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Environmental Effects on Yellow-cedar Pollen Quality

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About the Forest Genetics Council of British Columbia

The Forest Genetics Council of BC (FGC) is a multi-stakeholder group representing the forest industry, Ministry of Forests, Canadian Forest Service, and universities. Council's mandate is to champion forest gene resource management in British Columbia, to oversee strategic and business planning for a cooperative provincial forest gene resource management program, and to advise the Chief Forester on forest gene resource management policies.

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Abstract

Deficiencies in high quality yellow-cedar (*Chamaecyparis nootkatensis*, (D. Don) Spach) pollen at pollination time may be one of the principal factors responsible for the failure of low elevation seed orchards to produce sufficient quantities of viable seed. Given the important consequence for location and management of yellow-cedar breeding and seed orchards, pollen development and its quality under various climate environments was examined. In a pollen quality study in 2001 and 2002, significant differences in pollen viability were observed between populations characterized by distinct climate conditions. There was a clear trend in the acceleration of pollen development and in the corresponding reduction of pollen quality as the elevation of the testing sites decreased and the mean monthly temperature increased. Furthermore, significant population, year, and population by year effects for pollen viability were observed. Results from this and other related investigations suggest that the production of poor quality pollen at low elevation is the result of several temperature related events. Since the final quality of pollen at pollination time appears to be regulated by temperature, the selection of the most favorable site for high quality pollen production hints at an optimum climate. Two possible candidate sites for the production of high quality pollen are characterized in this report.

Introduction

Yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach.) grows naturally in cold and humid climates in the Pacific Northwest, occurring at high elevations in the southern areas of its range and from low to high elevations in the central to northern extent of its range (Fowells 1965). It is the first of its associates to free itself of snow and to undergo pollination when heavy snow load on the ground and severe temperature fluctuations are common early in the spring (Fowells 1965; Owens and Molder 1975).

Yellow-cedar seed production in natural stands can be minimal and sporadic; consequently, seed is in short supply for reforestation (Russell 1993). Seed orchards were established at low elevations with the idea that the influence of warmer climate will promote earlier and increased quality cone production. To the contrary, seed orchards produced lower quantities of viable seed than wild stands (Anderson et al. 2002). Climatic differences between high elevation natural stands and low elevation seed orchards could be one of the main causes for reduced viable seed.

Sexual reproduction of yellow-cedar, adapted to cold climates and short growing seasons, may be negatively affected when grown at warmer, low elevations with longer growing seasons. Typical maturation of yellow-cedar pollen in one growing season, for example, is uncommon in temperate conifers but it may be a reproductive adaptation to the harsher climates the species experiences (Owens et al. 1980). Accelerated maturation allows pollen to be readily available for pollination when female flowers become receptive early in the spring. However, this adaptation may have an adverse effect on pollen development under warmer conditions.

The objective of this study was to investigate the effect of environment on yellow-cedar pollen development and quality.[1] It has been documented in the literature that extreme temperature conditions during pollen development, with meiosis being the most sensitive stage, play a significant role in pollen quality in several tree species (Eriksson 1968, Jonsson 1974, Luomajoki 1977). Comparable effects on yellow-cedar pollen have not been documented in the literature. Examining and comparing pollen quality among various geographic and climatic environments may provide insight into which conditions are beneficial for healthy pollen development and maturation for yellow-cedar.

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[1] Pollen quality was based on pollen viability where pollen with good germination vigour, and germination tubes equal to or greater than three times the hydrated pollen grain diameter was considered viable.

Experimental Design

This study was conducted at seven sites in southwestern British Columbia. Four sites were located on Vancouver Island at Mt. Washington (850 m), Jordan River high (750 m), Jordan River low (150 m), and Mt. Newton Seed Orchard (Timber West Ltd.) near Victoria (50 m). Three sites were situated on the mainland at Whistler high (1100 m), Whistler low (660 m), and Coquihalla Mountain (1100 m).

The study included two trials. In the first trial, pollen from all seven locations was sampled at shedding to determine if geographic and climatic descriptors of population-origin influenced yellow-cedar pollen quality. The second trial involved sampling a subset of three populations varying in mean annual temperature to examine the influence of temperature on pollen-cone development and pollen quality over time.

1. Environmental effects on pollen quality

Branches with pollen-cones were collected from five to 11 trees at each of the seven sites in the spring of 2001 and 2002. Collected branches were kept separate by tree and placed overnight into a room with high humidity (75% RH) and moderate temperature (17°C) to promote pollen shed. The following day, pollen was extracted, cleaned, and let dry in a room with low humidity (40% RH) for approximately two hours. Dried pollen was then dusted onto a solid germination medium (0.3% agar and 10% sucrose) in a petri-dish, covered with a tight-fitting lid, and placed into a germinator with uniform temperature of 26°C. Pollen was allowed to germinate for eight days, beyond which the incidence of fungal contamination was too high for accurate germination assessments. Germination percentage was based on a count of 200 grains per dish, with two dishes per tree. Only pollen grains with good vigour (i.e., germination tubes equal to or greater than three times the hydrated pollen grain diameter) were considered viable. Temperature was recorded every four hours throughout the year at each site using a Hobo XT Temperature Logger (Onset Computer Corporation) mounted on a tree. Analyses of variance and Fisher's LSD means separation test were used to test for significant population and year effects (Proc GLM SAS Inc.) on pollination germination.

2. Environmental effects on pollen maturation and quality during development

The second trial followed the development of pollen-cones from the initiation stage in the summer of 2001 to the shedding stage in the spring of 2002, as well as pollen viability at specific stages. A subset of three sites were selected from the first study based on sampling across the six site mean annual temperatures: Mt. Newton, Mt. Washington, and Whistler Mt. Monthly observations of the developing pollen-cones morphological stage were made from June 2001 at both Mt. Newton and Mt. Washington. Pollen developmental data were recorded every month until shedding time in 2002 at the Mt. Newton site, while observations at the Mt. Washington site were interrupted during the winter months, but were continued in the spring. Pollen developmental data were collected at Whistler Mt. on three occasions: when pollen development stopped in October 2001, during dormancy in December, and at shedding in May 2002. The developing cones were classified into six morphological stages (J.N. Owens, University of Victoria, pers. comm.) (Table 1). Morphological stages were used to track pollen-cone development from its initiation to the shedding time and to determine the occurrence of meiosis at each site. Meiosis occurs between morphological stages 3 and 4, when rapid enlargement of pollen sacks causes them to become exposed, giving pollen-cones a distinct yellow colour (Owens and Molder 1974).

Table 1. *Morphological stages of developing yellow-cedar pollen from initiation to maturation*

Stage	Morphology
1	Modified leaves (microsporophylls) assume pollen-cone shape ≥ recognizable cones
2	Pollen-sacks (microsporangia) with green colour visible in <50% of cone
3	Pollen-sacks with green and light yellow colour visible in >50% of cone; high moisture
4	Pollen-sacks, all with yellow colour, visible on whole cone; pollen wet
5	Pollen-sacks swollen, dark yellow colour; pollen dry, powdery ≥ mature pollen
6	Pollen shedding

Assessments of pollen viability at Mt. Newton started at pollen stage 5 in fall 2001 and then continued approximately every six weeks until shedding in spring 2002. Assessments at the other two sites were limited during the winter, with only one assessment done in December 2001 at Whistler Mt., and in February 2002 at Mt. Washington. Assessments recommenced in the spring of 2002 at both sites. Procedures for pollen viability assessment were similar to those described for the first trial. However, without natural shedding, pollen was extracted by mechanical means (pollen-cones were pressed lightly through a sieve to break the pollen sacks and to release pollen grains). Analysis of variance was used to test for significant population and time effects for pollen viability and population mean differences were estimated using Fisher's LSD means separation test (Proc GLM, SAS Inc.).

Results

1. Environmental effects on pollen viability

Pollen viability at shedding time varied between years, with a higher overall viability in 2002 than 2001(Fig. 1). There were also significant population and population by year effects for pollen viability (all at p <0.001). There were no population rank changes between years (r = 0.92) indicating that the significant population by year interaction was a scale effect. Combining the results from the two years (for ease of presentation and because of a lack of significant rank changes), pollen collected at high elevations of Whistler and Coquihalla Mts., where the mean temperatures of the coldest month were the lowest (-8°C), had the highest average viability (70%) (Fig. 2). Pollen viability then decreased linearly with a decrease in elevation (r = 0.85, p <0.0001) and corresponding increase in mean temperature of the coldest month (r = -0.91, p <0.0001), reaching the lowest level (16%) at Mt. Newton, where the mean temperature of the coldest month was the highest (3°C).

Visual observations revealed substantial differences in germination media contamination by fungus between population pollen samples that had an apparent elevation trend. Germination media with Mt. Newton pollen had the highest levels of fungus contamination; Jordan River high and Jordan River low had moderate levels; Mt. Washington, Whistler high and Coquihalla had low levels.

2. Environmental effects on pollen maturation and quality during development

Pollen-cone development in 2001 was accelerated by approximately one month at Mt. Newton when compared to Mt. Washington, correlating with higher average monthly temperatures at the lower elevation site throughout the year (Fig. 3). At Mt. Newton, developing pollen cones were recognizable in the third week of July (morphological stage 1), while at Mt. Washington, the same stage was reached in the third week of August. In general, all early stages of pollen-cone development (i.e., stages 1 to 3) at Mt. Newton occurred when the mean monthly temperatures reached their peak in July and August (15°C and 16.5°C respectively), while only the last two stages, 4 and 5, occurred when the high temperatures started to decrease. The last stage, 6, which is the shedding stage, was completed the next spring. At Mt. Washington, on the other hand, only the first stage occurred at the peak of the mean monthly temperature in August (14°C), while the rest of the pollen-cone development (stages 2 to 4.5) occurred under more moderate temperatures in September and October

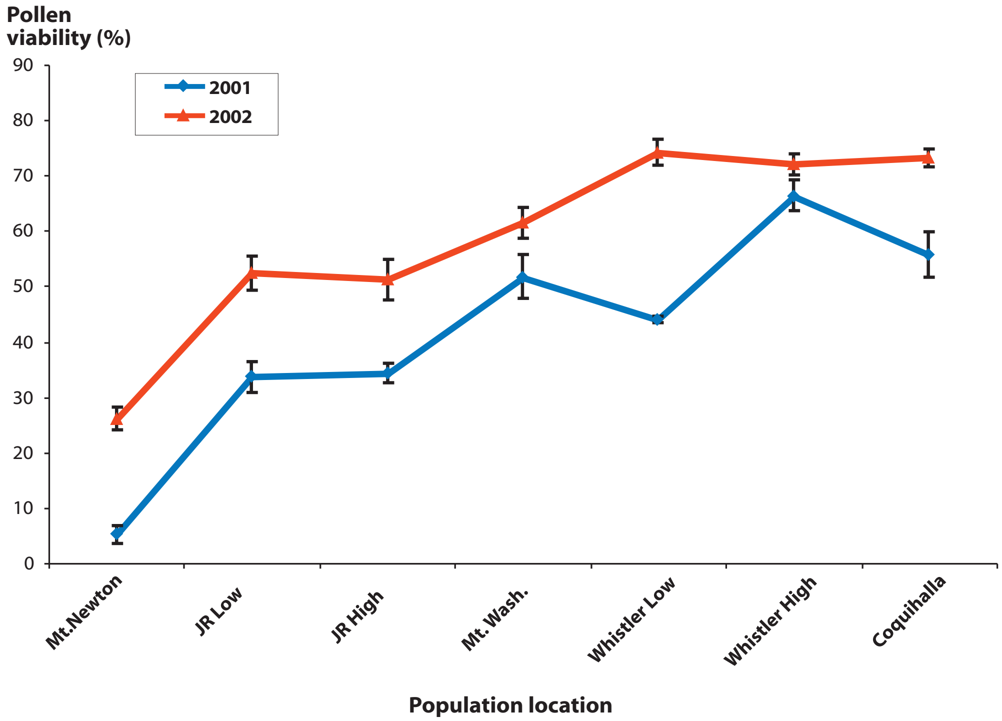
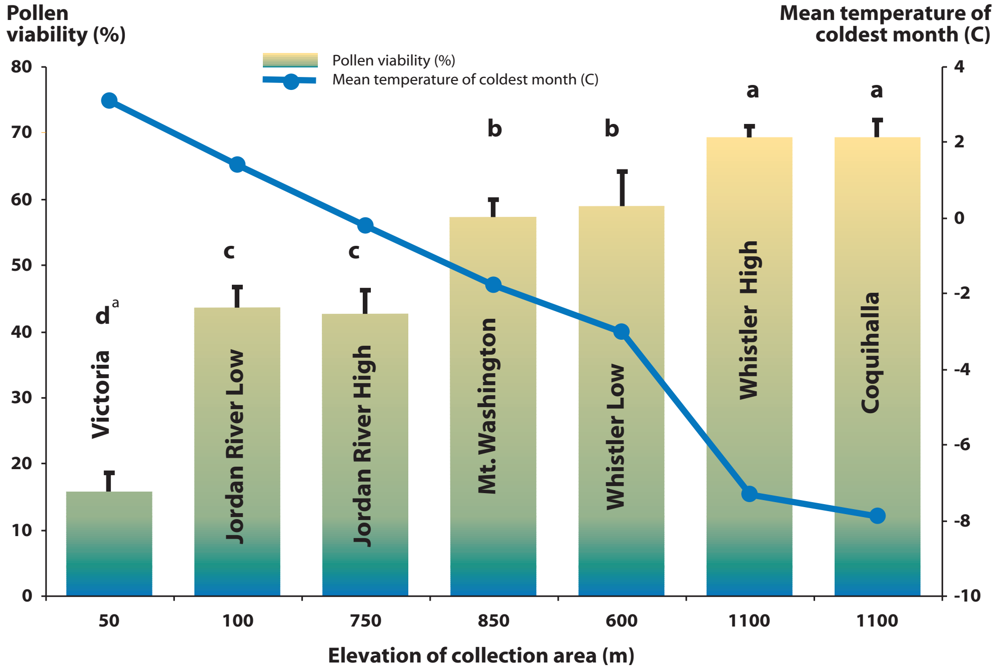


Fig. 1. *Yellow-cedar pollen viability (+/- s.e.) during pollination from two collection years by population.*



a Different letters indicate significance at $p < 0.05$ according to Fisher's LSD means separation test.

Fig. 2. *Relationship between yellow-cedar pollen viability at pollination*[2] *and population-origin elevation and mean temperature.*

[2] Population mean based on 2 years of data (2001 and 2002).

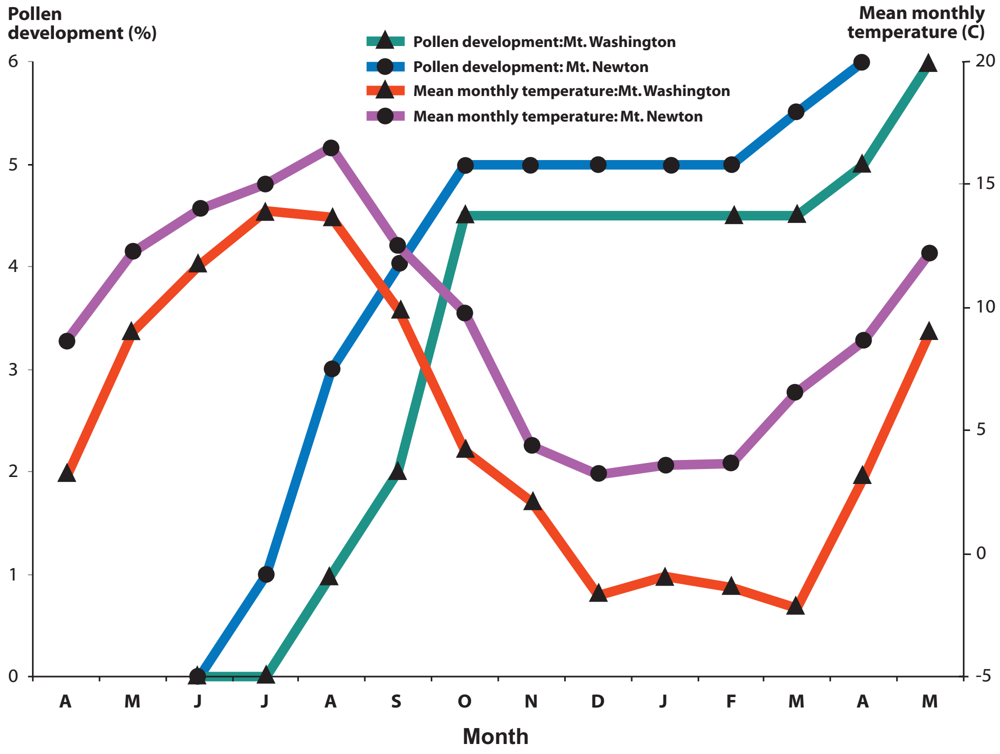


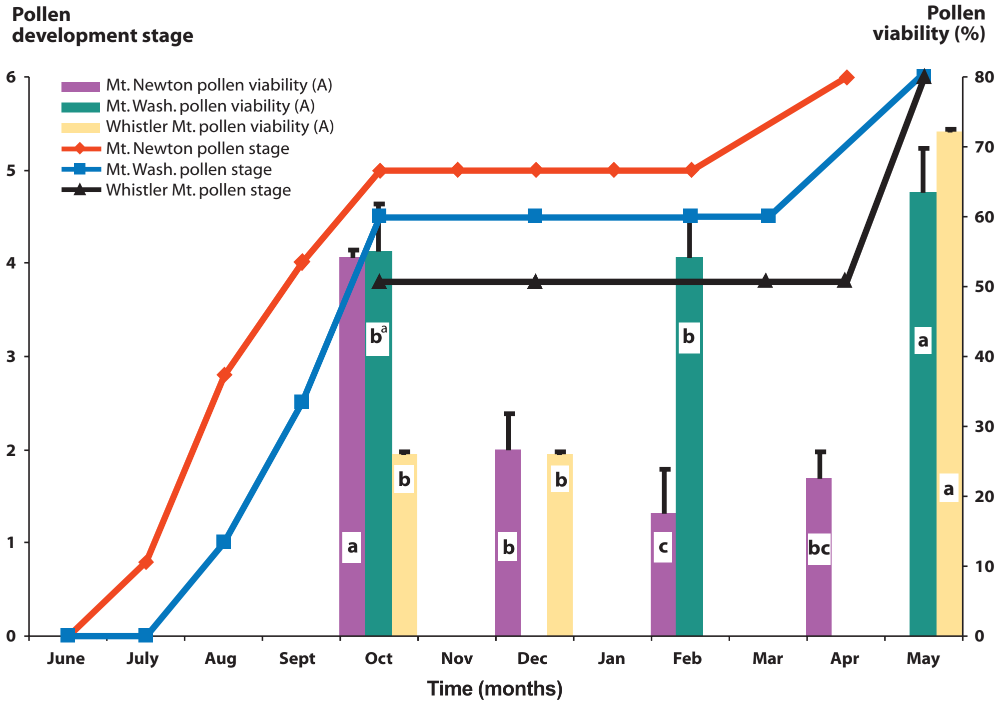
Fig. 3. *Relationship between yellow-cedar pollen development and mean monthly temperature at two contrasting sites, from spring 2001 to spring 2002.*

(10°C and 4°C respectively). All the pollen-cones at both elevations interrupted their development by the end of October. At Mt. Washington, the final pollen-cone maturation stage before winter dormancy was only slightly lower (stage 4.5) than at Mt. Newton (stage 5.0). The average stage of pollen-cone development before winter dormancy at Whistler Mt., however, was substantially lower (stage 3.5) (Fig. 4). There was a greater tree-to-tree variation at Whistler Mt. during the pre-dormancy pollen-cone developmental stages (from 3.0 to 4.5) compared to the Mt. Newton and Mt. Washington sites (from 4.5 to 5.0).

Average pollen viability before winter dormancy at Mt. Newton and Mt. Washington was comparable (54% and 55% respectively) but at Whistler Mt., pollen viability during the same period was much lower (26%), reflecting lower levels of pollen maturation at that time (Fig. 4). Mt. Washington and Whistler Mt. pollen remained viable at the pre-dormancy level throughout the winter, while the viability of Mt. Newton pollen decreased significantly from 54% to 17%. At pollination time in spring 2002, Mt. Newton pollen viability remained low at 22%, while there was a moderate increase in viability at Mt. Washington from 55% to 63%. The most significant change occurred in now mature Whistler Mt. pollen, with viability increasing from 26% to 72%.

Discussion

There was a significant trend in the acceleration of pollen development, and in the corresponding reduction of pollen quality, as the test site elevation decreased and the mean monthly temperatures increased. There were substantial differences in pollen development and quality between warm, low elevation sites and colder, high elevation sites. However, this trend was less apparent at lower elevation sites characterized by cooler summer microclimates. Cooler summer temperatures at these sites had a possible modifying effect on pollen development and its quality. Summer temperatures at the Jordan River low site, for example, were moderated by partial shade and frequent morning and afternoon fog developing



a Different letters denote significant differences ($p < 0.05$) between times, within populations, according to Fisher's LSD means separation test.

Fig. 4. *Yellow-cedar pollen development and viability (+/- s.e.) by population over time (2001 to 2002).*

from the nearby ocean. This possibly resulted in improved pollen quality when compared to Mt. Newton. As well, pollen quality from Jordan River low was comparable to the Jordan River high site. Both the Mt. Newton and Jordan River high sites were fully exposed to mid-day summer sun and experienced frequent temperature extremes that could have caused higher pollen abnormalities and sterility. Similar circumstances existed at the shaded Whistler low site that had comparable pollen quality to the more exposed, higher elevation site at Mt. Washington. Furthermore, pollen viability testing during 2001 and 2002 pollination showed that variations in pollen quality between years existed, and that these variations could be a direct reflection of the previous summer temperature variations. The growing season in 2001, which produced higher quality 2002 pollen, was shorter with a colder spring and fall than in 2000, which produced lower quality 2001 pollen.

The effects of temperature on yellow-cedar pollen quality are not known and may be varied. One possibility is that pollen in its initial stages of development is more susceptible to temperature damage than during its mature dormant stage. Several investigators have concluded that extreme temperature conditions during pollen formation, with meiosis being the most sensitive event, play a significant role in the occurrence of pollen abnormalities in several tree species (Eriksson 1968, Jonsson 1974, Luomajoki 1977). Jonsson (1974) further concluded that temperature extremes, both low and high, have a negative effect on the pattern of meiotic cell division with the consequence of abnormalities or pollen sterility. The level of meiotic irregularities therefore depends on the incidence of temperature extremes and their timing in relation to the meiotic stage of pollen development. The probability of pollen damage during the summer months was quite high at the Mt. Newton site since all the early developmental stages occurred when the temperatures were at their maximum in July and August (mean monthly averages 15°C and 16.5°C respectively). Luomajoki (1977) reports that continuous temperatures exceeding 15°C is too high for regular meiosis in *Picea abies*. On the other hand, the likelihood of similar damage to pollen at high elevation sites may be low because meiosis occurred under more moderate temperatures in September and possibly early October (mean monthly averages 10°C and 4°C respectively). Likewise, warmer temperatures and a longer growing season in 2000 than in 2001 may have accelerated pollen development and shifted its meiotic stage further into the warmer period at all sites. This may have caused lower overall pollen viability at pollination in 2001 as compared to 2002.

Observations in this study confirm earlier findings (Owens and Molder 1974; Owens at al. 1980) that yellow-cedar pollen at both low and high elevations enters winter dormancy in its mature stage (i.e., after meiosis). However, this study also revealed that pollen at some high elevation microsites, characterized by low sun exposure and northern aspect, entered dormancy at earlier stages of development (i.e., before or possibly during meiosis). For example, visual observations revealed that trees with the least exposure to sun at Whistler Mt. had the lowest developmental stage before winter dormancy (3.0) while trees with the greatest exposure to sun reached the highest stage of 4.5. Immature pollen from these shadier microsites completed its development in the spring resulting in the highest pollen quality produced among all the trees tested. Owens and Molder (1974) also proposed that pollen over-wintering in the immature pre-meiotic stage may be less susceptible to cold damage than more developed pollen. On the other hand, results in this study showed that the quality of fully developed pollen was not adversely affected by freezing temperatures, and its viability remained unchanged throughout the winter. Since high elevation yellow-cedar pollen can enter winter dormancy at both the pre-meiotic and the post-meiotic stage, it is then highly probable that a certain amount of pollen was still in its meiotic stage when the winter freezing temperatures set in. Given the high sensitivity of meiosis to temperature extremes, it is possible that the majority of the damage to high elevation pollen occurred at that time. Low temperatures have been shown to slow down meiotic progress in several *Larix* species, causing meiotic irregularities, and consequently resulting in abnormalities and pollen sterility (Eriksson 1968; Luomajoki 1977).

Another possible factor affecting pollen quality is the influence of temperature on mature pollen during winter dormancy. Mature pollen at low elevation was exposed to warmer temperatures during winter dormancy than high elevation pollen. The mean temperature of the coldest month at low elevation was only 3°C and the mean monthly winter temperatures stayed above 3°C. Warm temperatures during late fall and winter may affect mature pollen in various ways. Firstly, pollen as a living organism uses its food reserves through respiration to stay alive. Under elevated temperatures, respiration rates increase, and the pollen food reserves are utilized more rapidly (Stanley and Linskens 1974). It is therefore probable that the dramatic decline in pollen quality during the late fall/winter at Mt. Newton was caused in part by higher levels of pollen respiration stimulated by warmer temperatures. In addition, pollen rich in nutrients and/or pollen already damaged by high summer temperatures may attract the establishment of insects and fungi that can cause further deterioration and pollen-cone abortion. Anderson at al. (2002) observed that pollen-cone abortion at low elevation was consistently associated with the presence of *Trisetacus* mites. The report also points out that mite damage to pollen-cones can be substantial and is likely to be influenced by environmental conditions that preside at a particular site or year. It is likely that freezing winter temperatures at colder sites kept pollen respiration and the potential insect and fungus damage to a minimum, and consequently preserved the quality of mature pollen.

Seed Production Implications

Observations from this and other related investigations suggest that the production of poor quality pollen at warmer environments may be the result of several temperature related events:

i) extreme summer temperatures during the meiotic stage of pollen development causing pollen abnormalities,

ii) absence of cold winter temperatures required to condition pollen before shedding,

iii) warm winter temperatures accelerating respiration and deterioration of mature pollen, and

iv) warm winter temperatures facilitating the establishment of pollen feeding insect and fungi on mature pollen.

The contrasting climatic events that occur at colder environments might facilitate the production of more superior quality pollen. However, moderate reductions in pollen quality at high elevation exist and may be the result of:

i) warmer spring temperatures accelerating pollen initiation and development and thus shifting the meiotic stage closer to the summer temperature extremes, and

ii) colder fall temperatures affecting the cold sensitive meiotic stage of pollen development.

Pollen quality appears to be regulated by temperature, and the selection of a site for high quality pollen production should therefore take into account the influence of potential temperature-related events. One possible site could be in an environment typified by cold winters and a shortened growing season, supported by a limited exposure to sun as a result of northern exposure and/or partial shade. These conditions would favour the development of pollen entering winter dormancy at its pre-meiotic stage and completing its development in the spring. Another option could be a site characterized by moderate summer temperatures, modified by partial shade or northern exposure, and a longer growing season with cooler winters, such as environments found at mid-elevations. This environment would promote full pollen development before winter dormancy, and also avoid high snow packs, which may be difficult to handle by persons interested in managing pollen events. Both environments have the likelihood of producing high quality pollen. More investigations are required to determine which environment is more readily available, accessible, practical to manage, and will produce sufficient quantities of pollen.

If poor quality pollen proves to be the main reason for the lack of viable seed at low elevation, then better quality pollen could be produced at selected colder sites. This pollen could then be stored and used the next spring for supplemental pollination at low elevation orchards. However, the plastic nature of yellow-cedar cone development (El-Kassaby 1995) still poses an orchard management challenge.

To date, all assessments of yellow-cedar pollen quality were based entirely on *in-vitro* testing using pollen germination. Therefore, these assessments should be interpreted only as an indication of pollen fertility. Studies are currently ongoing to examine the relationship between pollen germination, conductivity, and respiration. Additional studies are needed to test the actual fertility of low elevation pollen using control pollinations in natural stands. Similarly, the fertility of females at low elevation should be tested through control pollinations, using high quality pollen collected from natural stands. Both studies would provide more definitive answers to questions on the viable production of yellow-cedar seed.

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