Western Hemlock

A Manual for Tree Improvement Seed Production

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BRITISH COLUMBIA
Ministry of Forests Research Program

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The Forest Genetics Council has laid out objectives for western hemlock seedling requirements up to the year 2007. These objectives include goals for both the number of seedlings and their **genetic worth**. The only current practical options for meeting these objectives are seed production and a limited amount of **bulking** through rooted cuttings. Rooted cuttings technology is more costly and relatively new. Performance of seedlings from cuttings remains largely untested although cuttings/seedlings comparisons are in place. For the near future, seed production will be our prime source of reforestation **propagules**. This manual compiles information from the Ministry of Forests research program as well as other allied programs to make recommendations for seed orchard production of high genetic gain seed lots.

With our current operational seed production capabilities, meeting requirements for numbers of seedlings will be a relatively straightforward task. Meeting goals for genetic worth is more problematic, and will require innovation in two directions:

- developing technologies based on what we have learned, and
- vigorously integrating these technologies into existing and future seed orchards.

Western hemlock lends itself easily to the first of these innovations because it is one our easiest conifer species to manage. The species (1) grafts well, (2) has crowns that are easily pruned for management as well as seed and pollen cone development, (3) is among the easiest species in which to induce flowering, (4) produces pollen that is relatively easy to handle and stores well for at least 3 years, and (5) does not suffer from severe competing pollen clouds (**contamination**) arising from stands surrounding most western hemlock seed orchards in British Columbia.

This manual includes research summaries for cone induction and crown management and actual research results for pollen management techniques that include:

- evaluation of pollen storage methods,
- comparison of germination techniques,
- development of three pollen viability tests,
- the relationships between viability tests and field fertility, and
- supplemental pollination (SP).

Our research has enabled us to formulate technologies that can be used in existing seed orchards and indicates directions for new orchards. However, our most severe limitation to developing technologies for producing seed of the highest genetic worth is determining supplemental pollination efficacy. At this time if we use pollen from quality lots of known **breeding value** we cannot accurately predict how much of the applied pollen will end up as seed.

A major unknown in this process is the effect of competing pollen. 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 gains in genetic worth are possible.

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1 INTRODUCTION

This manual is intended to help orchard managers and tree breeders of western hemlock produce adequate quantities of seed with optimal genetic worth. Both published and unpublished information based on field studies in coastal container- or soil-based seed orchards is presented. Most of the material is on research involved with **cone induction** and **pollen management**, and there are also shorter sections on **crown management** and orchard strategies. It is by combining management of crowns, cones, and pollen with orchard strategies that optimum genetic worth can be achieved.

Protocols presented in this manual are not to be accepted uncritically. While it is reasonable to assume that results from a limited number of coastal orchards are applicable in a general way to other orchards in the Pacific Northwest, different site factors (e.g., meteorological and soil conditions) may vary enough between orchards to make direct application of specific protocol inappropriate. This is especially true for dates associated with the **phenology** of pollen or seed cones and to dates for cone induction treatments. Where an orchard manager is concerned that these protocol do not apply to their orchard or conditions, then we recommend that the protocol be used as a reference point to test and develop orchard-specific procedures.

This manual is written with the assumption that the reader has a fundamental knowledge of the reproductive biology of western hemlock. A detailed account of this subject is outside the scope of this manual, but a brief summary of the essential points along with references for further reading is presented in Appendix 1.

Appendix 2 provides technical details of laboratory viability assays and Appendix 3 provides a glossary of technical terms used in this manual. The first time a term appears it is in **bold**.

1.1 Forest Genetics Council of British Columbia

The responsibility of managing the genetics of forest trees lies with the Chief Forester of British Columbia. It is the Forest Genetics Council (FGC), formerly known as the Tree Improvement Council, that is charged with the responsibility of developing an organizational structure for the efficient delivery of the Forest Genetics program. The approach of the FGC is stated within the strategic business plan with specific objectives for the next decade. This plan is published as Forest Genetics Council Strategic/Business Plan 1998–2007, and is hereafter referred to as the FGC Plan.

The overview in this plan is that "forest gene resource management is the conservation, controlled use, and enhancement of the genetic diversity of B.C. tree species which have, or may acquire, commercial value. Included are stewardship functions of gene conservation, monitoring, and setting **seed transfer guidelines**, as well as genetic improvement to obtain higher value from the resource."

1.2 Objectives

The objectives for western hemlock orchards are the same as for most conifer seed orchards: to supply abundant quantities of seed with the optimal genetic worth. Two objectives of the FGC Plan that have direct effects on western hemlock seed orchard production are:

- doubling the average gain of genetically improved seed from 6 to 12% by the year 2007, and

- increasing the proportion of genetically improved seed used for sowing to 75%.

Within the FGC Plan are a "Genetic Improvement Strategy Timeline," a "Gene Resource Management Plan," and an "Activities and Costs" schedule for low and high elevation western hemlock. These plans specify the timeline for developing the high gain parents and briefly mention the principal source of propagules. The FGC Plan does not provide details about how this elite breeding material can be utilized to increase average gain or increase the percentage of genetically improved seed. It is the purpose of this manual to provide this detail.

2 OVERVIEW

2.1 Distribution and Economic Importance

Western hemlock (*Tusga heterophylla* (Raf.) Sarg.) grows along the Pacific Coast from Alaska to northern California with scattered pockets in the interior of British Columbia and the northwestern states. Its light colour and relatively straight grain make it an attractive wood for finishing and pulping. It is an important commercial species for both coastal and interior British Columbia, making up about 40% of the coastal harvest and about 60% of the province's export market. Sowing requests for 1997 were 7.9 million, with the bulk of planting (about 5.5 million) occurring on low elevation, south coast sites.

Because of its prolific regeneration capacity, many western hemlock areas could be allowed to reforest naturally. However, this regeneration capacity combined with the biological diversity displayed by western hemlock also makes it a good candidate for tree improvement.

2.2 Economics of Genetic Gain

The FGC Plan has devised "Business Plan Value Rankings" and ranks the investment opportunity for south coast (south maritime, low elevation) western hemlock fifth among all tree improvement species in British Columbia (FGC Plan 1998). As a program, the incremental value of tree improvement for western hemlock is ranked third behind coastal Douglas-fir and Prince George lodgepole pine. The return on the investment for improving western hemlock is clear. Furthermore, the economic return of using improved seed for reforestation is additionally compensated for by faster green-up and evenly spaced plantations at rotation.

2.3 Tree Improvement Research

In British Columbia, tree improvement of western hemlock began in the early 1970s and has now grown to include about 1400 selected trees (King et al. 1997). This program is part of the larger Western Hemlock Tree Improvement Cooperative (HEMTIC), an organization that includes about 14 government and private forest company agencies throughout the Pacific Northwest (King et al. 1997). The stated objective of this program is to identify elite parents that will be used for seed production of well-adapted **progeny** that can provide optimal **genetic gain** for wood quality and quantity. Breeding programs both in British Columbia and co-operatively throughout the Pacific Northwest (King and Cress 1991) are now fully in place. Results from the HEMTIC program will not be realized for several more years. However, production from earlier established seed orchards has contributed to 41, 48, and 44% of the annual sowing requests in British Columbia for 1996, 1997, and 1998, respectively (SPAR nursery extract files, B.C. Ministry of Forests).

2.4 Genetic Worth

The genetic quality of orchard seed lots is comprised of three components: genetic worth, **adaptation** to the planting zone, and **genetic diversity**. Genetic worth is a quantifiable estimate of average breeding value of contributing orchard parents weighted by clonal gamete contribution and adjusted for contamination and supplemental pollination. For untested seed lots, adaptation of an orchard seed lot is measured by seed transfer guidelines. For parents under progeny tests, adaptation is measured as the mean latitude, longitude, and elevation of the contributing orchard parent **ortet** location. Genetic diversity is a reflection of the number of contributing parents to the seed lot and is measured by effective population size (Ne). The detailed approach for seed lot rating of genetic worth is provided by Woods et al. (1996).

2.5 Orchard Production

In British Columbia, we currently distinguish three seed zones for western hemlock:

1. maritime low elevation (<600 m)
2. maritime high elevation (>600 m)
3. Queen Charlotte Islands low elevation (<600 m).

According to the species plans for western hemlock (FGC Plan 1998) the current level of genetic worth for Maritime low elevation western hemlock is between 5 and 10%, with anticipated levels rising to 15% by 2002 and 20% by 2007. For high elevation hemlock in the Maritime zone, the corresponding values are about 2% currently and are expected to rise to about 5% by 2007. For the Queen Charlotte Islands, only 2% gain is noted for all years up to 2007.

According to the Fifth Progress Report of the Coastal Tree Improvement Council (Reid and Crown 1995), there are 11 western hemlock orchards registered for seed production. However, the more recent species plans for western hemlock (FGC Plan 1998) list eight orchards for the Maritime low seed zone and five for the Maritime high seed zone.

All are currently soil-based orchards. However, at least two orchards were operated as container orchards (orchards 150 and 163, MacMillan Bloedel). These latter two orchards were dismantled because production requirements for both seed quantity and genetic gain were being adequately met in the less costly soil-based orchards. More recently, second-generation orchards have been established at TimberWest (Mount Newton), Canadian Forest Products (Sechelt), and Western Forest Products (formerly Pacific Forest Products) (Saanichton), with the latter designed as a clonal-row orchard. These orchards will be the main source of improved seed when production begins in a few years.

With the current number of orchards, overproduction of lower genetic worth seed lots is likely. As higher breeding value parents are developed, incentives to increase genetic worth using alternate delivery systems (e.g., vegetative bulking and **clonal deployment**) could further reduce our reliance on orchard seed. If seed orchards are to be more than just a vehicle for generating elite controlled-pedigree seed lots, then management principles must be directed towards the optimization of both the quantity and quality of production seed lots.

There are a number of options available to the orchard managers, which we will discuss in the section "Orchard Strategies." For all strategies, orchard managers must stress the genetic value of production crops. Seed lot rating protocols are being used to evaluate the genetic worth of all crops, and their value will be directly related to either maintaining or improving genetic worth. We will not discuss the rating protocol (Woods et al. 1996) further but rather emphasize those aspects of the protocol (e.g., supplemental pollination) that can be manipulated to improve the genetic worth of existing and newly established orchards.

2.6 Improvement and Potential Gain

Western hemlock is known to be both a good cone producer and a prolific seeder, at least for low elevation sites (Fowells 1965; Owens and Molder 1984). Cone crops occur yearly, with good crops occurring every 3 or 4 years (Fowells 1965). Reports of millions of viable seeds per hectare being released from mature western hemlock trees are common. Obviously, reproductive potential in western hemlock is relatively easy to attain.

The objective is to utilize this reproductive potential to achieve genetic gain, and this can be done by adopting a management strategy that includes some form of plantation forestry. Whether the source of improved propagules is vegetative propagation (rooted cuttings) or sexual reproduction (seed orchards) will be determined by the development of appropriate technologies and costs. Certainly western hemlock's potential for flowering and seed production makes seed orchard production a very attractive option.

Our current seed orchard strategies can produce adapted plantation stock with a genetic gain potential approaching the average breeding value of the orchard parents. With the inclusion of intensive seed orchard management and higher breeding value parents, seed yields and their genetic worth will increase.

3.1 Introduction

Seed orchard managers will be challenged to incorporate better breeding value parents into their current orchards while keeping production schedules at target levels. In general, current orchard designs do not lend themselves to these objectives.

Initially, orchard design and size were determined by projected planting requirements and expected yields based on relatively conservative estimates of production. In almost all cases, orchard production began sooner and produced more per **ramet** than estimated. As a consequence, orchards tended to be larger than necessary. Also, as new seed orchard planning zones were developed and potential seed production estimates updated, the size of and number of parents within these orchards became considerably smaller (Hanson 1986). Wind pollination within these smaller orchards could not be relied upon (Woessner and Franklin 1973) and intensive management practices, including pollen management, were required.

These management practices add costs, but with the introduction of incentives (seed lot price, annual cut allotments, and access to adjacent stands) interest in producing high genetic worth seed lots has increased. Now, decisions about orchard systems and their design, as well as orchard management, will play a more important role. Orchard systems involve choices between a field-based or a container-based system and orchard design involves choices about location and spacing of ramets. Orchard management involves decisions about crown management, cone induction, and pollen control. Orchard strategies involve the interplay between orchard design and orchard management.

Under seed orchard conditions, western hemlock can flower early and abundantly and responds well to crown management techniques. Some of these strategies are currently being used for hemlock seed production and others should be considered for use. Orchard design is probably the least appreciated strategy for facilitating orchard production, while orchard management techniques have been fully employed. We will briefly summarize information about orchard systems, their design, and their management. The remaining part of this manual will then focus on orchard management practices.

3.2 Orchard Systems

The two options for seed orchard production systems are container- and soil-based. Ross et al. (1986) and Eastham and Ross (1988) offer a detailed discussion of the potential advantages of the container seed orchard. These include better production (earlier and more abundant flowering with fewer pest problems) over a smaller land area and improved genetic efficiency (isolation from contamination and increased control over parental contribution). Also, new breeding material (seed cone trees and pollen) can be infused rapidly into the production population as it becomes available.

There are risks, however. Container technology has been available for a number of years but orchard managers have been reluctant to use this orchard approach. Potted trees are more sensitive to rapid changes in their environment and are less forgiving of mismanagement or lack of attention. Container seed orchard technology has been used for western hemlock seed production (MacMillan Bloedel orchards 150 and 163) but the added cost of production was not justified by the low breeding value of available parents. This may change as better breeding value parents become available.

Container seed orchards are being used extensively in the United States for western larch and to a limited extent for western hemlock. Container seed orchard technology for seed production of western hemlock was the most successful for all species tested and still remains a viable option for limited seed production needs in British Columbia. However, before considering this option for seed production, managers should be well versed in the basic horticultural technique of pot culture, cone induction procedures, and seed cone maturation techniques.

3.3 Orchard Designs

Soil-based orchards of traditional design will continue to be our principal source of seed for the foreseeable future and they likely will continue to be predominantly traditional designs. Traditional orchards were designed to maximize **out-crossing** with the assumption that management of crowns, cones, and pollen would be minimal. Ramets of clones were placed randomly throughout the orchard, and wind pollination was allowed to proceed. Initial spacing varied from 2 to 16 m depending on the long term objectives of orchard production. Each unit contained 50 or more clones and ramets were placed in several randomized designs (Giertych 1975). The initial strategy for managing randomized block designs was to minimize inbreeding but it placed no emphasis on controlling out-cross pollen. However, because the timing and amount of seed and pollen production differs from clone to clone, random-design orchards tend to partition into discrete populations due to different dates (phenology) of receptivity (e.g., early, mid, and late).

As the number of orchard parents become fewer, this partitioning and the lack of control over out-crossing makes sustained production of high genetic worth seed lots more problematic and costly. Our seed production priorities are now shifting towards capturing gain. Traditional orchard designs are difficult to improve and do not lend themselves to intensive orchard management. Orchard ramets are being top pruned to give better access to the crop but it is the clonal row orchard design that offers more flexibility in controlling the parental structure (pollen) of production seed lots.

Clonal-row orchards are simply orchards designed with all ramets in a row to facilitate intensive orchard management practices. Normally, they are more closely spaced (2–4 m) since crown pruning controls crown spread. They are more like a breeding orchard in design but are managed for production as well. At least one clonal-row orchard for western hemlock has been established by El-Kassaby (1997) at the Western Forest Products (formerly Pacific Forest Products) Saanich Seed Orchard site. One of the major concerns with clonal-row orchards is that their design promotes **self-fertilization**. El-Kassaby (1997) compared mating patterns and seed yields to a traditional random-designed orchard and found that both orchard designs produced similar yields but that the out-crossing rates were lower for the clonal-row orchard (0.899 versus 0.997), suggesting that selfing was higher. Supplemental pollination was not considered in this test but El-Kassaby (1997) recommends it as a necessary practice to reduce selfing.

3.4 Current Trends

There has been considerable interest in the developing vegetative bulking-up procedures of specific crosses with exceptionally good breeding values. Weyerhaeuser's Douglas-fir program has been bulking-up about 1 million elite propagules yearly (Ritchie 1993). However, the added cost of this procedure limits its application to only the highest genetic worth seed lots.

In British Columbia, we are also developing bulking-up techniques for most species, including western hemlock. We are still at the testing stage, and full-scale deployment will not be considered until costs and seedling performance are fully evaluated. However, it is likely that vegetative amplification will become an integral part of western hemlock propagule production and we should begin to prepare our orchard production strategies to include **controlled-pedigree orchards**. Clonal-row and crown-pruned orchards should also be seriously considered.

3.5 Orchard Management

The major components of orchard management are crown management, bloom delay, cone induction, and pollen management (supplemental pollination). These four components are defined briefly in the following two paragraphs and three of them are discussed in detail in Section 4.

Crown management involves pruning crowns back to manageable size while maintaining adequate seed and pollen cone development. Western hemlock is very amenable to crown management. With other conifer species (in particular coastal Douglas-fir) it is necessary to delay seed cone development in order to reduce pollen contamination. This is not a concern for most western hemlock orchard sites, so slowing seed cone development is not discussed in this manual. Cone induction is possible with a number of treatments; many include using mixtures of the plant hormone **gibberellins** (specifically a mixture of gibberellin A4 and A7 or GA4/7). Such treatments can cut many years off the time required for naturally induced seed cone crops.

The most versatile tool for orchard management of western hemlock is pollen management. This can involve either **supplemental pollination** (**SP**) in which relatively large amounts of pollen are used or **controlled pollination** (**CP**), which utilizes smaller amounts of pollen and normally involves some form of seed cone protection (e.g., bagging).

The increasing emphasis on maximizing yields with the highest genetic worth has turned our attention towards novel orchard designs and intensive seed orchard management. Of the various management techniques we have mentioned, crown management, flower induction, and supplemental pollination are the most useful. By far the most versatile of these techniques is supplemental pollination. The path to successful supplemental pollination is pollen management, and most of the following discussion is given to this topic. Experimental results are given in order to provide managers with the background research upon which we made the recommendations for management decisions. Technical details for testing pollen viability are given in Appendix 2.

4.1 Crown Management

Normally, grafted stock does not flower profusely for 4–6 years after establishment. By this time the ramets are 3–4 m tall. Trees this large have the advantage of producing large seed crops, but the crop is difficult to manage. It is now common practice to at least top prune large trees or in some cases to prune for specific types of branches with a higher potential to produce seed and pollen cones. This pruning is less detrimental for western hemlock than for most conifer species because western hemlock typically has sparse crowns with few potential cone sites. When used in conjunction with routine cone induction, the cone-forming part of the crown can be easily maintained within 2–3 m of the ground.

Studies with other conifer species have shown that removing 40–60% of the live crown can effectively control height without significantly losing cone production (Matheson and Willcocks 1976; Nienstaedt 1981; Masters 1982; Philipson 1985). Ross (1989) has shown this to be true with western hemlock as well. At age 5–6 years, rooted cuttings of a clonal western hemlock orchard were either severely (50% of the live crown removed) or moderately (25% of the live crown removed) pruned. Crop production over the next 6 years showed no effect on either pollen or seed cone production.

Clearly, crown forming is a practical technique for intensive management. Removing the strongly vegetative terminal and lateral shoots encourages more shoot development in the upper crown where seed cones are borne on moderate to vigorous shoots (Owens and Molder 1974). Repeated pruning also encourages the production of less vigorous shoots in the mid and lower crown, which become high potential pollen producing shoots (Ross 1989). Furthermore, Ross (1989) did not find any effect of pruning on induction response. Both seed and pollen cone buds were significantly enhanced the years following spray application of gibberellins.

Similar results have been shown for potted western hemlock stock as well. In fact, for younger orchards, container stock can out produce their soil-based counterparts (Bower et al. 1986; Eastham and Ross 1989), especially if production is based on orchard area and not on individual trees. One of the important reasons increased production can occur is the ability of maintaining crown form and greater control over annual flower induction.

For container stock (Bower et al. 1986), crown forming began the year after grafting and simply involved removing about 50% of the vigorous terminal and lateral shoot growth (February/March) to encourage further lateral and secondary shoot growth. It is far less severe to prune as part of

graft establishment than to remove approximately 50% of the live crown in older material. In the Ross (1989) study, 25 or 50% of the live crown was removed in February, depending on the severity of the pruning regime. Trees were re-pruned in subsequent years to remove stronger up-turning shoots and any vigorous lateral shoots by removing 25 or 50% of the previous year's growth. Thereafter, severely pruned trees were maintained at a height of 2 m.

4.2 Cone Induction

Under natural conditions, western hemlock can remain in its vegetative growth phase for 20–30 years (Puritch 1972; Owens and Molder 1984). However, extensive flowering research under controlled cultural and environmental conditions has shown that western hemlock responds well to several cone induction procedures. Most of these involve a mixture of gibberellins known as GA4/7. This mixture of gibberellins has been used in conjunction with treatments that include: fertilizer (Pollard and Portlock 1983), temperature (Pollard and Portlock 1981a, 1981b), water stress (Ross et al. 1981; Brix and Portlock 1982), and photoperiod (Pollard and Portlock 1984). The treatments have been tested alone and in various combinations. Girdling as an induction technique has not been used extensively. In British Columbia, Parkinson (1989) tested girdling operationally and found that it did not improve either pollen or seed cone production, but Rayonier's Washington State seed orchard has used girdling successfully (C. Cartwright, pers. comm.).

Much of the success of induction treatments has been attributed to timing of treatments in relationship to seasonal patterns of reproductive bud development. A summary of these patterns is shown in Appendix 1, Figure 20. (See also Owens and Molder 1984.)

Both foliar spray and stem injection techniques for GA4/7 application are available. The latter is more commonly used at present. Treatments generally begin at vegetative bud burst, and foliar spray treatments continue for 6 weeks. Owens and Colangeli (1989) suggest that spraying should start at the earlier stage of vegetative bud swell. This is considerably before the start of cone differentiation (Owens and Molder 1974). In another timing of application study, Harrison and Owens (1992) showed that GA4/7 spray application had its greatest effect on seed and pollen cone production when applications began before the onset of cone bud differentiation. Earlier treatments also led to increased cone bud production (especially pollen buds) but the optimal increase occurred at the time of vegetative bud burst.

The techniques for foliar spray are outlined by Ross (1989) and Harrison and Owens (1992). Typically, about 200 mg/L GA4/7 is used in a 0.05% solution (weight/volume) of the aqueous **surfactant** Aromox C12/W. The GA is mixed with the Aromox first and then diluted to the final concentration with warm water. The solution is applied to the foliage to run off followed by a fresh water wash the next day (to reduce the **phytotoxic** effects of the surfactant). Treatments are applied weekly for 4–6 weeks beginning around vegetative bud burst.

Stem injection of GA4/7 is a one-time treatment. Normally, the concentration of GA4/7 applied is adjusted to tree size (stem diameter or crown diameter). Harrison and Owens (1992) used 4 mg of GA4/7 in 0.1 mL of 95% ethanol for 7- to 8-year-old ramets. The solution is pipetted into a 4–5 mm hole drilled about 2 cm deep into the stem at a downward angle about 25 cm above ground level. For larger trees, the dose of GA4/7 can be increased proportional to stem diameter. The same concentration can be used but the number of holes is increased by one for every 5 cm of stem diameter.

There are some problems arising with the supply of GA4/7. Currently our only supplier is Abbott Laboratories (1401 Sheridan Road, North Chicago, Ill. 60064-4000, USA). They have recently formulated the gibberellin mixture into a proprietary product called ProCone™. The active ingredient of ProCone™ is GA4/7 at a concentration of 4% but we do not know what the inert ingredients (carriers) are. Thus, the given protocol for flower induction in western hemlock will only be useful as long as crystalline GA4/7 is in stock. If ProCone™ is to be a meaningful substitute, we will have to test its induction efficacy before recommending it as an operational substitute. Furthermore, we will have to deal with Canadian import laws governing the import of GA4/7 as a registered pesticide in the United States.

4.3 Pollen Management

Because pollen is relatively easily to handle and contributes 50% of the genetic worth of seed lots, effectively managing pollen offers orchard managers a powerful tool to manipulate the genetic structure of their seed lots. Managing pollen is also essential for breeders to complete mating designs in an orderly and timely fashion. Pollen management refers to the collection, extraction, storage, testing, and re-application of pollen. Pollen can be applied either fresh or after storage. Controlled pollination (CP) is the application of small quantities of selected pollen while eliminating other sources of pollen. Supplemental pollination (SP) (also referred to as supplemental mass pollination or SMP) is the application of large volumes of pollen under open pollination conditions.

The extent to which any artificial pollination program is successful depends largely on the viability of the applied pollen. Therefore, maintaining pollen viability during handling and storage is the principal goal of pollen management, and viability testing is an essential part of the process.

Pollen is a living organism, and its activity *ex situ* is controlled primarily by its moisture content and temperature. When pollen is shed from its cone, it is particularly vulnerable to high humidity (>60%) and high temperatures (>30° C). Pollen development leading up to pollen shed can also be adversely affected by environment.

When dealing with pollen it is important to be able to recognize stages of pollen development in the field and to understand the biological significance of these stages. A brief account of these stages is given in the following paragraph, and there is a more detailed presentation in Appendix 1.

Pollen buds are initiated and differentiated the year prior to shedding and over winter at the **pollen mother cell** stage. The pollen sacs (**microsporangia**) borne on bract-like structures (**microsporophylls**) begin to swell and break their bud scales in late winter. This process is very temperature-dependent and occurs from February to early March. The first appearance of the pollen cone as it breaks its bud scales is the purple cluster of microsporophylls. 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. Pollen shed will occur 1–2 weeks after the microsporophylls begin to separate from the cones. On the coast this begins towards the end of March to early April.

4.3.1 Collection/Extraction Pollen management requires a sound knowledge of both the **phenology** and **cytology** of pollen development. A summary of this development in Appendix 1 is based on the work of Colangeli and Owen (1988). They use nine stages of pollen cone development.

For our purposes we find the following six stages adequate:

Stage 1 Dormant bud; pollen mother cells (Plate 1A)
Stage 2 Initial pollen cone swelling; meiotic cells (Plate 1B)
Stage 3 Bud burst; one-celled pollen (Plates 1C and D)
Stage 4 Elongation of the pollen cone axis; one-celled pollen to four-celled pollen (Plates 2A and B)
Stage 5 Pollen shed; five-celled pollen (Plate 2C)
Stage 6 Spent pollen cone (Plate 2D)

Pollen cones can be picked at stage 4 but it is always better to wait until the first signs of pollen shed begin. We pick the pollen bud rosette and dry the buds at 30° C at an average humidity of 30%. This procedure is consistent for several other conifer species (e.g., Douglas-fir, interior spruce and lodgepole pine). Most of our initial experiments with pollen drying were done with a dryer designed to control temperature and monitor both temperature and humidity during the drying cycle. This system, described in Webber and Painter (1996), can reduce about 25–30 L of wet buds to a moisture content between 5 and 10% of the extracted pollen in about 36–48 hours. The only difference between drying western hemlock and Douglas-fir pollen is the mesh size for the bags used to contain the drying pollen buds and shedding pollen. The diameter of western hemlock pollen is about 60 microns, ranging between 59 and 71 microns (Owens and Simpson 1986). The pore size of the extraction bags is about 45 microns. Average yield is about 45 mg pollen per mL of buds, or approximately 10% (volume of dried pollen buds to volume of pollen extracted).

Although good quantities of pollen can be picked in reasonable time, it may not be cost-effective for large scale pollination programs. A good alternative, vacuum collection of the shedding pollen, may be considered. We have successfully used a small portable generator and a backpack vacuum with good success. We replace the vacuum bag with a mesh screen (silk screen material) with a pore size of about 45 microns. Obviously the problems of supplying power to a field operated machine and vacuuming shedding pollen under wet or high humidity weather is a detraction. However, the alternative of not being able to produce sufficient quantities of high gain seed lots may be the incentive needed to develop cost-effective pollen collection systems.

The other problem with vacuum collection of shedding pollen is drying extracted pollen. It is far more easy to dry pollen buds and then extract pollen at a moisture content suitable for storage. However, when shed pollen is collected and needs further drying, then one of the following techniques may be employed (details provided in Webber and Painter 1996). For small lots (<10 mL), we use a desiccator cabinet with a fully charged (dry) desiccant. Pollen is spread thinly and evenly in a 10 cm diameter Petri dish and exposed to the desiccant until moisture content is in the desired range (5–10%). Rate of drying will depend on the initial pollen moisture content, the size and numbers of lots, and the size and charge of the desiccator chamber. For a chamber about 30 x 40 x 50 cm and a volume of desiccant of about 500 mL, about 15 Petri dishes with about 150 mL of pollen can be dried to 5–10% MC in a few hours.

For drying larger volumes of pollen (few hundred mL to several L), a drying column, such as described in Webber and Painter (1996; p. 14), can be used. These columns are not commercially available, so their use will be limited to those capable of building one. If this cannot be done, then drying small

Photographic Plate 1
Pollen cone development; stages 1, 2, and 3

A. Stage 1 *Dormant buds; females in terminal positions and males in axial positions.*

B. Stage 2 *Initial pollen cone swelling due to meiotic activity.*

C. Stage 3 *Bud burst resulting from meiotic activity and cone axis elongation.*

D. Late Stage 3 *Bud burst.*

Photographic Plate 2
Pollen cone development; stages 4, 5, and 6

A. Early Stage 4 *Maturing pollen cone resulting from elongation of the pollen cone axis, separation of the microsporophylls, and pollen maturation.*

B. Late Stage 4 *Mature pollen cone; pollen cone can be picked.*

C. Stage 5 *Shedding pollen cone.*

D. Stage 6 *Spent pollen cone.*

batches in the desiccator is still possible. A third but less recommended option is drying in a warm room. Pollen is spread out on paper sheets and left exposed to the room environment until drying is complete. The room should be kept at 25–30° C and the humidity reduced to about 35–40 % RH. Be careful to avoid exposing the pollen to both high temperatures (30° C) and high humidity (70 % RH). Under these conditions, pollen viability can deteriorate quickly.

It is also possible to accelerate pollen development in western hemlock. Colangeli and Owens (1991) placed cut branches into water at room temperature. This technique was effective only if the buds were already at an advanced stage of development. Buds that had elongated 50% beyond the bud scales produced normal yields and viability. Buds forced before this stage produced less pollen and increased the number of abnormally developed pollen grains and lower seed yields in field pollination trials. Accelerated pollen development in potted western hemlock can also be accomplished by simply bringing potted stock into a warm shelter house (J.E. Webber, unpublished data). This is a more effective treatment because the stage of pollen development was not important. Pollen shed occurred about 10–14 days sooner than their outdoor counterparts and the pollen was the same in both yields and fertility potential.

4.3.2 Storage The importance of pollen moisture content on retaining viability has been stressed. If pollen is to be used soon after collection (e.g., for pollination in the same year) then pollen moisture content is less important. However, if pollen is to be stored for any length of time (more than 4 weeks), it is important to know the pollen moisture content before storing. The other two important factors affecting pollen fertility in storage are storage temperature and storage atmosphere. Main factor effects (pollen moisture content, storage temperature, and storage atmosphere) on 1- and 3-year storage of western hemlock pollen is shown in Figure 1. The interaction of these main factor effects for 1- and 3-year pollen storage are shown in Figures 2 and 3, respectively.

The results for 1- and 3-year storage trials for western hemlock are similar to those of most other conifers tested. Pollen reduced to a moisture content below 10% and stored in a container with the air removed (vacuum) at standard freezer temperatures (-25° C) retains fertility, expressed as mean filled seed per cone, for the 3 years tested.

Based on data presented in Figures 1, 2, and 3, we recommend that western hemlock be stored in evacuated containers (no air) with a pollen moisture content not exceeding 10% (preferably between 4 and 8% MC). The lowest freezer temperature we tested was -25° C (operating temperature for a normal commercial freezer). If hemlock behaves similarly to other conifer species, storage at temperatures lower than -25° C can be recommended. Data shown in Figures 2 and 3 suggest that western hemlock pollen could be successfully stored at +5° C for 3 years if air is removed from the sealed container. However, where a freezer is available, pollen should still be stored in evacuated containers at freezer temperatures.

4.3.3 Pollen viability tests The extent to which any applied pollination program is successful depends principally on the viability of the pollen. Therefore, viability testing is a core step of any pollen application program. Laboratory viability tests are available to test potential fertility in most conifer species, including western hemlock. The procedures for hemlock

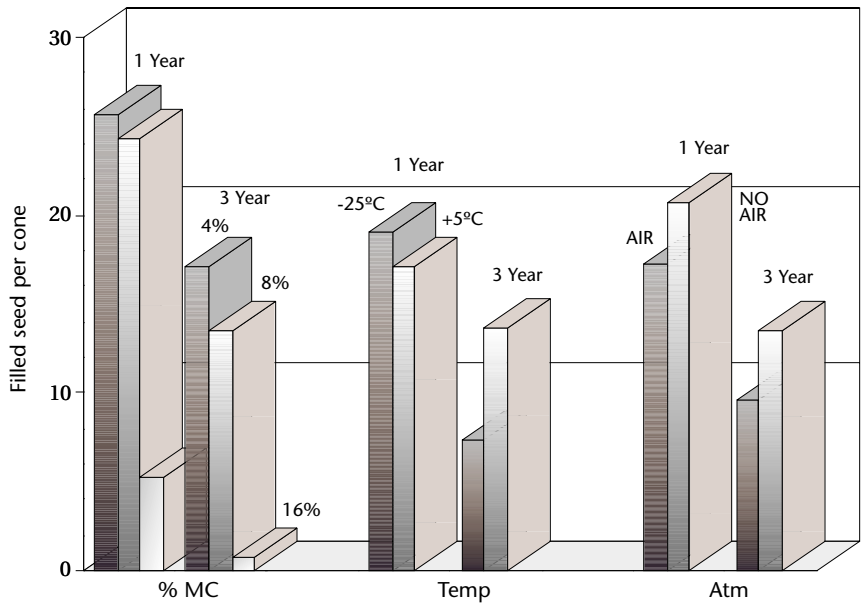


FIGURE 1 Effects of moisture content, temperature, and atmosphere on pollen storage — 1 and 3 years.
Main factor effects of pollen moisture content, storage temperature, and storage atmosphere on fertility (expressed as mean filled seed per cone) of western hemlock pollen stored for 1 and 3 years.

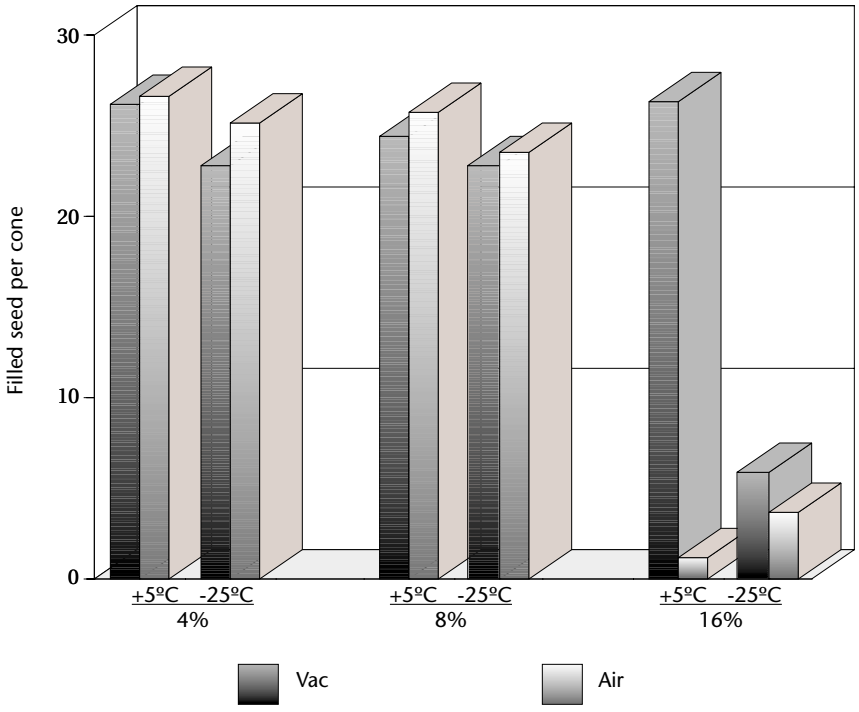


FIGURE 2 Interaction between moisture content, temperature, and atmosphere on pollen storage — 1 year.
The interaction between pollen moisture content, storage temperature, and storage atmosphere on the fertility (expressed as mean filled seed per cone) of western hemlock pollen stored for 1 year.

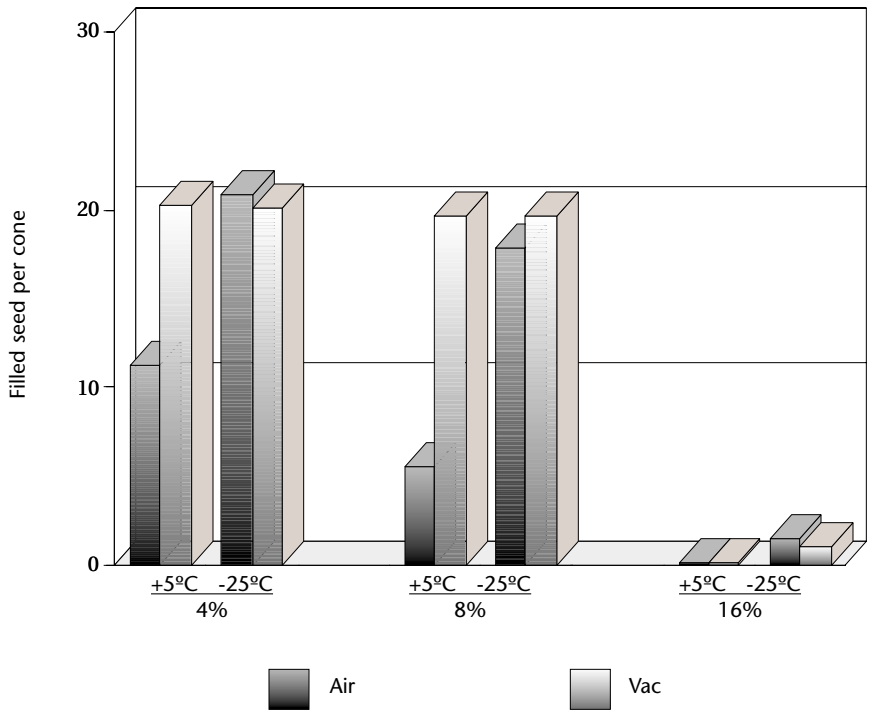


FIGURE 3 Interaction between moisture content, temperature, and atmosphere on pollen storage — 3 years.
The interaction between pollen moisture content, storage temperature, and storage atmosphere on the fertility (expressed as mean filled seed per cone) of western hemlock pollen stored for 3 years.

are adopted primarily from those developed for Douglas-fir, which are summarized in Webber and Bonnet-Masimbert (1993) and Webber and Painter (1996).

The procedures we developed can estimate pollen viability, relative vigour (rate of pollen tube growth), and potential fertility. These tests were developed primarily to estimate the effects of storage on pollen fertility. However, they are also useful for estimating potential fertility of pollen at all stages of handling (e.g., pollen collection and extraction), as well as estimating the yearly variation in pollen viability attributed to environmental effects.

Another important application of viability tests is in formulating specific pollen mixes for improving the genetic worth of production seed lots. Paternal success of individual lots within the mix is affected by its viability. If any particular pollen lot has a significantly lower fertility potential, then the contribution from that lot will be less or not realized at all. Previously, we recommended formulations of pollen mixes with no fewer than 15–20 lots. In this way, probability would dictate whether a low viability pollen lot is in competition with a higher viability lot. More recently, Stoehr et al. (1999) showed that formulating pollen poly-mixes on the basis of viability rather than of volume is an effective way to balance paternal contribution and therefore improve effective population size (Ne).

Under open pollination conditions, where competition from high viability pollen occurs, the estimates of potential parental contribution from pollen lots with a low viability may not be realized. The pollination mechanism (see Appendix 1) of western hemlock is such that pollen competition could be a more important factor than in other species. Because pollen tubes must grow over relatively long distances to reach the nucellus, any reduction in a pollen

lot's vigour will make it less competitive and less likely to contribute to the genetic worth of the seed lot.

We have not studied the pollination mechanism of western hemlock as a factor in manipulating the genetic worth of seed lots, especially where pollen lots of varying levels of viability are placed in competition for fertilizing. We do not recommend that low viability pollen lots be used. Specific pollen viability values, below which low viability pollen lots should not be used where competition will occur (e.g., under open pollination conditions), are offered in the subsection, Assay response and fertility (p. 25).

There are many types of laboratory assays to indicate pollen viability but three are most useful for the conifers we have tested. These are:

1. respiration: a measure of oxygen uptake under controlled conditions
2. conductivity: a measure of the conductivity of an aqueous pollen leachate, and
3. germination: a measure of pollen grain growth under controlled conditions.

The following five subsections contain a discussion on pollen preconditioning, which is a necessary first step for testing most conifer pollen lots, a general discussion of each of the three assays, and a final section on the correlation of assay response to field fertility. Additional details on each of these five topics are given in Appendix 2.

Pollen preconditioning When pollen is taken from storage it is in a dormant, dehydrated state, and, before metabolic activity is restored, uptake of water is essential. Imbibition of water depends to a large extent on the osmotic status of the pollen grain and its surrounding environment. Under natural conditions, conifer pollen is shed in a hydrated state, and further water uptake to complete germination and tube growth readily takes place within the micropyle. The same is true for most conifer pollen being tested for viability. Pre-hydration just prior to the assay improves both the assay response and its relationship to field fertility.

The effect of pre-hydration on assay response for western hemlock pollen is shown for each of the three assays described below. For respiration and germination procedures, pre-hydration did not improve assay response or the relationship to field fertility. For conductivity, however, pre-hydrating pollen at 100% RH at 20° C for 16 hours significantly improves the correlation coefficient (r), which is a measure of the strength of the relationship between assay response and seed yields. The proportion of variability in seed yields explained by the assay response is determined by calculating the coefficient of determination (r^2).

The actual time to hydrate varies by species. For western hemlock, pollen moisture content will rise from a dehydrated state of about 8% to about 25% in about 16–18 hours under an atmosphere of 100% RH at 20° C. Figure 4 shows the relationship between pollen moisture content and exposure time to 100% RH at 20° C for two good and two poor viability pollen lots. Where pre-hydration is used prior to assay, we recommend exposure for 16 hours (overnight) at 100% RH and 20° C.

Conductivity Conductivity is a direct measure of pollen membrane stability. When pollen is dehydrated, pollen membranes lose their structural integrity and viability. The extent of this effect depends on the species and the level of moisture reduction. As pollen moisture content is reduced, the membranes become more porous, resulting in reduced membrane activity

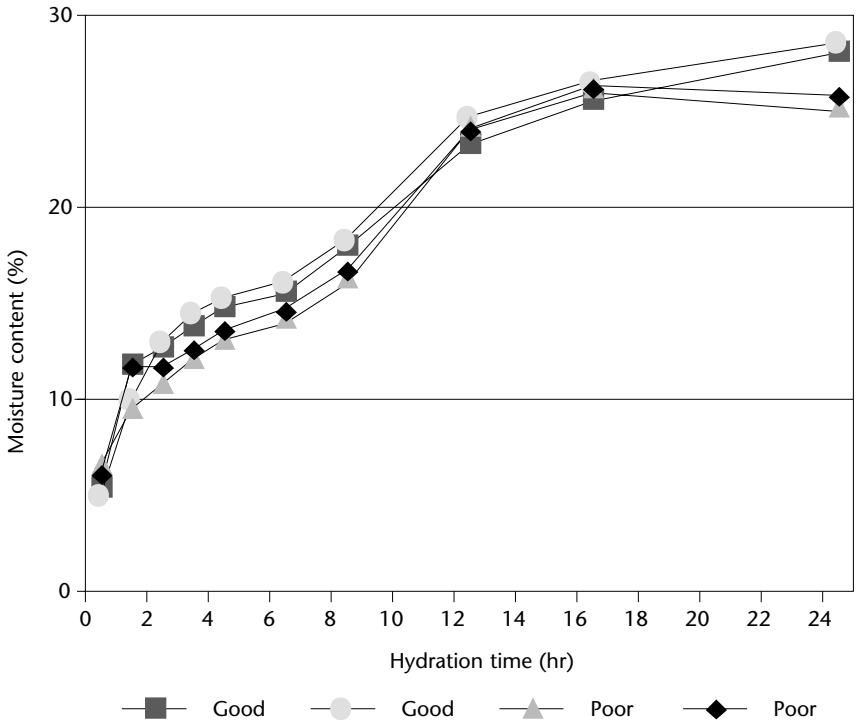


FIGURE 4 Pollen moisture content and hydration.
The relationship between pollen moisture content and hydration interval for two good and two poor viability western hemlock pollen lots.

and an increase in pollen cellular content that can be leached. Since this leachate carries an electrical charge, simple conductivity measurements of the leachate provide a direct estimate of membrane damage and reduction in viability.

The procedure is relatively simple and the assay can be completed in less than 2 hours. However, as for all laboratory assays, more reliable estimates of potential fertility can be made if conductivity is assessed with either germination or respiration results.

Conductivity results can be obtained by simply leaching the pollen in cold water with constant agitation and measuring the electrical conductivity of the leachate. However, variability in the total leached material between pollen lots does occur and can be accounted for by including a hot leachate step. Thus, two analyses are used: cold conductivity and hot conductivity. Percent conductivity is then calculated as the ratio of cold leachate to hot leachate. In most cases, regression coefficients (r) for percent conductivity and filled seed per cone are higher than between cold conductivity and filled seed per cone (Webber and Bonnet-Masimbert 1993; unpublished data for western hemlock).

Both prior hydration and leaching time will affect the amount of leachate and therefore the conductivity results. Figure 5 shows the effect of pollen moisture content on cold conductivity for two good and two poor western hemlock pollen lots. As moisture content rises, cold conductivity values drop for both good and poor viability lots, although to a lesser extent for the poor lots.

The detailed procedures for estimating both cold and hot conductivity in western hemlock pollen are given in Appendix 2. The relationship between

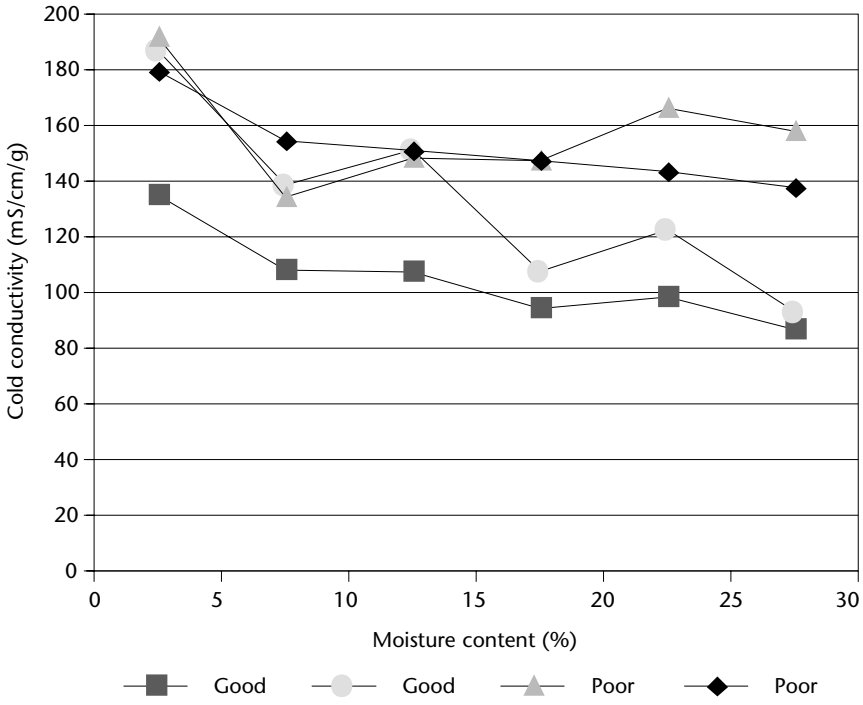
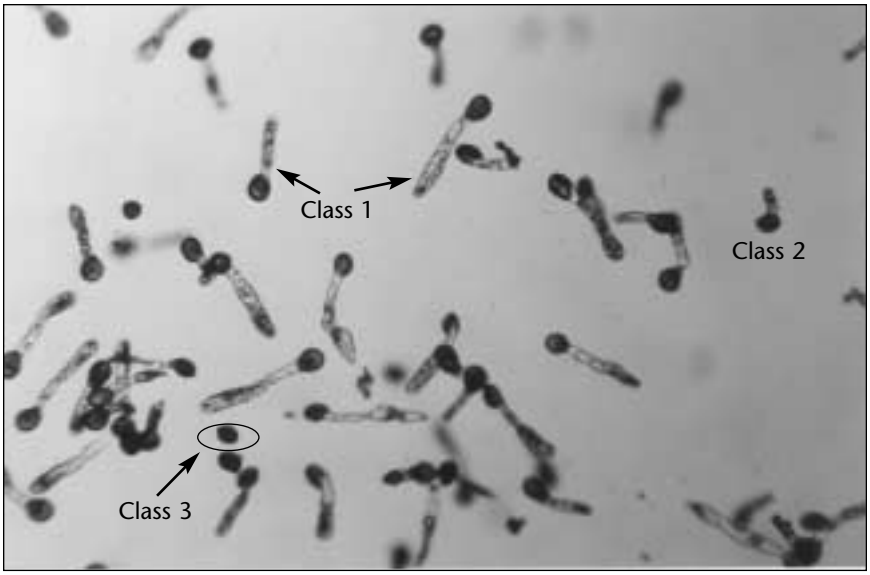


FIGURE 5 Pollen moisture content effects on cold conductivity.
The effect of hydrating good and poor viability western hemlock pollen lots to various moisture contents on cold conductivity.

percent conductivity assay response and field fertility is described in the subsection on Assay response and fertility and shown graphically in Figure 15.

Germination Pollen grains of most conifer species will germinate and grow a pollen tube when cultured in a suitable medium. Of all the conifer species, western hemlock forms the longest tubes both under natural conditions and in culture. This unique feature of western hemlock pollen and its mechanism for being captured is explained further in Appendix 1. Although germination response to laboratory and field conditions may be different, the techniques we have developed for assessing laboratory response are still a good measure of a pollen lot's potential fertility.

Most conifer pollen grains can readily germinate in aqueous solutions with little or no added nutrition. However, in water alone germination is often incomplete. Tube lengths are shorter and in many cases pollen grains will burst if the osmotic potential of the pollen grain and the solution are considerably different. Mature pollen grains normally carry sufficient food reserves (free sugars, starch, lipids, and protein) to enable them to be independent identities from the time of pollen shedding through germination and early tube growth. In general, conifer pollen can be cultured on relatively simple media. Several factors affect pollen response in culture, among which hydration state and medium composition are the most important.



Photographic Plate 3 Classes of western hemlock pollen germination

Germination of western hemlock pollen is classified into three categories (see Figure 6):

Class 1 elongating pollen tube is >2x original pollen grain diameter.
Class 2 elongating pollen tube is <2x original pollen grain diameter.
Class 3 grain is hydrated (original diameter) but no pollen tube is present.

The total number of pollen grains scored in each of Class 1 and 2 determines the overall germination rate expressed as a percentage of Class 1+2 grains to total grains counted.

The effect of hydration state and media composition on germination of western hemlock pollen can be seen in the following figures. Based on laboratory tests, pre-hydrating pollen for germination assay is not necessary for germinating western hemlock pollen since the correlation coefficients (r) did not improve the relationship between assay response and field fertility (see subsection on Assay response and fertility)

The germination medium for western hemlock started with the results of Ho and Sziklai (1971) who used an aqueous solution of Brewbaker and Kwack's (1963) solution. However, by adding other components to this medium, we improved both assay time and response. The media components we tested were: (1) Brewbaker's solution (Brewbaker and Kwack 1963), (2) polyethylene glycol (PEG, molecular weight 4000) as an osmoticum, and (3) sucrose as either an osmoticum or carbon source. These components were tested in either an aqueous solution or a semi-solid agar medium.

The effects of three concentrations of sucrose and PEG in an aqueous solution, with and without Brewbaker's solution, for hydrated and unhydrated western hemlock pollen lots, are shown in Figures 6, 7, 8, and 9. Figure 10 shows the effect of three concentrations of agar (solid medium) without any added components. Note that the response of Class 1 grains is better with all three agar concentrations alone than with any other aqueous media types. We did try various combinations of agar with sucrose and

PEG and Brewbaker's solution. Our maximum germination response (Class 1+2 grains) was obtained with a medium containing 0.25% agar, 5% sucrose, and 10% Brewbaker's solution (Figure 11).

Pre-hydration of pollen improved assay response for aqueous solutions of media (see Figures 6, 7, 8, and 9). However, for the solid media containing agar, the effect of pre-hydration is less important (see subsection on Assay response and fertility). It is clear that media affects assay response. The 0.25% agar containing 10% sucrose and 10% Brewbaker's solution yielded the highest response of Class 1 gains under the conditions we used. However, it may well be that other media types are better. For now, the germination techniques described in Appendix 2 will adequately indicate the pollen lot's potential fertility. The relationship between germination assay response as well as conductivity and respiration to actual field fertility (filled seeds per cone) is described in the subsection, Assay response and fertility below and is shown graphically in Figure 13.

Respiration Respiration is a measure of oxygen uptake that provides an overall estimate of the metabolic status of pollen. The laboratory procedures used to measure oxygen uptake by pollen are straightforward but require rather sophisticated equipment. Because of the technical requirements to maintain, calibrate, and operate the equipment, respiration analysis may not be suitable for operational use. However, from extensive experience with other species, the rate of oxygen uptake has consistently given us the best estimate of pollen fertility. Furthermore, the actual assay procedure is quick, and estimates of pollen fertility can be made in less than 10 minutes. Specific details on determining the oxygen uptake of pollen is given in Appendix 2.

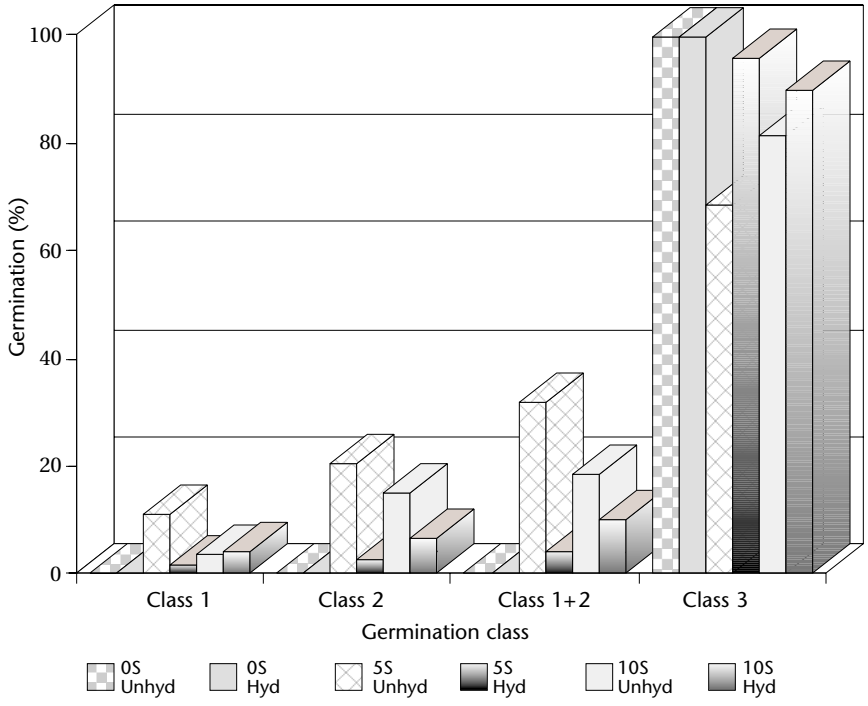


FIGURE 6 Sucrose concentration and hydration effects on germination. *The effect of three concentrations (0, 5, and 10%) of sucrose (S) on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.*

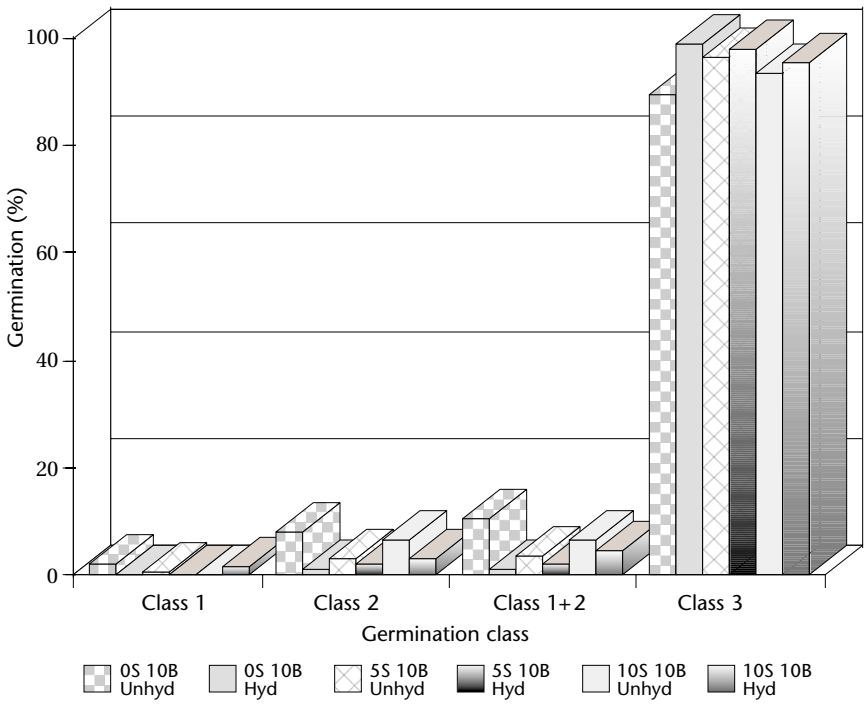


FIGURE 7 Sucrose concentration, hydration, and Brewbaker's solution effects on germination.
The effect of three concentrations (0, 5, and 10%) of sucrose (S) with 10% Brewbaker's solution (B) on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.

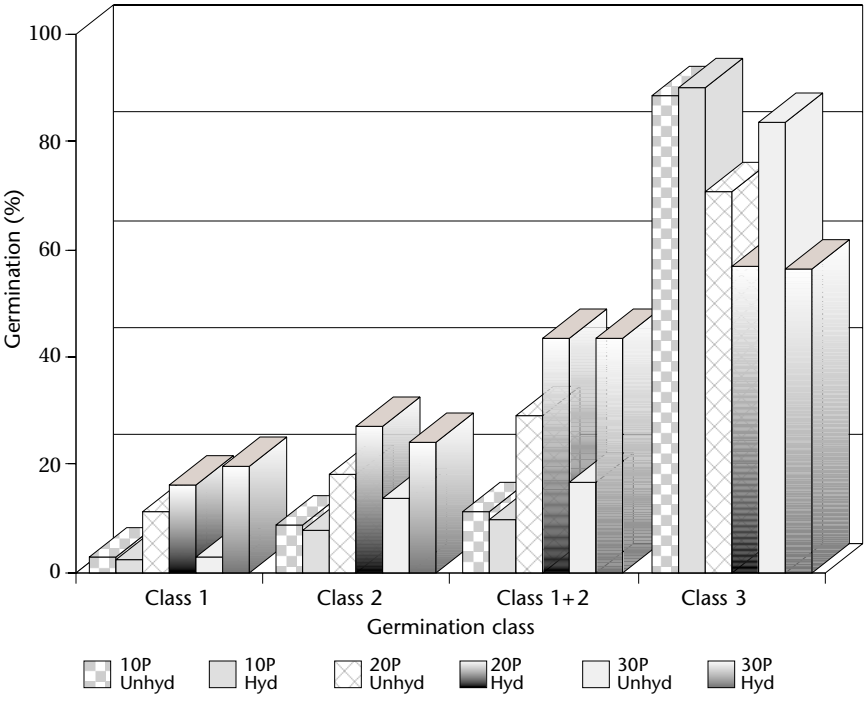


FIGURE 8 Polyethylene glycol and hydration effects on germination.
The effect of three concentrations (10, 20, and 30%) of polyethylene glycol (P) on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.

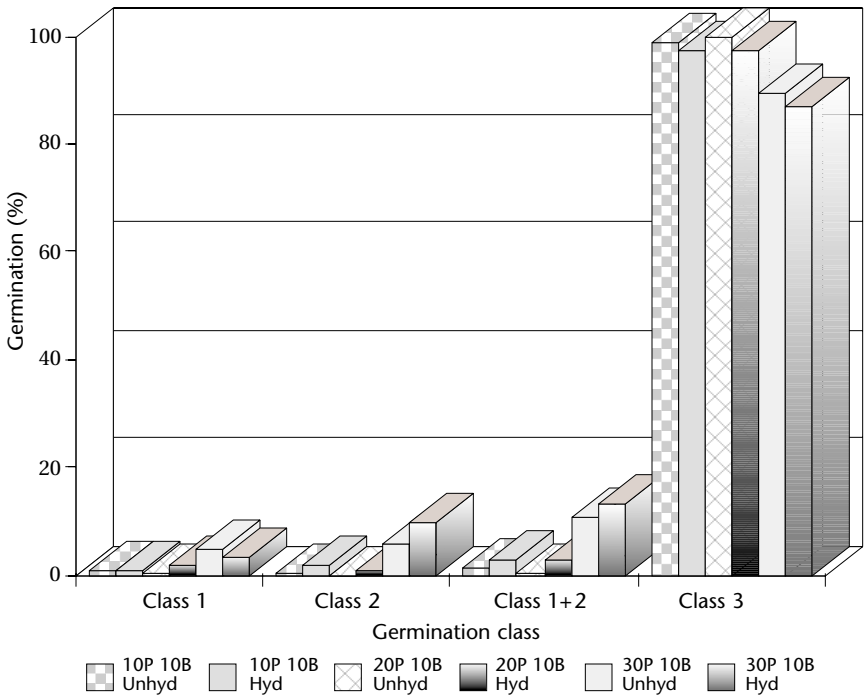


FIGURE 9 Polyethylene glycol, hydration, and Brewbaker's solution effects on germination.
The effect of three concentrations (10, 20, and 30%) of polyethylene glycol (P) with 10% Brewbaker's solution (B) on the germination of hydrated and unhydrated western hemlock pollen.

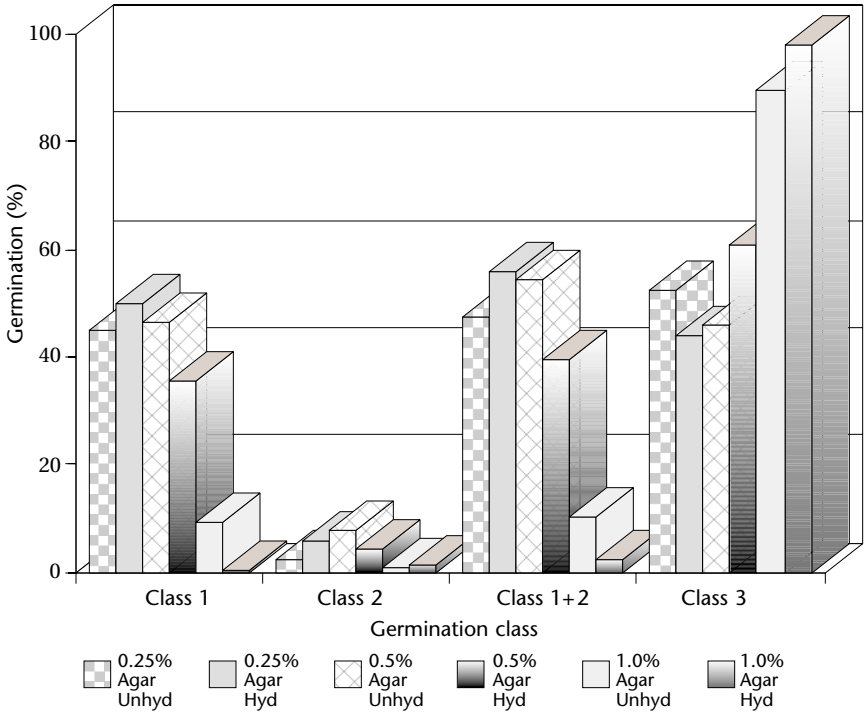


FIGURE 10 Agar and hydration effects on germination.
The effect of three concentrations (0.25, 0.5, and 1%) of agar on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.

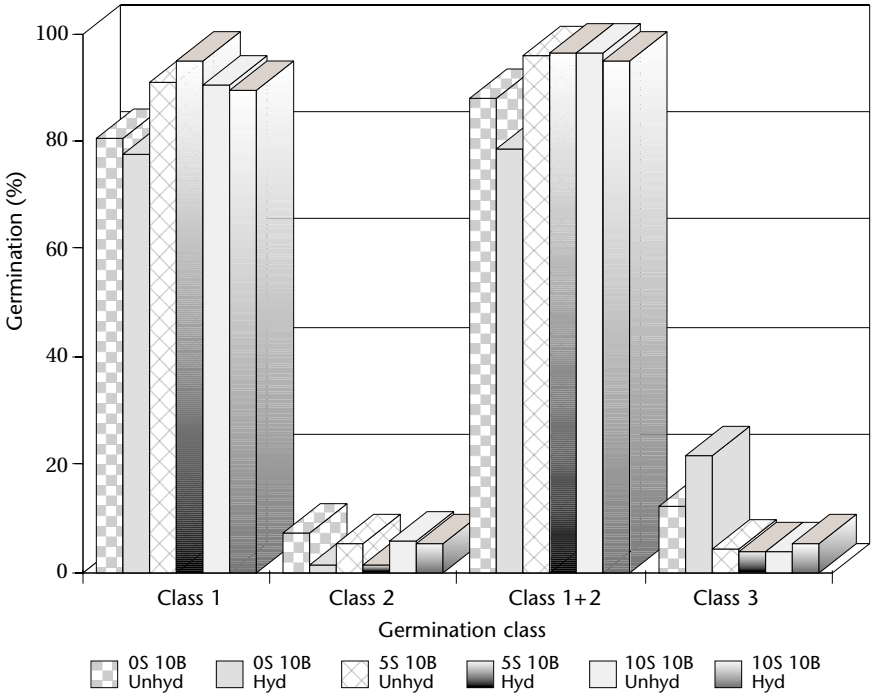


FIGURE 11 Agar, hydration, and Brewbaker's solution effects on germination. *The effect of three concentrations (0, 5, and 10%) of sucrose (S) with 10% Brewbaker's solution (B) in 0.25% agar on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains. The recommended medium for germinating western hemlock pollen is 0.25% agar containing 10% Brewbaker's solution and 5% sucrose.*

Figure 12 shows the oxygen uptake for two good and two poor pollen lots each hydrated and dehydrated. The effect of pre-hydration is more apparent for the poor lots than for the good lots. Hydration does improve assay response but its effect on the correlation coefficient (r) is not significant. The relationship between respiration assay response and field fertility (filled seed per cone) is described in the following section and is shown graphically in Figure 14.

Assay response and fertility Laboratory tests of any pollen lot are of little use if they cannot be related to field fertility or competitive ability relative to other lots. The viability assays we use for estimating fertility potential have been correlated to actual fertility using controlled crossing conditions. There are several factors that produce variability in fertility potential and, therefore, effect the ability of a specific assay response to predict potential seed yields. These factors include, but are not necessarily limited to:

- level of competing pollen cloud density,
- timing of pollen application,
- atmospheric conditions,
- variation in the ability of seed cone parent trees to produce yields, and
- pollen and seed cone parent interactions.

For single lots used under controlled crossing conditions, assay response is more meaningful. When a pollen lot is used in a poly-mix its assay

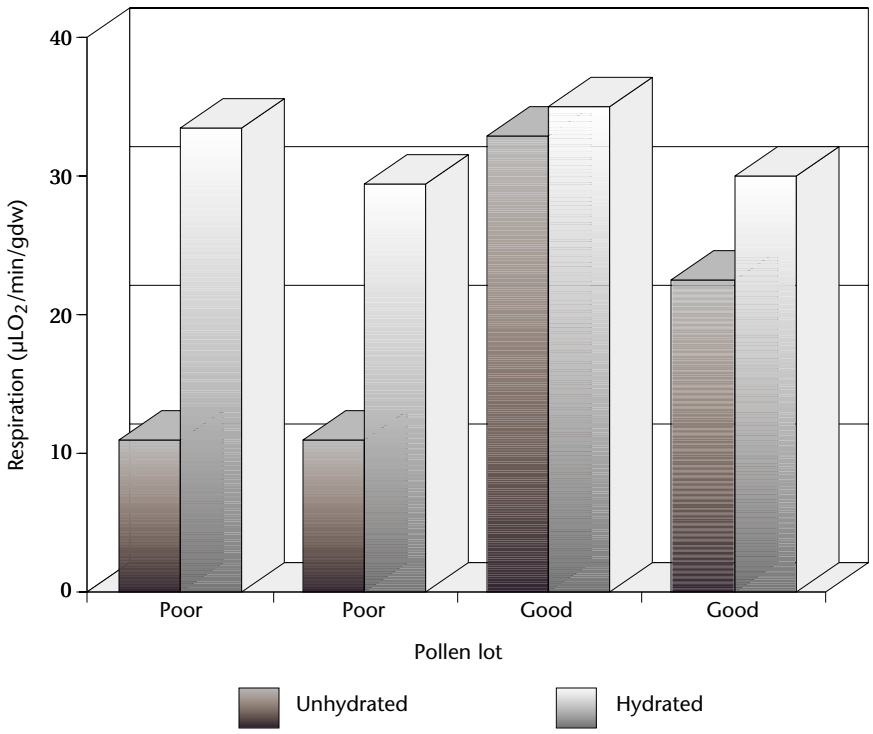


FIGURE 12 Pollen viability and hydration effects on respiration. *Oxygen uptake for hydrated and unhydrated poor pollen lots and hydrated and unhydrated good pollen lots.*

response should give a relative ranking of fertility potential for lots used in a pollen mix. For Douglas-fir we have used relative assay responses to formulate the mix, resulting in a better paternal contribution in the resulting seed lot (Stoehr et al. 1999). Even for open pollinated conditions, we strongly recommend testing lots before they are applied. Eventually, we will be able to define limits of pollen viability that can be reliably used to include or exclude any particular pollen lot from a pollen mix.

Figures 13, 14, and 15 show the nonlinear relationship between assay response and field fertility (seed yields) defined as the percent filled seed per cone. The data scatter seen in these figures is typical of the variability associated with pollen testing and field seed yields. The curves generated from these data are also typical. Seed yields are seen to rise as pollen viability (respiration and germination) increases but there is a point (the asymptote) beyond which any further increase in pollen viability is not associated with a corresponding increase in seed yields (see data shown in Figures 13, 14, 15, and 19). The asymptote is generally found around the 50% seed yield value. This is also typical for other species (Webber and Bonnet-Masimbert 1993). On average, our best pollination technique (controlled crossing) produces only 50% of the potential seed available. We are not certain why our filled seed percentage is not higher. The limitation to full yields is more likely related to seed cone development (incomplete ovule formation, which still forms a complete seed coat), cone morphology (limited or restricted access to the ovule), and incomplete pollination than to pollen viability. For our purposes then, 20–25 filled seeds per cone should be considered an operational limit.

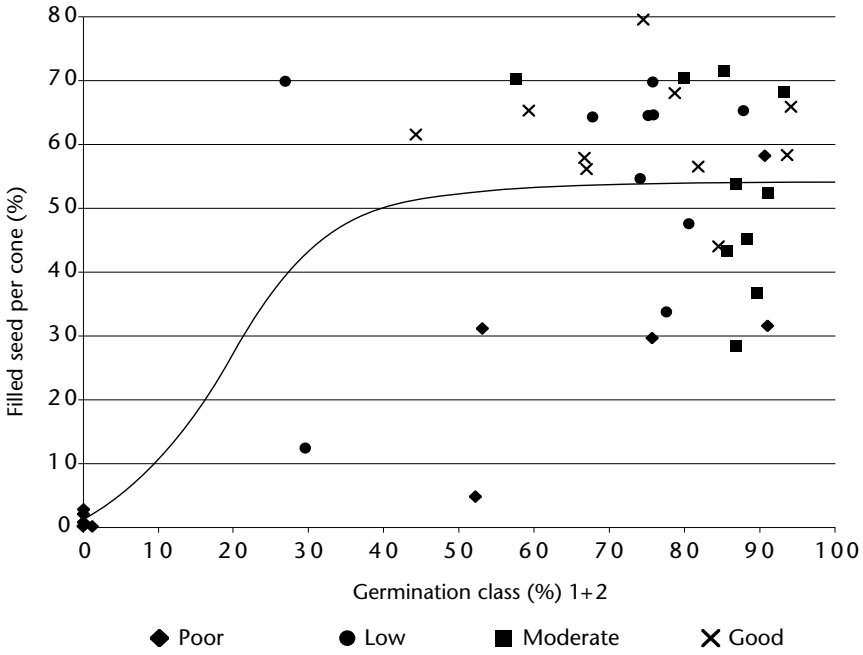


FIGURE 13 Relationship between filled seed per cone and germination.
The relationship between percent filled seed per cone and percent germination (Class 1+2 grains) for 40 dehydrated western hemlock pollen lots segregated into four viability classes (poor=diamond, low=circle, moderate=square, and good=cross). The parameters for the logistic function are a=53.8, b=-0.13, and c=2.61. The coefficient of determination (r^2) is 0.528.

The curves shown in Figures 13–15 were obtained by classifying pollen lots as poor, low, moderate, and good. We used respiration values to assign pollen lots to these four classes. Actual assay response for percent germination (Class 1+2 grains), percent conductivity (ratio of cold to hot conductivity), and respiration (μL O_2/min/gdw) were determined for each pollen lot from each of the four classes (10 lots per class). Pollen lots were tested both hydrated and dehydrated. Seed yields were determined as average values from controlled crossing using all 40 pollen lots each crossed with seven seed cone parent trees (separate clones). Seed yields were expressed as percent filled seed per cone, which represents the ratio of filled seed per cone divided by potential seed per cone. Potential seed per cone was defined as the number of seeds with a mature seed coat. Regression analyses between assay response and percent filled seed per cone were completed using SAS (1988) nonlinear procedures and various logistic functions. For percent germination and respiration, the form of the logistic function was $y=a/(1+e^{c+bx})$ and for percent conductivity, a hyperbolic function was used of the form $y=ae^{bx}$. Parameters a, b, and c were estimated and then iterated to the best fit by SAS nonlinear procedures.

Figures 13, 14, and 15 show the relationship between percent filled seed per cone and percent germination (Class 1+2), percent conductivity, and respiration (μL O_2/min/gdw), respectively. The values for respiration and germination are shown for dehydrated pollen. The coefficient of determination (r^2) is a direct measure of the variation in seed yields explained by the assay response. For respiration and germination, the r^2 for dehydrated/hydrated pollen are 0.697/0.595 and 0.528/0.523, respectively.