.
- 5 Leaching: transfer the weighed and hydrated pollen directly into the leaching tube. Pipette 30 mL of de-ionized water into the tube. Accuracy in both weighing and pipetting will reduce errors and improve assay results. Replace the cap and ensure that it is leakproof.

To ensure effective leaching, the tubes must be vigorously shaken. Maintain leaching temperature between 22° and 27° C. After 1 hour, remove the tubes from the shaker and allow the pollen to settle for 5 minutes.

Conductivity values will vary with leaching time. Therefore, keep the time between leaching, settling, and measuring constant. If a number of samples are batch tested, then start the measurements on the sample that was pipetted first and proceed in order.

- 6 Measurement: leachate conductivity can be determined with any commercial meter with a range of 10^{-6} (micro) to 10^{-3} (milli) Siemens (some meters may still use the unit mho but these are not SI units). There are several types of conductivity cells (immersion, flow through, or pipette type), and probes have various ranges of sensitivity (cell constants).

Cell constant refers to the relationship between the cross-sectional area of two parallel plates (electrodes) and the distance between them. Since conductivity results will vary with the cell constant, it is important to first calibrate your system with a standard solution before proceeding.

A 0.01 normal solution of KCl is the standard reference solution for electrical conductivity and has a value of 0.001412 Siemens/cm (S/cm) at 25° C. If your conductivity meter and cell do not produce a value of 1.412 milliSiemens/cm (mS/cm) for the standard KCl solution, then the cell constant is not 1.00. To standardize values, calculate the cell constant ratio K_r :

$$K_r = (\text{EC Std})/(1.412)$$

EC Std = electrical conductance (EC) at 25° C for a 0.01 normal KCl solution using your cell

$$1.412 = \text{EC (mS/cm) value for 0.01 normal KCl where } K_r = 1.00.$$

The temperature of the solution will affect the value obtained. The electrical conductance of an aqueous salt solution will increase by about 2% per Celsius degree. Ensure that all solutions are at 25° C. After shaking, place the tubes in a 25° C water bath to allow the pollen to settle and the solution to equilibrate. The settling time (5 minutes) should be consistent between runs.

- Recording: comparison between lots from different orchards and species is possible by standardizing the results as follows:

% MC = dehydrated pollen moisture content
 Wt_d = oven dry pollen weight (gram dry weight [gdw])
 Vol = leaching volume (mL)
 K_r = cell constant ratio
 EC = electrical conductance (mS/cm)
 EC_s = standardized EC
 T₁ = leaching temperature
 T₂ = solution temperature

Thus:

$$EC_s = (K_r \times EC) / (Wt_d) \text{ mS/cm/gdw}$$

Hot conductivity

- The total leachate can be estimated by heating the pollen for 1 hour in a boiling water bath (an oven at 90–95° C will also work), cooling the pollen to 25° C, and determining the electrical conductivity value. If the solution used to determine the cold leachate does not change volume (i.e., no water is lost because of evaporation), then the same sample can be used to determine the hot leachate value.
- The results are expressed as cold ($EC_c = COND_c$), hot ($EC_h = COND_h$), and the ratio of cold to hot ($EC_c/EC_h \times 100 = \%COND$).

Germination We have tested several germination media for western hemlock. The essential ingredients for germinating pollen tubes are sucrose, inorganic nutrients (notably calcium and boron), and an osmoticum (to prevent rapid imbibition of water in the germination medium). The addition of sugar to the medium may promote further osmotic regulation and/or provide a source of carbon. Regardless, for western hemlock the addition of sugar to the culture medium improves its germination response.

The medium that provides the best germination response for western hemlock pollen is a 0.25% agar containing a 10% Brewbaker and Kwack's solution (boric acid, calcium nitrate, magnesium sulphate, and potassium nitrate) and 5% sucrose (see Figure 11).

1	Brewbaker's solution compounds	mg/100 mL
	Boric acid (H ₃ BO ₃)	100
	Calcium nitrate (Ca(NO ₃) ₂ *H ₂ O)	300
	Magnesium sulphate (MgSO ₄ *7H ₂ O)	200
	Potassium nitrate (KNO ₃)	100

Add de-ionized water to make 100 mL of solution.

2 Working solution

The working solution is a 10% dilution of the stock solution. Thus, 10 mL of the working solution are diluted to 100 mL with a solution of 5% sucrose.

3 Agar medium

There are several types and purity of agar. We use a microbiological source, Bacto-Agar product supplied by Difco. The medium is made by weighing 0.25 g of the agar and placing it in 100 mL of 10% Brewbaker's and 5% sucrose (let the water sit overnight if you do not have access to purified water). The solution is then heated to boiling to dissolve the agar and set the gel.

4 Assay Method

About 3 mL of the warm agar solution is plated into a 35 mm diameter petri dish and allowed to cool. Approximately 10 mg of pollen is sprinkled evenly over the agar. The lid is replaced and the petri dish placed in a larger (95 mm) petri dish lined with water-saturated filter paper. The lid of the larger petri dish is replaced, ensuring that the fit is tight. The pollen is incubated for 48 hours at 25°C (on the work bench is also acceptable, but avoid drafts). It is not necessary to control either light intensity or photoperiod.

5 Scoring germination

After 48 hours, pollen germination is scored using a 40x power microscope for a quick view of the germination results, and 100x power for scoring the classes of germinating pollen grains.

Germination of western hemlock pollen is classified into three categories:

Class 1 elongating grain is >2x original diameter

Class 2 elongating grain is <2x original diameter

Class 3 grain is hydrated (original diameter) but not elongating.

The total number of pollen grains scored in each of Class 1 and 2 determines the overall percent germination. Since we are not certain that Class 1 grains are actually more potentially fertile than Class 2 grains, we combine the two for the total percent germination. However, by separating the two for counting purposes, we do get an estimate of the more vigorous lots determined under the specified incubation conditions. Knowing the percentage of high vigour grains with any particular pollen lot may have implications for pollination technique and fertilization potential when several pollen lots are used within a pollen poly-mix.

Respiration Respiration is a measure of oxygen uptake and provides an overall estimate of the metabolic status of pollen. Strictly speaking, the term respiration may not be appropriate, since oxygen uptake can occur in nonrespiratory cellular functions (oxidation). If we can assume that the nonrespiratory oxygen uptake is minor in comparison to respiratory uptake, then respiration is a more useful term and is a direct estimate of the rate of oxygen uptake and mitochondrial activity.

1 Equipment

Measurement of oxygen uptake in an aqueous solution uses a YSI (Yellow Springs Instrument Co., Yellow Springs, Ohio 45387, USA) model 5300 (dual channel) oxygen monitor, a model 5301 standard bath assembly, two model 5331 oxygen probes (Clark-type polarographic electrodes), a constant-temperature, circulatory water bath, and a dual-channel chart recorder.

2 Calibration

The heater/circulatory pump is turned on and set at 30°C. The oxygen monitor is also allowed to warm up for about 30 minutes before calibration or measurements are attempted. The oxygen probes are covered with a gas-permeable membrane to protect the electrode tip. These should be changed regularly, especially if they are scratched or do not meet specifications in the probe test (see YSI manual).

When changing membranes, it is important to ensure that there are no trapped air bubbles between the membrane and probe tip and no wrinkles or holes in the membrane. Change the membrane after 20 or 30 measurements. Under heavy use, this is every 2 days. When the probe is not being used, store the tip in de-ionized water. Refer to probe handling instructions if extended storage is required.

The Model 5300 monitor has a self-zeroing feature but the chart recorder must be zeroed before the probe is activated. The 100% output value is set by immersing the probe into 3 mL of de-ionized water equilibrated at 30°C, ensuring that all the air bubbles have been removed and the stirrer is on. The function switch on the monitor is set to "AIR" and, after unlocking, the calibration channel control knob ("CAL") is rotated to 100% on the display. Relock the "CAL" control knob. Set the recorder to 100%. The monitor output at 100% is 1.000 volt. The stability of the system is indicated by the recorder trace. The trace should be noise-free and not drift more than 0.5% over a 15-minute period.

3 Measurement

About 100 mg of dry pollen is weighed. Record the actual weight of pollen used and its moisture content. Add 3 mL of de-ionized water to the sample chamber and allow the water to reach bath temperature by stirring for 3 minutes. The pollen sample is then added to the water and allowed to reach bath temperature for about 2–3 minutes. Stirring is stopped and the probe is inserted. Make certain that all air is removed (it may take a few stops and starts with the stirring magnet to exclude all air bubbles). The monitor is then switched to "AIR" and the chart recorder turned on (a chart speed of 1 cm/min is suitable). Uptake is allowed to occur over a minimum of 5 minutes. If the pollen lot is particularly active the curve on the chart may reach 0 uptake in less time, in which case a slower chart speed could be used or time as a factor in calculating respiration values changed. The rate of oxygen consumption is calculated from the slope of the trace and read as a change in percent O₂ consumption over time. To complete this, a best-fit straight line is drawn along the trace. A point on the line is selected and the percent O₂ recorded. The percent O₂ for 5 minutes later is also recorded. The difference between the two recordings is the actual percentage of O₂ uptake by the pollen from the aqueous solution.

The respiration rate (i.e., oxygen consumption) is calculated based on the solubility of oxygen in water at 30° C at 1 atmosphere pressure at sea level. Changes in oxygen solubility due to changes in atmospheric pressure are assumed to be small and therefore are ignored. Thus, the solubility of oxygen in water at 30° C is 5.48 $\mu\text{L O}_2/\text{mL}$. The overall respiration rate is then calculated as follows:

$$R_r = (\%O_2 \text{ change})(5.48 \mu\text{L O}_2/\text{mL})(3 \text{ mL})/(5 \text{ min})/W_{td}$$

where: R_r = respiration rate ($\mu\text{L O}_2/\text{min/gdw}$)

W_{td} = dry weight of pollen (gram dry weight)

Field testing (controlled cross-pollination) Viability assays are best completed in the spring, before the pollination season, when field testing of pollen lots can be completed. For single lot testing, use the controlled cross-pollination technique.

- 1 For each lot, use two pollination bags (make sure that all pollen cone buds have been removed) containing three or four seed cone buds on each of four clones (more if time allows) before bud burst. If it is not possible to replicate all pollen lots on the same ramet/individual (clone/family), then use single ramets (individuals) as a replicate. Because male–female interactions are important, do not apply some lots to one clone/family and other lots to a different clone/family. Make certain that all lots are applied to the same clone/family and repeat this on a minimum of four separate ramets/individuals.
- 2 Pollinate the seed cone buds at their most receptive stage (stage 4, see Plate 5A) with about 0.2–0.5 mL of pollen per bag. If a compressed air pollinator is not available, then use a syringe device and apply the pollen directly to the receptive seed cone buds. We always add a small cube (1 cc) of No-Pest Strip containing 18.6% dichlorvos to each bag to protect against insect damage. The white paper pollination bags can be left on the tree until the cones mature. However, in windy areas bags can tear or damage to the branches can occur so lighter, mesh insect bags may be used as an alternative.
- 3 For each pollination, a minimum of two cones are sampled and the seed is hand extracted for each cone separately (two replicates per pollination). The number of **potential seed per cone** (PSPC) is determined by counting the number of seeds with fully developed seed coats. The number of seeds with fully developed embryos is determined by either squashing the seed or by X-ray analysis. Results are expressed as filled seed per cone (FSPC).
- 4 X-ray analysis is the best way to determine the presence or absence of an embryo. We use Kodax Industrex 620 paper and a Hewlett-Packard Faxitron series (Model 43855A) X-ray machine operating at 15 Kv for 2 minutes. Seed yields are expressed as either filled seed per cone (FSPC) or percent filled seed per cone (%FSPC) calculated as the ratio of filled to potential seed per cone ($FSPC/PSPC \times 100$). Regression analysis between the viability response and filled seed per cone or percent filled seed per cone determines the strength of the correlation between the two variables.

adaptation	a general measure of the ability of an organism to change and cope with the physical and biotic elements of a particular environment, enabling it to reach its potential for growth and reproduction. For orchards, adaptation refers to the degree to which seedlings derived from orchard seed will cope with the environment of the target plantation zone.
adjacency	the mature stand of timber adjacent to the cutblock being planted with improved seed.
alleles	alternative forms of a gene, but with differing DNA sequence, that controls the same character.
anthesis	the stage of full expansion of the flower structures, or—in the case of conifers—strobili. Bud burst is the anthesis of the seed cone (female strobili), and pollen shedding is the anthesis of the pollen cone (male strobili).
archegonium	the flask-shaped female sex organ that contains the egg cell. In most conifers, there are more than one archegonium per ovule.
breeding orchard	an orchard where (usually) controlled pollination is used to produce progeny for testing. Many more parents are usually maintained in a breeding-orchard than in a seed-orchard.
breeding population	the base population of trees in a breeding program that contains the parent material with sufficient genetic variation for long term improvement. For seed orchards, production population is a more specific term and refers to all parent trees within the seed orchard.
breeding value	the expected increase of a clone above the mean of the base population for a selected trait. It can be measured in trait units or percentage gain.
bulking	(sometimes called vegetative multiplication) clonal reproduction of embryos or seedlings by rooted cuttings, tissue culture, grafting, or somatic embryogenesis.
chromosome	rod-like bodies within the cell nucellus that carry the units of inheritance (genes). The number of chromosome sets varies with species and tissue; for western hemlock there are 12 sets for sexual cells and 24 for vegetative cells.
clonal deployment	the use of propagules derived from clones for reforestation.
clone	a group of plants derived from a single individual (ortet) by asexual reproduction such as rooting, grafting, or tissue culture. All members (ramets) of a clone are genetically identical and can reproduce by mitotic division.
conductivity	within the context of pollen viability testing, this is a technique that relates viability with the conductivity of an aqueous solution of pollen leachate.
cone induction	bringing about seed cone development by manipulating the environment, cultural practices, or specific plant hormones (e.g., gibberellins).

contamination	the incorporation of pollen external to orchard sources and generally considered to be deleterious to genetic worth either as poor growth performance and/or introducing poorly adapted genes into a production population.
controlled-pedigree orchard	an orchard in which controlled pollination is used for the purpose of producing elite full-sibling crosses.
controlled pollination (CP)	the transfer of pollen from a known source to known seed cone parents while excluding all other pollen sources.
crown management	controlling the size and shape of a tree's crown while maintaining or increasing seed cone and pollen cone development.
cytology	study of cells.
diploid	vegetative cells that have twice the number of chromosomes as the sex cells (haploid) cells.
embryogenesis	a laboratory technique utilizing specific cultures to produce many embryos from a single seed. These are developed into seedlings that are genetically identical to the original parent seed. Seedlings derived by this process are called emblings.
family	seedling offspring from a particular tree. Half-siblings have one parent in common among family members and full-siblings have both parents in common.
fertility potential	the potential for a particular pollen lot to fertilize an egg cell.
fertilization	the union of the male gamete (sperm) with the female gamete (egg) to form a zygote from which a new plant will form.
filled seed per cone (FSPC)	the number of seeds per cone with a fully developed embryo.
gamete	a mature sex cell, either male (sperm) or female (egg). For conifers, there are two sperm cells (derived from the body cell) within each pollen grain.
gene	a hereditary unit that consists of a specific portion (length) of one of the organism's chromosomes.
genetic diversity	tree breeders use this term to refer to both the variability of traits within a species and the variability of genetic material for these traits.
genetic efficiency	the extent to which orchard seed genetic quality and genetic variability reflects the parental composition of the orchard.
genetic gain	the average (heritable) change from one generation to the next that results from selection.
genetic variability	a general term that reflects both the number and the genetic heterogeneity of the contributing parents to orchard seed.

genetic worth	the average breeding value of all seed from contributing parents weighted for the proportion of their contribution.
genotype	an individual characterized by a specific genetic constitution. The manifestation of a genotype interacting with the environment produces a phenotype.
germination	within the context of pollen viability testing, this is a technique that relates viability with the growth of pollen grains under controlled conditions.
gibberellins	a class of plant hormones, which number over 100, that occur in most plants including conifers. The mixture of gibberellins A ₄ and A ₇ (GA _{4/7}) is the active component for inducing flowering in western hemlock as well as most other Pinaceae species.
haploid	cells that have undergone reduction division (meiosis) and carry one-half the number of chromosomes (gametes) of the vegetative (diploid) cells.
hedge-orchard	an orchard of hedged plants that produces cuttings or other tissue for vegetative propagation. Hedge-orchards have also been used in New Zealand for controlled crossing seed production. In these orchards ramets are placed in clonal rows and tightly spaced (500 sph), crowns are pruned for height control, and induction begins the year after grafting.
hedging	the repeated clipping of an ortet and/or some of its ramets to produce a low hedged plant. The purposes of this procedure are to slow or halt maturation of the plant, and to control its size and shape for efficient operations.
heterogeneity	a qualitative term that describes the extent of variability for a number of traits within a species. For example, genetic heterogeneity describes differences between individuals as a result of the combined action of all genes.
homozygosity	presence of identical alleles (either dominant or recessive). Homozygous individuals breed true for the trait in question when mated with the same genotype.
imbibition	the activity of water uptake in which water moves from an area of high potential (e.g., pure water for germination) into an area of low water potential (dry pollen).
inbreeding	intercrossing of related clones (including self-pollination) of a sexually reproducing population. In general, inbreeding decreases genetic variability and increases homozygosity.
incremental value	the value of tree improvement that is beyond past and current investment.
integument	the tip of the ovule structure surrounding and derived from the nucellus. During early (stage 2) seed cone elongation the integument forms a wide funnel-shaped structure (the micropyle) surrounding the nucellus.
meadow orchard	a variant of the traditional orchard design where ramets are planted at considerably higher density (5000 stems per hectare), cone induction begins the year after establishment, and trees are pruned as required to keep tree height under 2 metres.

meiosis	nuclear divisions leading to the formation of gametes (either egg or sperm cells). The first division results in reducing the number of chromosomes in half (haploid) and the second division results in the formation of four haploid daughter nuclei.
meristem	an undifferentiated plant tissue, often with rapidly dividing cells, from which new tissues or organs arise.
micro-orchard	an orchard in which the ramets are established within clonal rows, crowns are maintained under 3 metres, and density of ramets is 400–500 stems per hectare.
micropyle	a wide, funnel-shaped structure arising from the integument and surrounding the nucellus through which the geminating pollen tube must pass to penetrate the nucellus to reach the egg cell.
microsporangia	two microsporangia are borne on each microsporophyll of the pollen cone. They contain the tissue that forms the pollen mother cells, which undergo meiosis and form the single-celled microspore that eventually develops into the 5-celled pollen grain.
microspore	the male pollen grain, which is mature at shedding and consists of 5 cells; two prothallial cells, a stalk cell, a body cell, and a tube cell.
microsporophyll	the bract-like structures of the pollen cone upon which the pollen sacs (microsporangia) are borne.
mitosis	mitosis is the division of a nucleus into two daughter nuclei by a process of duplication and separation of chromosomes sets. Cell division and cell enlargement then occur, resulting in vegetative growth.
mother cell	the tissue cells from which sex cells develop.
nucellus	at the base of the micropylar canal, the nucellus is the first tissue mass that a pollen tube must penetrate to reach the egg cells.
open pollination	pollination where exposed and receptive seed cone buds are pollinated by any pollen source. In wild stands, wind is the principal vector, whereas in seed orchards, wind is augmented by artificial methods (supplemental technique).
ortet	the original parent tree in wild stands from which clonal copies (ramets) are produced using either grafting, rooting, or tissue culture techniques.
osmotic potential	the solute potential of the medium and cell, which affects the direction and magnitude of water movement in and out of the pollen grain.
osmoticum	a solute of polyethylene glycol added to a germination medium to adjust the osmotic potential of the medium to that of the pollen grain.
out-crossing	breeding between parents that are not related (selfing or inbreeding).
outcross pollen	pollen that is available for fertilization (either airborne or applied supplementally) that is unrelated to the seed cone parent tree.

ovuliferous scales	the scales of the seed cone buds that carry the ovules and then the seeds. They are analogous to the microsporangia of the pollen cone bud.
percent filled seed per cone	seed efficiency defined as the ratio of filled seed per cone divided by the potential seed per cone.
percent gain	the percent improvement of a selected trait for an orchard population in comparison to some unimproved population.
phenology	study of the relations between climate and periodic biological phenomena.
phenotype	the observable characteristics of an organism produced by the interaction of its genotype with the environment.
phytotoxic	the damage resulting from application of chemicals, such as gibberellins and surfactants, to plant tissue (foliage).
pollen cloud density	a relative measure of the number of airborne pollen grains. The actual value is dependent on the technique used, in which case the term could be relative pollen cloud density.
pollen load	a relative measure that indicates pollen cloud density. One hundred percent pollen load is the value beyond which increases in density do not produce corresponding increases in seed yields.
pollen management	the use of various pollen handling techniques to aid or control pollination. See also controlled pollination and supplemental (mass) pollination.
pollen mix	the application of mixed clonal sources of pollen parents either under controlled crossing or open pollinated conditions.
pollen tube	an elongated outgrowth of the germinating pollen grain that penetrates the nucleus and ovule and through which the sperm is transferred to the egg cell.
pollen vigour	a quantitative measure of the actual response of a pollen lot to laboratory assays (e.g., high germination or respiration rates refer to a high vigour pollen lot).
pollination efficiency	the degree to which pollination or supplemental pollination effectively delivers pollen to the seed cone, resulting in seed formation. For most conifer species, pollination efficiency of 100% would be considered to be three to five pollen grains reaching the micropyle but for western hemlock this may only mean pollen deposited on the bracts and scales.
potential seed per cone (PSPC)	the number of seed per cone with fully developed seed coats. For western hemlock, this number is an estimate of the actual number of viable ovules at pollination.
progeny	the offspring of sexual reproduction.
progeny trial	a trial in which the performance of pedigreed progeny from different parents are compared, usually over several progeny sites. Results from progeny trials are used to backward-select among the parents or to forward-select among the offspring.

propagule	a young plant produced by any one of a number of propagation techniques.
ramet	a genetically copied individual from a parent tree clone.
receptivity	the stage of phenological development of the seed cone that permits the entry and capture of pollen on exposed bracts, scales, and micropyle.
respiration	within the context of pollen viability testing, this is a technique that relates viability to oxygen uptake in an aqueous solution.
roguing	the systematic removal of undesirable clones or individuals from the orchard breeding population because of low breeding value or poor flower production.
scales	ovuliferous scales.
seed orchard	a plantation of clones or seedlings from selected trees, cultivated for early and consistent flowering and managed to produce seed crops with a desirable genetic quality and variability.
seed set	the culmination of all steps of sexual reproduction from pollination to seed maturation. For purposes of evaluating field pollination trials, we use filled seed per cone as the sampling unit for seed set.
seed transfer guidelines	the restrictions put on the deployment of any seed lot to specific seed zones and elevation bands to ensure that the seedlings are well adapted to the plantation site.
selection trait	a specified trait (e.g., growth) that is the criterion used to compare performance of offspring of selected parents (clones).
self-fertilization	fertilization by self-pollination.
self-pollination	pollination of female strobili of a tree with that tree's own pollen.
strobilus	the reproductive structures of conifers containing the micro-gametophytes (pollen cones) and the mega-gametophytes (seed cones).
supplemental pollination (SP)	(also called supplemental mass pollination [SMP]) the broadcast spraying of large amounts of pollen (several litres) used to both increase orchard yields and/or genetic worth.
surfactant	an agent (e.g., Aromox) added to aqueous solutions to help "wet" foliage when applying foliar sprays (for cone induction).
total seed per cone	the total number of fully developed seed coats (from fully developed ovules) and rudimentary seed (from immature ovules).
vegetative multiplication (bulking)	when seeds of desired families or populations are in short supply, embryos or seedlings are clonally reproduced by rooted cuttings, tissue culture, grafting, or somatic embryogenesis.

viability the ability of a pollen grain or pollen lot to set seed as determined by its assay performance in laboratory tests (viability assays). For western hemlock, three assays are used: respiration, conductivity, and germination. Correlation analyses are used to determine the strength of the relationship between laboratory assay response and field fertility (seed set).

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