Final Report of the Lodgepole Pine Seed Set Task Group

Interior Technical Advisory Committee,

Forest Genetics Council of British Columbia

February 2002

FGC Forest Genetics Council of British Columbia

FOREST RENEWAL BC

Executive Summary

Among its goals for the provincial forest gene resource management program, the Forest Genetics Council (FGC) has two production targets to be achieved by 2007:

- to double the average volume gain of select[1] seed produced, from 6% to 12%
- to increase the amount of select seed used to 75% of total provincial sowing.

Each year more volume is harvested and more trees are planted of lodgepole pine (Pli) than of any other species in the province. About 75 million lodgepole pine trees are planted annually, almost 40% of the provincial total. Clearly, Council's seed production goals for the province cannot be achieved without achieving the Pli seed production targets.

Many Pli orchards were sited in the north Okanagan beginning in the mid 1980s so as to take advantage of the relatively warm, dry climate for purposes of increasing production of both male and female strobili. North Okanagan sites are also free of contamination from local Pli pollen, a problem with many other seed orchard locations in the province. All species other than Pli at the Kalamalka Forestry Centre site (Si, Fdi, Lw) have responded well to the warm, dry location in terms of flower and seed production. Something has been interfering with seed set in Pli in the north Okanagan that is not affecting other species. Proposed theories attribute the problem to heat, insects (*Leptoglossus* spp.) and other causes.

Research investigating this phenomenon began around 1997 and accelerated in September 1999 when the Interior Technical Advisory Committee (ITAC) formed the Pli Seed Set Task Group[2]. Research teams from the University of Victoria and the MoF undertook projects that were a combination of continuing work started years before and new initiatives directed at reproductive developmental biology and environmental (cultural) manipulations to ameliorate high temperatures and low humidity during pollination, fertilization and cone enlargement stages.

Two years (reproductive seasons) of work by the two research teams were reported at a meeting of the Pli Seed Set Task Group on 17 January 2002. Discussion following the presentations showed task group members were confident that the various factors affecting Pli seed production were understood adequately and could be controlled sufficiently through manipulation of environmental variables, that we should proceed with locating new Pli orchards in the north Okanagan and vicinity.

After considering the evidence presented at the January 17 meeting, the Task Group passed a motion recommending to ITAC that additional lodgepole pine seed orchards be established in the north Okanagan area. Also agreed was that research continue to improve our understanding of these phenomena and to guide operational methods of ameliorating extremes of humidity and temperature, controlling *Leptoglossus* damage, and managing crown shape and size.

The Interior TAC formally adopted the task group's recommendations on 31 January 2002.

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[1] "Select" refers to seed and vegetative materials exhibiting genetic gain over wild-stand material.
[2] Gertzen, D. Tech Coord 1999. Interior Lodgepole Pine Seed Set Task Group Presentations. proceedings of a meeting held in Vernon B.C., 15 October 1999. Extension Services, Tree Improvement Branch, British Columbia Ministry of Forests, Surrey BC.

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1.0 Introduction

Among its goals for the provincial forest gene resource management program, the Forest Genetics Council (FGC) has two production targets to be achieved by 2007:

- to double the average volume gain of select[3] seed produced, from 6% to 12%
- to increase the amount of select seed used to 75% of total provincial sowing.

Each year more volume is harvested and more trees are planted of lodgepole pine (Pli) than of any other species in the province. Almost 75 million lodgepole pine trees are planted annually, which is about 38.5% of the provincial total (Table 1). Clearly, Council's seed production targets for the province cannot be achieved without achieving the Pli seed production targets.

Table 1 Numbers of forest trees currently planted annually in British Columbia, by species.

Species	Annual planting (millions)	% of total
Lodgepole pine (Pli)	74.7	38.5
Interior spruce (Sx)	72.1	37.1
Western Red Cedar (Cw)	8.9	4.6
Coastal Douglas-fir (Fdc)	8.2	4.2
Interior Douglas-fir (Fdi)	7.2	3.7
Western white pine (Pw)	5	2.6
Western hemlock (Hw)	4.4	2.3
Western larch (Lw)	3.8	2.0
Subalpine fir (Ba)	3	1.5
Sitka spruce (Ss)	3	1.5
Interior /Sitka spruce transition (Sx/Ss)	2.5	1.3
Yellow cedar (Yc)	1.1	0.6
Grand fir (Bg)	0.3	0.2
All species	194.2	100.0

Many Pli orchards were sited in the north Okanagan beginning in the mid 1980s so as to take advantage of the relatively warm, dry climate for purposes of increasing production of both male and female strobili. North Okanagan sites are also free of contamination from local Pli pollen, a problem with many other seed orchard locations in the province. All species other than Pli at the Kalamalka Forestry Centre site (Si, Fdi, Lw) have responded well to the warm, dry location in terms of flower and seed production. Something is interfering with seed set in Pli in the north Okanagan that is not affecting other species. Proposed theories attribute the problem to heat, insects (*Leptoglossus* spp.) and other causes.

Our experiences and observations of Pli behaviour over the past decade led to many hypotheses to explain the low filled-seed per cone numbers despite large cone and pollen cluster numbers and healthy orchard tree appearance. Research investigating this phenomenon began around 1997 and accelerated in September 1999, when the FGC's Interior Technical Advisory Committee

[3] "Select" refers to seed and vegetative materials exhibiting genetic gain over wild-stand material.

(ITAC) formed the Pli Seed Set Task Group[4]. The task group held brain-storming sessions to gather all that was known about this problem and propose directed research projects to find answers as quickly as possible.

Research teams from the University of Victoria and the MoF submitted proposals for research projects of 2–4 year durations. Work began in the winter of 2000 at various orchard sites in the Vernon and Prince George areas with funding from the Operational Tree Improvement Program (OTIP). These projects were a combination of continuing work started years before and new initiatives directed at reproductive developmental biology and environmental (cultural) manipulations to ameliorate high temperatures and low humidity during pollination, fertilization and cone enlargement stages.

Two years (reproductive seasons) of work by the two research teams were reported at a meeting of the Pli Seed Set Task Group on 17 January 2002. Discussion following the presentations showed task group members were confident that the various factors affecting Pli seed production were understood adequately and could be controlled sufficiently through manipulation of environmental variables, that we should proceed with locating new Pli orchards in the north Okanagan and vicinity.

After considering the evidence presented at the January 17 meeting, the task recommended to ITAC that additional lodgepole pine seed orchards be established in the north Okanagan area. Also agreed was that research continue in 2002 and beyond with apparatus in place to continue to improve our understanding of these phenomena and to guide operational methods of ameliorating extremes of humidity and temperature, controlling *Leptoglossus* damage, and managing crown shape and size.

The Interior TAC formally adopted the task group's recommendations on 31 January 2002.

2.0 First Task Group Meeting, 15 Oct 1999, Vernon

On Friday 15 October 1999 about 35 people met at the Prestige Inn in Vernon to consider the phenomenon of low seed-set in lodgepole pine long observed in the Vernon area. Discussion resulted in identification of three general areas for subsequent research:

Research Area	Research Team Leaders
pest management	Ward Strong, Sarah Bates
orchard culture & reproductive biology (macro)	Joe Webber, Michael Stoehr
reproductive biology (micro)	John Owens, Vivienne Wilson, Jordan Bennett

The information presented made at this meeting was compiled by Diane Gertzen[5], and included presentations by David Reid, Chris Walsh, Hilary Graham, George Nicholson, Tim Lee, Clare Hewson, Dave Kolotelo, Michael Stoehr, Vivienne Wilson, Jordan Bennett, Ward Strong, Sarah Bates, Roger Painter.

[4] Gertzen, D. Tech Coord 1999. Interior Lodgepole Pine Seed Set Task Group Presentations. Proceedings of a meeting held in Vernon B.C., 15 October 1999. Extension Services, Tree Improvement Branch, British Columbia Ministry of Forests, Surrey BC.
[5] Interior Lodgepole Pine (*Pinus contorta var. latifolia*) Seed Set Task Group Presentations, October 15, 1999, Vernon BC.

Subsequently, research teams from the University of Victoria and the MoF submitted proposals for research projects of 2–4 year duration. Work began in the winter of 2000 at various orchard sites in the Vernon and Prince George areas with funding from the Operational Tree Improvement Program (OTIP). These projects were a combination of continuing work started years before and new initiatives directed at reproductive developmental biology and environmental (cultural) manipulations to ameliorate high temperatures and low humidity during pollination, fertilization and cone enlargement stages.

3.0 Second Task Group Meeting, 17 Jan 2002, Vernon

By January 2002 ITAC and the Council were facing a difficult decision. Achieving the Council's seed production goals required installing a significant area of lodgepole pine seed orchards immediately. Trees were ready for planting and being maintained in holding beds. SelectSeed Company was ready to begin tendering contracts for orchard location and management. But we had to decide where to install these orchards – should the north Okanagan locations be used, or should sites be found elsewhere?

A meeting of the Task Group was convened on 17 January in Vernon, with about 20 people in attendance (Table 1).

Table 1 Participants in meeting of Pli Seed Set Task Group, 17 January 2002, Vernon BC.

Name	Affiliation
Vicky Berger	MOF (Kalamalka)
Michael Carlson	MOF (Kalamalka)
Terry Carter	MOF
Keith Cox	MOF (Skimikin)
Dan Gaudet	VSOC
Diane Gertzen	MOF (Tree Impr. Br.)
Gary Giampis	MOF (Kalamalka)
Hilary Graham	PRT
Barry Jaquish	MOF
Tim Lee	VSOC
Mark Montville	PRT
John Murphy	MOF (Kalamalka)

Name	Affiliation
George Nicholson	Riverside FP
Greg O'Neill	MOF
John Owens	University Victoria
Greg Pleper	Riverside FP
Lynette Ryrie	MOF (Kalamalka)
Ward Strong	MOF (Kalamalka)
Jordan Tanz	FGC Secretariat
Chris Walsh	MOF (Kalamalka)
Joe Webber	MOF (Research. Br.)
Jack Woods	FGC/SelectSeed
Alvin Yanchuk	MOF

Michael Carlson welcomed everyone, and explained the purpose of the meeting. He explained that something has been interfering with Pli seed set in the north Okanagan that has not affected other species. Theories proposed relate to heat, insect problems (Leptoglossus spp.), and others. Achieving FGC goals for Pli seed production requires that large areas of new orchards had to be installed very soon. ITAC had to decide soon whether to locate these orchards in the north Okanagan or elsewhere. The Task Group's responsibility was to consider the evidence presented at the meeting and to make a recommendation to ITAC.

The Task Group heard presentations by Drs. Webber and Owens, summarizing the results of their research over the preceding two years. Summaries are presented below. Their full reports are included in Appendices B and C.

Summary of Presentation by Joe Webber

Over the past two years of this study, we found no evidence to suggest pollen supply, pollen quality or pollen uptake was limiting. Misting (crown cooling) during June/July of 2000 (fertilization and embryo development) did significantly improve seed yields at Kalamalka (PRT misting not activated). Our improved irrigation technique did significantly increase first-year cone numbers, cone size, and seed weight but there was no effect on seed yields. Meteorological conditions in 2001 were mild in April/May (except for a brief period of hot weather at the end of May) and June/July. Results clearly show that good seed yields (may be the highest on record) can be obtained at both orchard sites. Significant seed losses due to *Leptoglossus* have been largely controlled but data collected for 2001 suggest losses still occur.

Summary of Presentation by John Owens

The following summary covers the studies carried out by my lab on Pli reproduction that started in 1980, resumed in 1987-1998 and again in 1999-2000. This has been followed by a formal OTIP study carried out at four seed orchards in 2000-2001 and at two orchards in 2001-2002. Final data will be collected in the spring and fall of 2002 for the experiments done in the spring 2001.

Basically, the problem was the low seed set per cone at Kalamalka (KSO) compared to Prince George Seed Orchards (PGSO). In a nutshell, our findings indicate that very early-on the trees were severely topped resulting in a complete intermingling of pollen cones and seed cone, which in turn resulted in very high levels of selfing. Our experiments and developmental studies show clearly that selfing in Pli, as in other pines, results in seed abortion at the early embryo stage soon after fertilization, about late-June to early-July in Pli. By the time of fertilization the seed coat and female gametophyte are fully developed. Embryo and female gametophyte abortion at that time results in an empty seed with a small shriveled female gametophyte and no embryo. In the recent years that my lab has done studies at KSO and PGSO, the filled seed/cone at KSO has increased from 8 in 1997, 12 in 1999, 15 in 2000 to 28 in 2001 and empty seed has decreased from 23 to 12 to 9 to 11 in the same four years, respectively. Filled seed at PGSO has remained in the low 20's and empty seed 6-8/cone.

So, while we have been busy studying many aspects of Pli reproduction, the KSO trees have out-grown their problem. Leader shoots are now 1-2 m long and female cones on these are separated from most male cones. These studies have provided a lot of useful information and solutions beyond the main selfing problem. These include the cause for cone drop, a problem at PGSO; misting or sprinkling to aid pollination and reduce protandry in very hot dry springs; methods of increasing the mix of pollen in all orchards, etc.

I predict that with proper mixing of pollen in the orchard (physically blowing it around every day during pollination with tractor-mounted blowers), sprinkling to aid pollination and reduce protandry, plus SMP of early and late clones and insect control that a goal of 30 filled seed/cone and a cone retention of 80% is possible at all orchards.

3.1 Summary Of Meeting Discussion

After the presentations by Webber and Owens, it seemed clear that the topping of the trees at Kalamalka did affect the amount of selfing. It was also clear that there had been two successive years in which climatic conditions were much more like the conditions naturally encountered by Pli. These had been the only two years like this since 1983. There was speculation that a hot dry spring in 2002 would permit testing the hypotheses developed by Jack Owens and Joe Webber.

During the discussion Roger Painter read an open letter urging the group not to make this important decision hastily, and to use B+ seed in the meantime.

A discussion involving everyone at the meeting ensued. The main points were:

The issue is to decide how confident we are about locating the Pli orchards in the north Okanagan. Better information is always desirable, but in this case a decision had to be made promptly to allow SelectSeed to determine the location for these orchards. We have a window of opportunity that can't be missed if we are to achieve Council's production goals.

There was discussion about whether a first phase could go in the north Okanagan, and the decision about a second phase be deferred until we have better information. Jack Woods said that proceeding in phases is both possible and planned. If ITAC supports the north Okanagan location, then phase 1 will begin within weeks, and phase 2 would likely begin next year.

The question was restated as: Given the information we now have, are we confident that we could achieve 15 seeds/cone in the north Okanagan? The program is based on assumptions of producing 150 cones per tree, and 15 filled seeds per cone. Production at Kalamalka is achieving 400 cones per tree in good years and over 20 filled seeds per cone. The orchard does not have to produce seed every year. Can we proceed, knowing that we will only have good crops in 2 to 4 years out of ten? Do we know of any other locations that are better than the north Okanagan? We have no information that would lead us to make a decision supporting a better location within the next few years? The information available now indicates that the north Okanagan can be made to work. Concern was expressed about pollen contamination in other less severe locations, which would be a much more difficult problem to manage.

Finally, confidence was expressed that the north Okanagan is the right place to locate these orchards, and that the north Okanagan sites can be managed to produce acceptably well.

3.2 Task Group Recommendation

The Task Group passed the following motion, and in so doing made its recommendation to the ITAC.

> **MOVED** (Carlson), that the Lodgepole Pine Seed Set Task Group for the establishment of additional lodgepole pine seed orchards in the north Okanagan area recommends that future orchard developments for this species be located on suitable sites, free of substantial contaminant pollen, in the north Okanagan area between Salmon Arm and Penticton, or in similar climatic types found in the Kettle Valley and Grand Forks areas. This does not preclude future consideration of other areas, pending receipt of new information or research results. **CARRIED** unanimously.
>
> Jordy Tanz is to prepare a package documenting the deliberations of the Pli Seed Set Task Group, and forward the recommendation to ITAC.

Also agreed was that research continue in 2002 and beyond with apparatus in place to continue to improve our understanding of these phenomena and to guide operational methods of ameliorating extremes of humidity and temperature, controlling *Leptoglossus* damage, and managing crown shape and size.

Following the meeting, the ITAC was to vote on whether to accept the Task Group's recommendation.

4.0 ITAC Decision

An e-mail ballot was distributed to members of the ITAC on 25 January 2002 regarding the Task Group's recommendation (Appendix A). Background information was attached to the e-mail ballot, including the reports of Drs. Webber and Owens.

At the conclusion of voting (3:00 PM, January 31, 2002), 15 out of 20 voting members of ITAC had voted to accept the Task Group's recommendation. No votes were opposed.

5.0 Conclusion

The decision to locate the new Pli orchards in the north Okanagan enabled SelectSeed Company to begin the process of tendering contracts to establish the new orchards.

In the coming year both research teams will continue their work investigating the causes of the seed set problem and exploring techniques for managing the orchards to increase see production.

Appendix A Interior TAC E-mail Ballot, 25 January 2002

From: Shiela O'Shea [mailto:soshea@cortex.ca]
Sent: Friday, January 25, 2002 4:19 PM
To: ITAC Listserver
Subject: FGC Interior TAC: 2 motions, 2 ballots on Pli Task Group's recommendations

Hello Members of ITAC:

At a meeting on 17 January 2002, the ITAC Pli Seed Set Task Group considered the establishment of additional lodgepole pine seed orchards in the north Okanagan, and passed the following motions recommending establishing future lodgepole pine orchards in the north Okanagan:

MOVED (Seed Orchard Establishment Decision)(Carlson), that the Lodgepole Pine Seed Set Task Group for the establishment of additional lodgepole pine seed orchards in the north Okanagan area recommends that future orchard developments for this species be located on suitable sites, free of substantial contaminant pollen, in the north Okanagan area between Salmon Arm and Penticton, or in similar climatic types found in the Kettle Valley and Grand Forks areas. This does not preclude future consideration of other areas, pending receipt of new information or research results. CARRIED unanimously.

MOVED (East Kootenay Pine Orchard)(Walsh), that the East Kootenay low seed orchard be established at Kalamalka. CARRIED unanimously.

The minutes of the January 17 meeting, the reports by Joe Webber and John Owens, and other background information on which the assessment was based are attached to this message for your information.

Upon notification of these recommendations, ITAC was to vote by email on whether to accept the Task Group's recommendations. The Forest Genetics Council indicated at its December meeting that it would accept ITACs recommendation in this regard. ITAC members are asked to cast a vote on each of the motions below.

Accordingly, Michael Carlson is making the following motion concerning the recommendations of the Task Group on the location of future lodgepole pine seed orchards:

MOVED by Michael Carlson and seconded by Hilary Graham, that the Interior TAC accepts the Pli Seed Set Task Group's recommendation of 17 January 2002, and recommends to the Forest Genetics Council that future orchard developments for lodgepole pine be located on suitable sites, free of substantial contaminant pollen, in the north Okanagan area between Salmon Arm and Penticton, or in similar climatic types found in the Kettle Valley and Grand Forks areas, without precluding future consideration of other areas, pending receipt of new information or research results.

TO VOTE ON THE FIRST MOTION:

Only voting members of the Interior TAC are eligible to vote.

If you are in favour of the motion, please indicate your support by sending an e-mail message to the ITAC listserver (L_for_itac@pop.gov.bc.ca) with the words, "I am IN FAVOUR of the motion of 25 January 2002 regarding acceptance of the Pli Seed Set Task Group's recommendations future orchard developments for lodgepole pine be located on suitable sites, free of substantial contaminant pollen, in the north Okanagan area between Salmon Arm and Penticton, or in similar climatic types found in the Kettle Valley and Grand Forks areas, without precluding future consideration of other areas, pending receipt of new information or research results."

If you oppose the motion, please indicate your opposition by sending an e-mail message to the ITAC listserver (L_for_itac@pop.gov.bc.ca) with the words, "I OPPOSE the motion of 25 January 2002 regarding acceptance of the Pli Seed Set Task Group's recommendations future orchard developments for lodgepole pine be located on suitable sites, free of substantial contaminant pollen, in the north Okanagan area between Salmon Arm and Penticton, or in similar climatic types found in the Kettle Valley and Grand Forks areas, without precluding future consideration of other areas, pending receipt of new information or research results."

Voting will close at 3:00 PM on Thursday, 31 January 2002. Ballots sent to the list server before that time will be counted. A majority is needed to carry the motion.

Regarding the second recommendation, Chris Walsh is making the following motion:

MOVED by Chris Walsh and seconded by Tim Lee, that the Interior TAC accepts the Pli Seed Set Task Group's recommendation of 17 January 2002, and recommends to the Forest Genetics Council that the East Kootenay low seed orchard be established at Kalamalka.

TO VOTE ON THE SECOND MOTION:

Only voting members of the Interior TAC are eligible to vote.

If you are in favour of the motion, please indicate your support by sending an e-mail message to the ITAC listserver (L_for_itac@pop.gov.bc.ca) with the words, "I am IN FAVOUR of the motion of 25 January 2002 regarding acceptance of the Pli Seed Set Task Group's recommendation that the East Kootenay low seed orchard be established at Kalamalka."

If you oppose the motion, please indicate your opposition by sending an e-mail message to the ITAC listserver (L_for_itac@pop.gov.bc.ca) with the words, "I OPPOSE the motion of 25 January 2002 regarding acceptance of the Pli Seed Set Task Group's recommendation that the East Kootenay low seed orchard be established at Kalamalka."

Voting will close at 3:00 PM on Thursday, 31 January 2002. Ballots sent to the list server before that time will be counted. A majority is needed to carry the motion.

I will announce the results of the vote after voting has finished. Don't hesitate to call for clarification if needed.

Thanks,
Jordan Tanz, RPF
Executive Secretary

Appendix B Research Report by Webber *et al*.

Seed Yields in North Okanagan Lodgepole Pine Seed Orchards - Summary of Research Data for 2000 and 2001.

Authors: Joe Webber, Clint Hollefreund, and Mark Griffin.

Over the past two years of this study, we found no evidence to suggest pollen supply, pollen quality or pollen uptake was limiting. Misting (crown cooling) during June/July of 2000 (fertilization and embryo development) did significantly improve seed yields at KAL (PRT misting not activated). Our improved irrigation technique did significantly increase first-year cone numbers, cone size, seed weight and but there was no effect on seed yields. Meteorological conditions in 2001 were mild in both April/May (except for a brief period of hot weather at the end of May) and June/July. Results clearly show that good seed yields (may be the highest on record) can be obtained at both orchard sites. Significant seed losses due to *Leptoglossus* have been largely controlled but data collected for 2001 suggest losses still occur (see Table 6D).

Introduction

The chronic problem of low seed set in north Okanagan lodgepole pine seed orchards has been attributed to three factors (Interior Lodgepole Pine Seed Set Task Group Group Summary, 1999):

- poor pollen uptake,
- seed cone bug (*Leptoglossus*),
- limiting culture regimes during seed cone maturation.

Lodgepole pine has a two-year reproductive cycle, which in the north Okanagan starts in April of year-one and finishes with cone maturation, the following year (August). The two important periods of seed cone development are pollen uptake and early pollen germination in year-one (May/June) and the resumption of pollen germination and the subsequent fertilization and early embryo development in June the following year. It is our contention that hot, dry weather (inadequate irrigation) during these two periods may be one of the principal causes of poor seed set.

The Experiment

Design

To test this hypothesis, we installed a larger capacity irrigation system in each of two orchards (Kalamalka and PRT Seed Orchards 307 and 308, respectively). We also installed overhead sprinklers to be activated when crown temperatures exceeded 24ºC. The experimental design for 2000 was a 2x2 factorial design in which four blocks were designated as either control, irrigation+misting, irrigation only, and mist only. The block size was 4 x 15 trees (2000) for each orchard and sampling was restricted to the inner rows (26 trees in total). Because of low line pressure at PRT, we could not activate the mist system.

In 2001, we used a split plot design at KAL only. Each block was partitioned into three plots: plot one treated in the first year, plot 2 treated in both years and plot 3 treated only in the second year) at KAL only. This was done to separate out one- and two-year treatment effects on second year cone yields. Sampling in all three plots was reduced to 12 trees. For 2001, we installed an auxiliary pump system at PRT and used the same field design (2x2 factorial). Irrigation began when the municipal water system was activated at KAL and when the pump system was installed at PRT (early April for both orchard sites). Misting was activated after pollen flight was completed (late May) at both orchard sites. Details of the irrigation and mist systems as well as the field set up are given in the 2000 final report.

Treatment Application

For irrigation, we used two spray-head nozzles (180º type) mounted midway between each tree at about 40 cm above the ground and directed toward the middle of the row. Each nozzle supplied about 1.7 litres/min. and covered about 80-90% of the plot area. Watering was automatically scheduled to provide 2 hours twice weekly. Both our irrigation system and the current drip system (used in the mist and control blocks) were scheduled for watering twice weekly. The drip system supplied between 70 and 145 litres/week of water to each tree but was limited to an area probably smaller than crown diameter. Our spray system supplied about 815 litres/week but was spread out over a greater area. Irrigation treatment began in early April and continued through to cone maturation (early August).

For crown cooling, we used overhead sprinklers applied to each tree per treatment block. The sprinkler heads had a volume output of 5.45 litres/min. They were adjusted to the height of each crown, and were activated by mid crown temperature. When temperatures reached 24ºC, the mist system was on for 10 min (2000) or 5 minutes (2001). Evaporative cooling was allowed to occur for 20 to 40 minutes, the length of time determined by the temperature range at activation. The mist system in both orchards was activated after orchard pollination was 95% completed and turned off at cone collection (early August).

Data Collected

For both orchards, we monitored spring pollen cloud density (PCD) and dates of pollen shed (20%, 50%, and 80% buds shedding) and seed cone receptivity (bud burst, maximum receptivity, end of receptivity, and bud closure) for each block. We also counted first- and second-year cones (2000) and followed through cone survival of first-years cones through to harvesting in 2001. In 2001, we also surveyed the new crop of first-year cones to determine if our treatments were affecting cone fecundity. At cone harvest, second-year cones were collected for cone analyses (seed yields, seed weight) and dry-mass determination. First-year cones were collected in mid October for dry-mass determination. Foliar samples were also taken at this time for nutrient analyses.

Results

I. Pollen Supply

Pollen quality and quantity were not limiting at either orchard. Pollen quality (2000) was tested using both *in vitro* assays and field fertility tests. Pollen samples were collected from each of 15 sample trees per block per orchard and bulked. We also tested a bulk Prince George Pollen lot used for our SMP treatments at each orchard. Table 1 shows the pollen assay response for each of the four blocks from each orchard site plus a bulk lot from PG (used for SMP trials in 2000). The three *in vitro* tests used were respiration, conductivity and germination as well as the corresponding seed yields from controlled crossing tests done at the Prince George Tree Improvement Station in 2000.

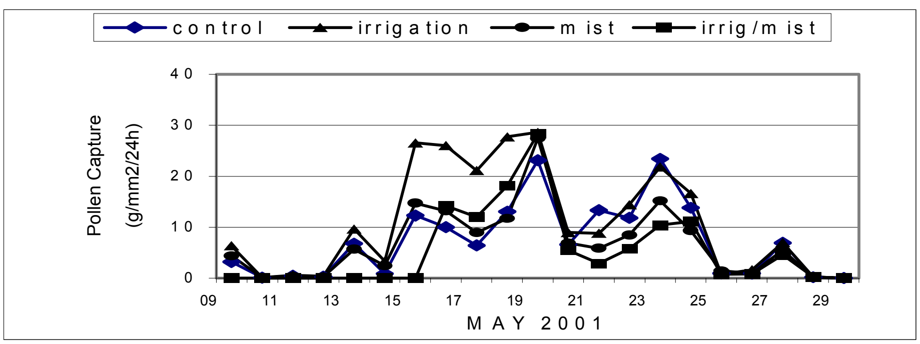
Respiration (µLO2/min/gdw) is a measure of oxygen uptake under controlled conditions. Conductivity is a measure of the electrical conductance of pollen leachate over a standard time interval (1 hour) and is expressed as a percentage of cold to hot conductivity. Germination is expressed as a percentage of pollen grains forming a pollen tube in specific medium and time interval (36 hours). Pollen quantity was assessed using 7-day pollen monitors for each of the four blocks per orchard site. We observed some of the highest pollen cloud density values ever recorded in any conifer seed orchard (Figures 1-2).

Table 1 **Response of pollen lots collected from each of four treatment blocks per orchard site (2000) plus a bulk lot from PG to three in vitro assays (n=3) and field fertility (n=6). A collection of wind pollinated cones from clones used for the 2000 pollination tests are also shown.**

	Kalmalka Seed Orchard 307				PRT Seed Orchard 308				PG	PG
	Control	Irr/Mist	Irrigation	Mist	Control	Irr/Mist	Irrigation	Mist	Poly-Mix	OP
RESP	25.9	21.2	20.7	21.3	24.0	18.7	20.9	19.5	20.4	
%COND	8.8	9.2	8.1	9.4	9.7	8.7	9.0	7.7	9.0	
%GERM	82.5	76.0	75.2	64.9	85.8	87.4	85.3	88.2	74.1	
TSPC	30.0	29.3	33.0	28.5	42.6	33.6	30.5	37.2	33.8	35.3
FSPC	23.7	23.3	27.4	22.0	35.5	28.0	24.8	29.9	27.5	25.3

Figure 1 **Pollen capture, expressed as grains/mm^2/24h, for the four treatment blocks over the period of A. May 5 to May 30, 2000 and B. May 09 to May 30, 2001 at the Kalamalka Seed Orchard 307.**

A. KAL-2000



B. KAL-2001

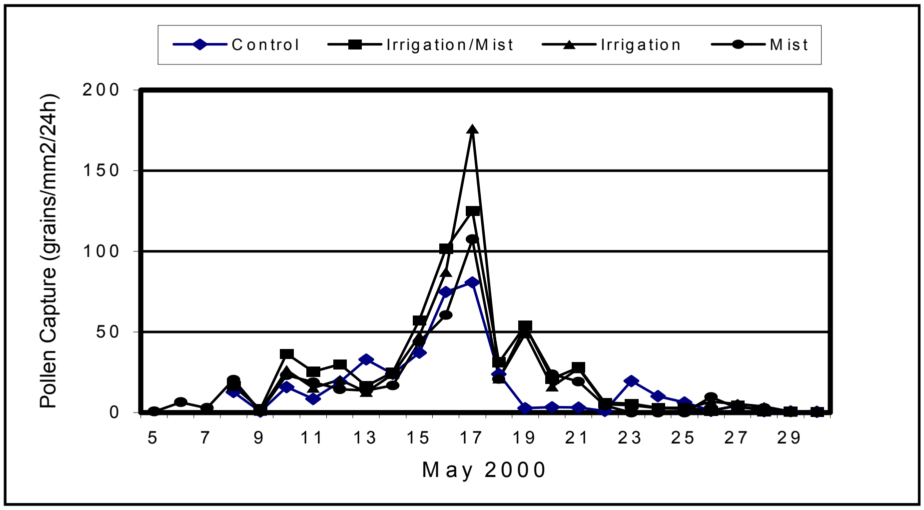
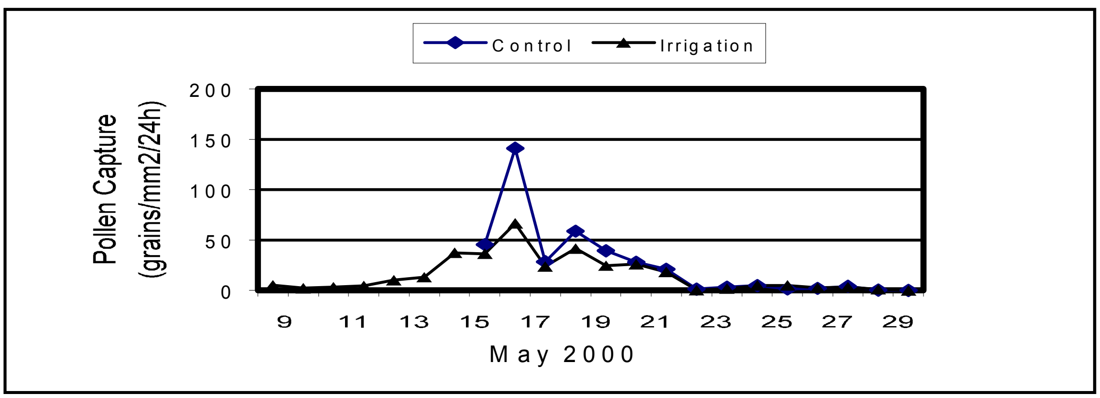
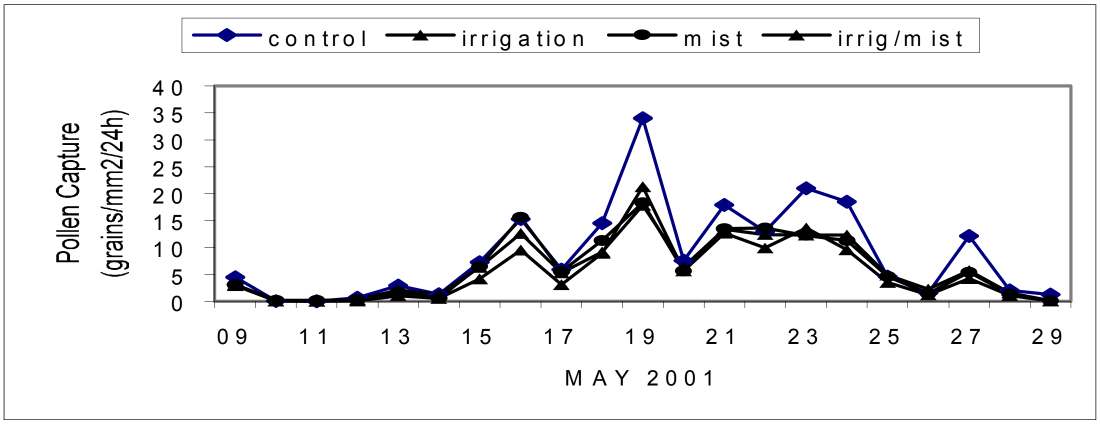


Figure 2 **Pollen capture, expressed as grains/mm^2/24h, for A. two treatment blocks over the period of May 9 to May 30, 2000 and B. four blocks over the period of May 09 to May 29, 2001 at the PRT Seed Orchard 308.**

A. PRT-2000



B. PRT-2001



Figures 1 and 2 show the range (dates and duration) of pollen flight to be similar in both years. However, at both KAL and PRT the bulk of the orchard pollen shed occurred over a shorter time period (about 1 week) in 2000 than in 2001 where pollen shed occurred over a longer period. This longer shed period in 2001 reflects the generally cooler wetter conditions experienced during the early part of the pollination period of 2001 at both orchards. Also, the amount of pollen captured in 2001 was about half that captured in 2000. For KAL, the average pollen capture over all blocks was 90 versus 30 grains/mm^2/24hour. At no time was pollen supply limiting.

II. Seed Cone Receptivity and Pollen Shed Synchrony

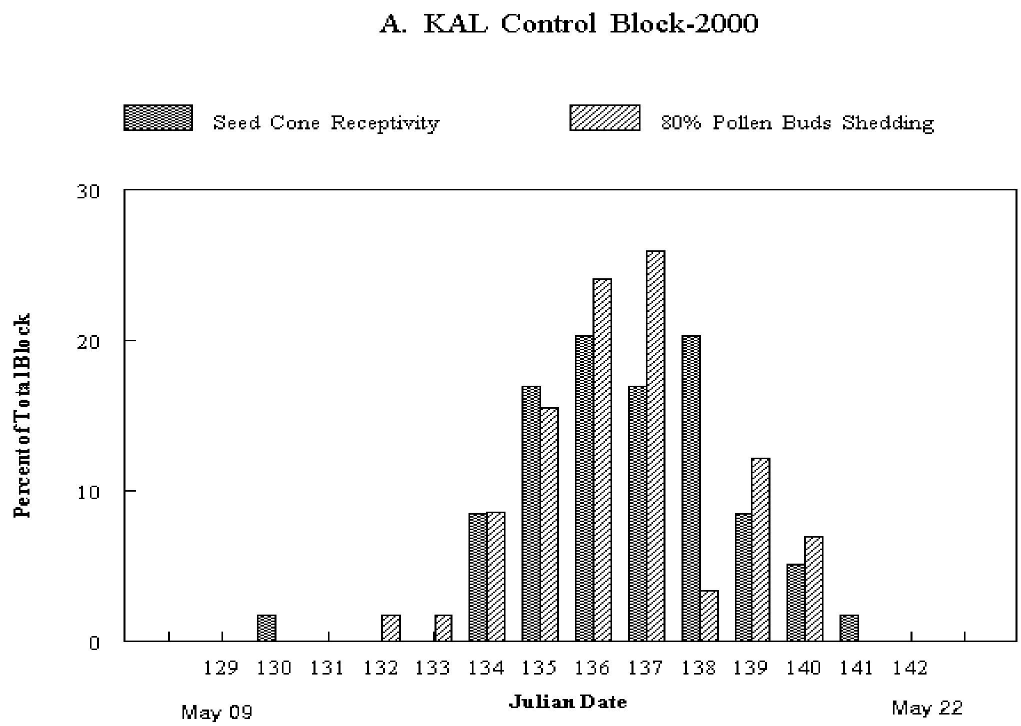
Mean Julian Date (JD) of seed cone receptivity and pollen shed at both KAL and PRT are shown in Tables 2 and 3 for 2000 and 2001, respectively. For 2000 data (Table 2), the average JD for 20% and 80% of pollen buds shedding on each tree are shown. For 2001, we also included the average JD when 50% of the buds were shedding and the average JD when receptivity was over (ovuliferous scales swollen).

The percentage of trees (clones) reaching receptivity and 80% pollen shed (2001) and receptivity and 50% pollen shed (2001) for both the control and irrigation blocks are shown in Figures 3-4 for year 2000 (KAL and PRT) and Figures 5-6 for year 2001 (KAL and PRT). Data for the Mist blocks are not shown because the misting treatment began after the pollination period in both years and would have no effect on seed and pollen cone phenology.

Table 2 Mean date for pollen shed (Julian Day) and seed cone bud burst, receptivity and bud closure for each of the four treatment blocks per orchard site in 2000.

Mean Julian Day of Pollen Shed and Seed Cone Receptivity by Treatment Block-2000									
	KAL Orchard 307					PRT Orchard 308			
	Control	Irr/Mist	Irr	Mist		Control	Irr/(Mist)	Irr	(Mist)
20% Pollen Shed	134	132	133	133		135	134	134	134
80% Pollen Shed	136	135	136	136		137	137	136	136
Bud Burst	133	132	133	133		135	134	133	134
Recptivity	137	136	136	137		139	138	138	138
Bud Closure	149	148	149	149		151	151	150	150

Figure 3 **The percentages of sample trees reaching peak receptivity and pollen shed in the A. Control Block and B. Irrigation Block at the at the Kalamalka Seed Orchard 307 for 2000.**



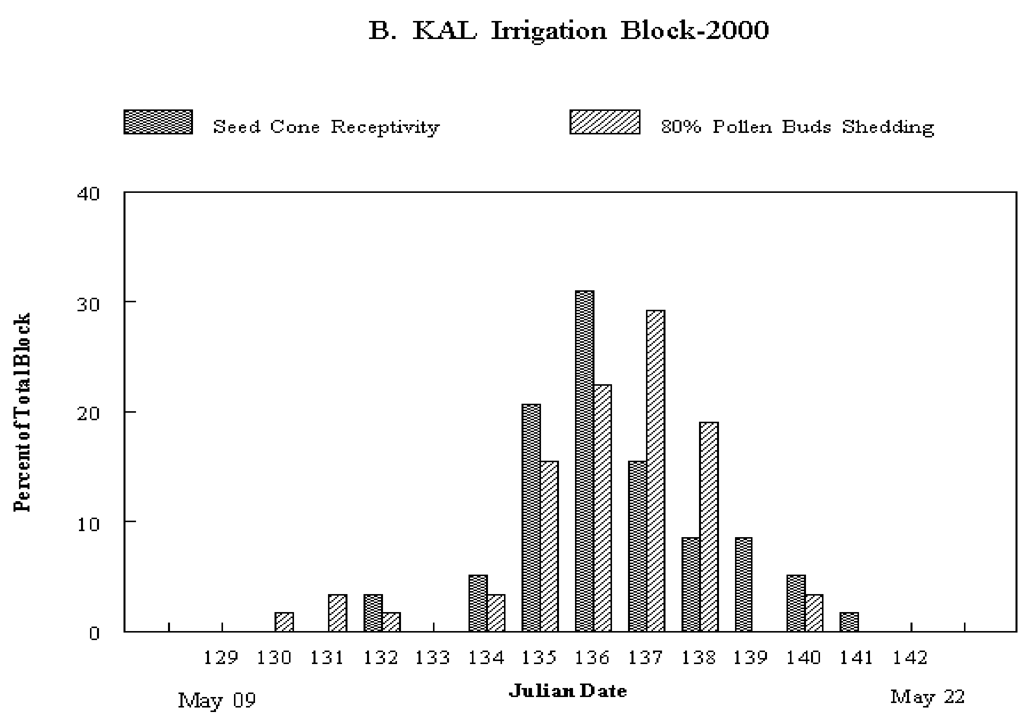
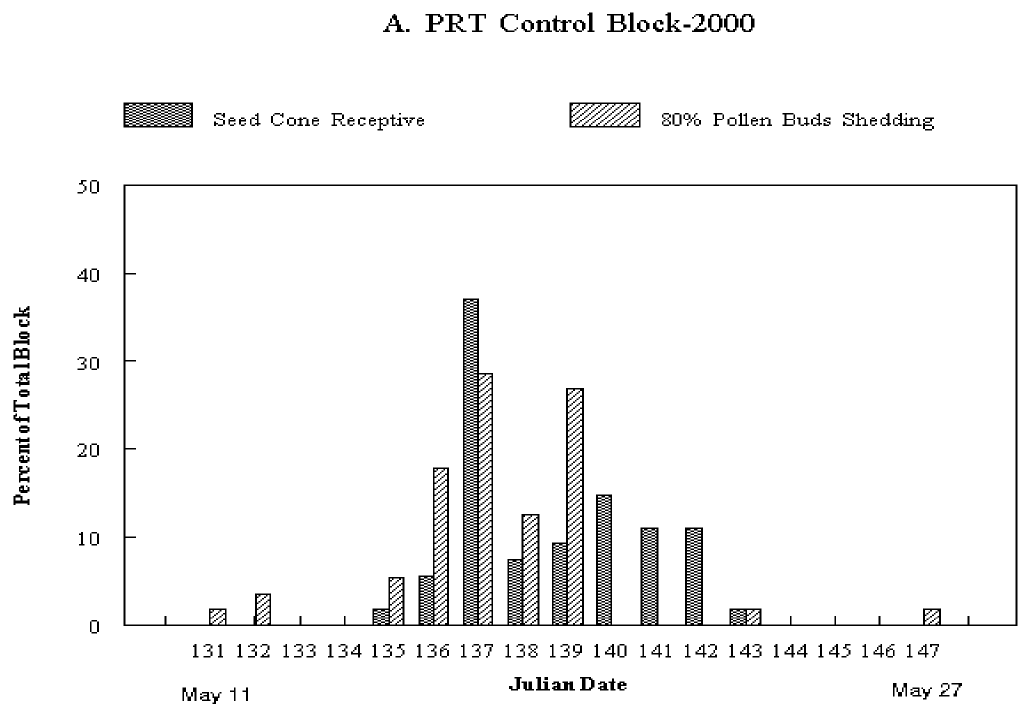


Figure 4 **The percentages of sample trees reaching peak receptivity and pollen shed in the A. Control Block and B. Irrigation Block at the at the PRT Seed Orchard 308 for 2000.**



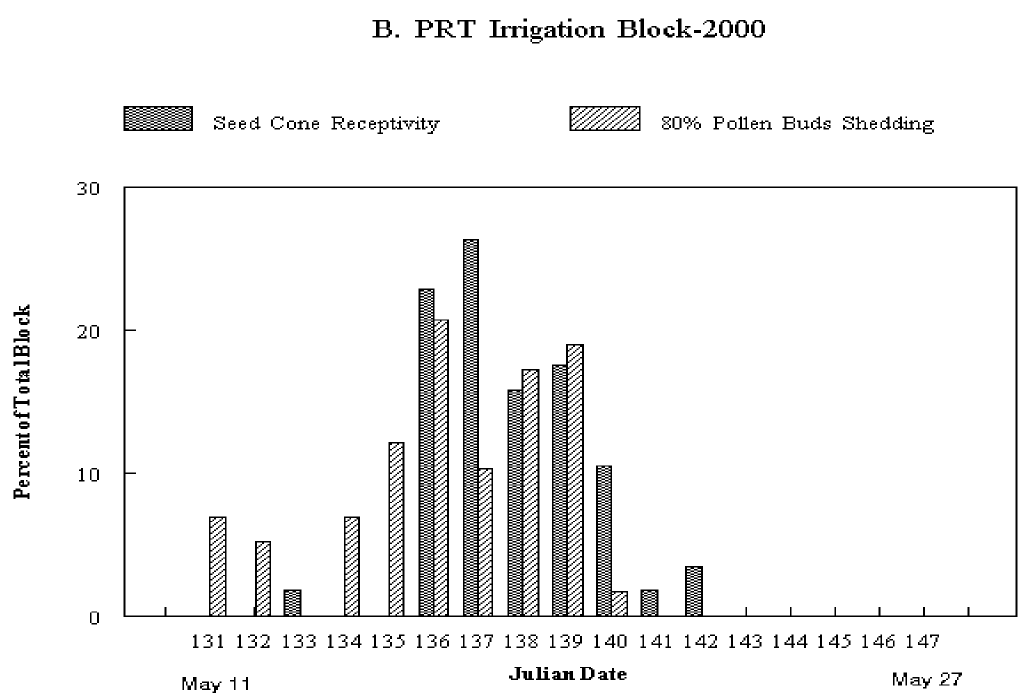
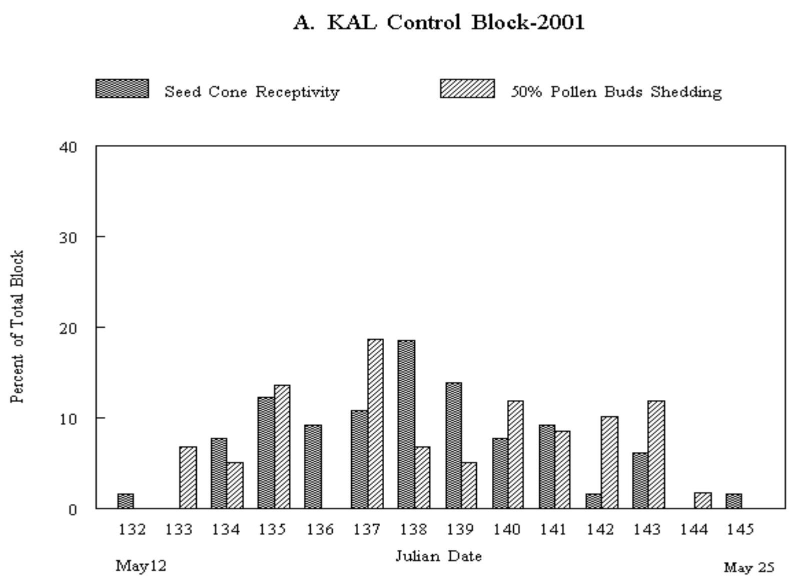


Table 3 Mean date (Julian Day) for pollen shed, seed cone bud burst, receptivity, end of receptivity and bud closure for each of four treatment blocks per orchard site in 2001.

	KAL Orchard 307				PRT Orchard 308			
	Control	Irr/Mist	Irr	Mist	Control	Irr/Mist	Irr	Mist
20% Pollen Shed	135	135	136	135	137	137	137	137
50% Pollen Shed	139	138	139	138	139	140	140	140
80% Pollen Shed	142	143	143	142	143	143	143	144
Bud Burst	135	134	135	135	138	137	137	137
Receptivity	138	137	137	137	141	140	140	140
End of Receptivity	145	144	144	144	146	145	145	145
Bud Closure	154	153	155	155	154	155	155	157

Figure 5 The percentages of sample trees reaching peak receptivity and pollen shed in the A. Control Block and B. Irrigation Block at the at the Kalamalka Seed Orchard 307 for 2001.



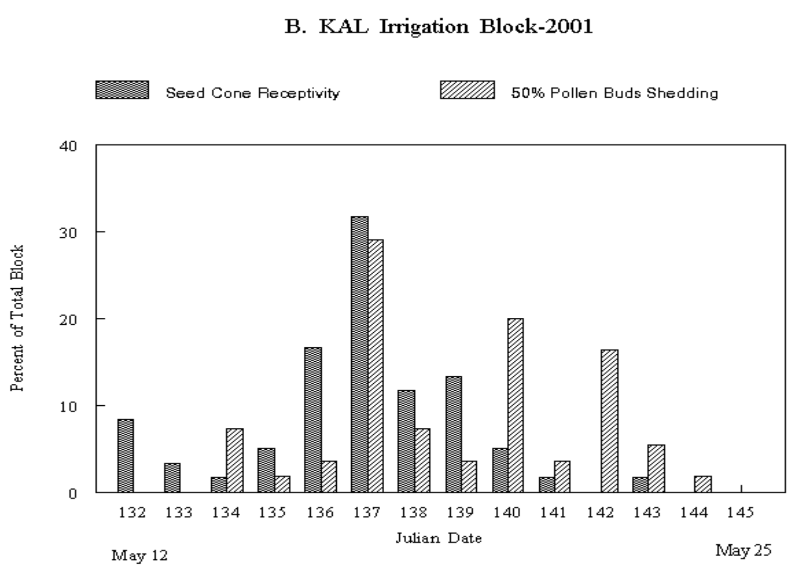
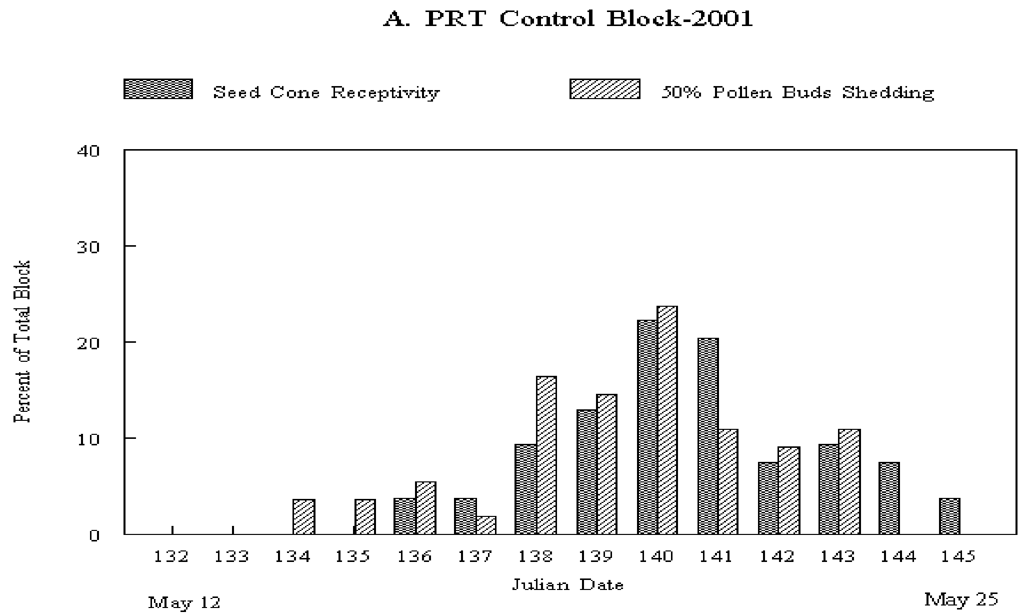
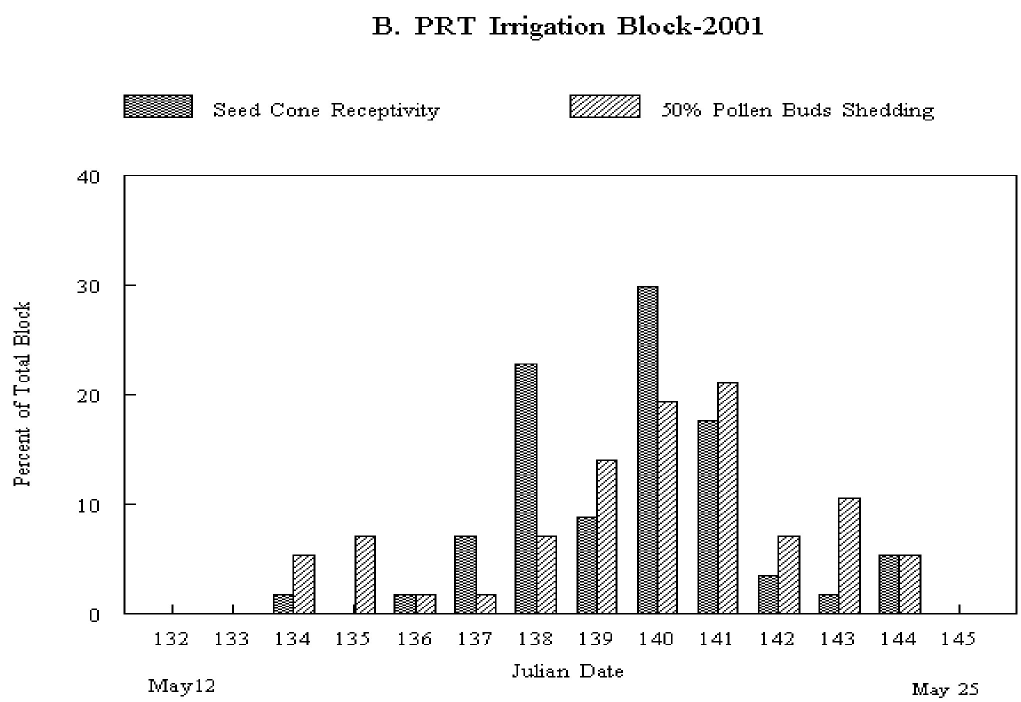


Figure 6 **The percentages of sample trees reaching peak receptivity and pollen shed in the A. Control Block and B. Irrigation Block at the at the PRT Seed Orchard 308 for 2001.**





III. Cone Analyses

Procedures for determining cone mass, cone numbers, and cone yields are detailed in year 2000 summary report. All data were analysed as a 1-way, completely randomized ANOVA with treatment as a fixed factor and trees as a random factor.

A. Cone Mass

Table 4 shows the oven-dry weights for first- and second-year cones from KAL (A) and PRT (B). Average values shown represent 4 cones per tree per block at KAL and 2 at PRT. For KAL, values shown are for plot 2 (treated in both years) of blocks 1, 2 and 3 only.

Table 4 Mean oven-dry cone weights (in grams +/- standard error) for first- (Yr1) and second-year (Yr2) cones for 2000 and 2001.

A. Kalamalka Seed Orchard (307).

2000 and 2001 Dry Cone Weights for Kalamalka Seed Orchard 307 By Treatment Block				
	Control	Mist/Irrigation	Irrigation	Mist
Yr1 Seed Cones (g)				
2000	0.229 (.015)	0.272 (.017)	0.223 (.013)	0.319 (.039)
2001	0.195 (.008)	0.276 (.021)	0.242 (.021)	0.245 (.023)
Yr2 Seed Cones (g)				
2000	6.54 (.445)	6.19 (.899)	5.46 (.624)	6.16 (.956)
2001	6.86 (.408)	6.95 (.658)	6.67 (.498)	6.64 (.308)

B. PRT Seed Orchard (308).

2000 and 2001 Dry Cone Weights for PRT Seed Orchard 308 By Treatment Block				
	Control	Irrigation/Mist	Irrigation	Mist
Yr1 Seed Cones (g)				
2000	0.232 (.018)	0.205 (.010)	0.193 (.013)	0.252 (.036)
2001	0.192 (.007)	0.178 (.008)	0.168 (.010)	0.242 (.027)
Yr2 Seed Cones (g)				
2000	4.92 (.327)	5.43 (.307)	3.50 (.222)	5.03 (.433)
2001	5.42 (.442)	5.02 (.376)	4.04 (.278)	6.30 (.493)

B. Cone Numbers

One of our concerns about irrigation was a negative effect on initiation and differentiation of pollen and seed cone buds for the following year. First- and second-year cone counts were done in the spring of each year by counting the total number of pollen and seed cone buds (first- and second-year) on each of 4 major whorl branches per sample tree per block per orchard (2000). Sampling was reduced to 2 major whorl branches in 2001. Averaged values for a major whorl branch for both orchards and years are summarized in Table 5.

Table 5 Cone bud surveys for study years 2000 and 2001.

A. Kalamalka Seed Orchard (307)

Lodgepole Pine Cone Counts Kalamalka Seed Orchard 307						
	2000				2001	
	Pollen Bud Clusters	First Year Conelets	Second Year Cones	%First Year Retention	Pollen Bud Clusters	First Year Conelets
Treatment						
Control	37.6	17.1	7.9	90.8	46.7	16.7
Irrigation/Mist	34.1	13.6	8.2	93.9	34.7	17.2
Irrigation	35.6	12.9	7.1	94.2	35.9	15.2
Mist	30.7	13.8	5.2	93.7	32.7	13.1
Mean	**34.5**	**14.4**	**7.1**	**93.2**	**37.5**	**15.6**

B. PRT Seed Orchard (308)

Lodgepole Pine Cone Counts PRT Seed Orchard 308						
	2000				2001	
	Pollen Bud Clusters	First Year Conelets	Second Year Cones	%First Year Retention	Pollen Bud Clusters	First Year Conelets
Treatment						
Control	22.9	5.0	7.1	90.8	32.2	8.1
Irrigation/Mist	21.0	7.7	6.1	91.0	30.7	11.8
Irrigation	31.0	7.4	7.2	85.4	36.5	12.4
Mist	25.1	6.0	6.3	90.2	32.9	8.4
Mean	**25.0**	**6.5**	**6.7**	**89.4**	**33.1**	**10.2**

Cone numbers varied by year and orchard site. AT KAL, the number of pollen clusters and first-year cones were about the same in both years but both mist and irrigation treatments significantly reduced the number pollen clusters (but not first-year cones) compared to the control block (2001). Both the number of pollen clusters and first-year cones increased at PRT in 2001. Irrigation treatment in 2000 had no effect on the number of 2001 pollen clusters but first-year cone numbers were increased (p=0.0767) in the irrigated block.

C. Seed Yields

Included in our cone analyses were seed yields (expressed as filled seed per cone) and seed weight. At both orchards, 8 cones (2000) and 6 cones (2001) per tree per block were hand extracted and the total number of seed counted. In 2000, only wind-pollinated cones were analyzed. For 2001, we also compared yields for cones treated to SMP in 2000 with wind-pollinated cones. Both these sets of cones were insect bagged in April 2001 and compared to seed yields from wind-pollinated cones (2000) that were not insect bagged in 2001. The number of filled seed was determined by x-ray analyses. Also, the mean weight of 10 filled seed was determined. At KAL, results are expressed for each of the three plots for the treatment blocks separately. Because there were no treatment effects on seed yields in 2001, we also show the results bulked by block.

Table 6 **Summary of total seed per cone (TSPC), filled seed per cone (FSPC), seed weight (mg) and standard errors (in parenthesis) for Kalamalka Seed Orchard 307 and PRT Seed Orchard 308 for years 2000 and 2001.**

A. Year 2000 results at Kalamalka Seed Orchard (307)

	OP - 2000		
	TSPC	FSPC	Seed Wt (mg)
Treatment			
Control	22.4 (2.4)	9.7 (1.6)	4.1 (0.14)
Irrigation/Mist	23.3 (2.2)	13.0 (1.5)	4.2 (0.16)
Irrigation	23.3 (1.9)	11.9 (1.4)	4.6 (0.24)
Mist	25.1 (2.5)	15.9 (1.9)	4.4 (0.10)

B. Year 2001 results at Kalamalka Seed Orchard (307) for cones wind pollinated in 2000 and insect bagged in April 2001

	Water Treatment			OP-Bagged - 2001		
	Plot	2000	2001	TSPC	FSPC	Seed Wt (mg)
Control		no	no	36.9 (2.6)	25.2 (2.9)	3.03 (0.17)
Irrigation/Mist	1	yes	no	38.2 (4.1)	26.2 (2.7)	3.61 (0.22)
	2	yes	yes	35.7 (2.3)	24.9 (2.0)	4.40 (0.22)
	3	no	yes	41.3 (3.6)	29.2 (3.7)	3.61 (0.30)
Irrigation	1	yes	no	38.7 (3.2)	24.5 (2.9)	3.72 (0.23)
	2	yes	yes	36.0 (3.6)	21.4 (3.6)	3.73 (0.39)
	3	no	yes	37.4 (4.7)	25.2 (3.6)	3.45 (0.20)
Mist	1	yes	no	37.8 (3.0)	27.2 (3.2)	3.61 (0.20)
	2	yes	yes	35.8 (2.5)	25.5 (2.8)	3.23 (0.19)
	3	no	yes	38.7 (3.6)	28.0 (3.0)	3.53 (0.23)

C. Year 2001 results at KAL for cones pollinated (SMP) in 2000 and insect bagged in April 2001.

	SMP-2000 Bagged-2001		
	TSPC	FSPC	Seed Wt (mg)
Treatment			
Control	39.4 (3.2)	29.2 (2.8)	3.79 (0.16)
Irrigation/Mist	41.1 (2.5)	29.7 (2.0)	4.41 (0.19)
Irrigation	43.6 (3.3)	31.3 (3.3)	4.90 (0.22)
Mist	42.4 (3.5)	29.2 (3.2)	3.98 (0.15)

D. Summary of year KAL-2001 seed yields (FSPC) comparing SMP-pollinated, wind (open)-pollinated versus for insect bagged and non-bagged cones.

	Filled Seed per Cone KAL-2001		
	SMP-Bagged	OP-Bagged	OP-No Bag
Treatment			
Control	29.2	25.2	18.8
Irrigation/Mist	29.7	26.8	19.5
Irrigation	31.3	23.7	18.5
Mist	29.2	26.9	21.5
Mean	**29.9**	**25.7**	**19.6**

Table 7 Summary of seed yields at PRT for 2000 and 2001

	PRT Seed Yields			
	2000	**2001**		
	OP-No Bag	SMP-2000/Bagged-2001		OP-2000
	FSPC	FSPC	Seed Wt. (mg)	noBag-2001
Treatment				
Control	8.1	29.6	4.13	9.0
Irrigation/Mist	5.2	21.5	4.31	11.5
Irrigation	6.4	21.7	3.74	6.2
Mist	7.5	26.5	4.38	15.0

IV. Foliar Analyses

We were not certain that nutrient status was limiting and may be a cause of poor seed set. We completed foliar analyses of micro- and macro-elements from each sample tree per block and orchard. Results are shown in Appendix B-4 and B-5. All elements were either at or above adequate levels and in some cases (Mn and SO_4) they were far above adequate levels, especially in the mist blocks. We also show the ratio of N to P, K, Mg and S. Except for N:Mg, all ratios are adequate. We see no reason to suspect a nutrient deficiency (or excesses) as a cause for poor seed set in these orchards.

Summary of Results

Our only significant treatment effect at either orchard was an increase in seed yields in the mist block (see Table 6A) at KAL for the year 2000 (15.9 filled seed per cone for mist and 9.7 for control). Irrigation did not increase seed yields but it did significantly increase first-year cone weight and seed weight (see Table 4A) in year 2001. Cone retention exceeded 90% at KAL and 85% at PRT.

We did not expect any significant treatment effects due to irrigation in the first year as root volume increased. However, in 2001 we did begin to see irrigation effects on first-year cone size (Table 4A) and seed weight (Table 6C) at KAL and first-year cone number at PRT (Table 5B).

We attribute the significant increase in seed yields of 2000 in the mist block to a reduction in temperature, increase in humidity and/or a lowering of the vapour pressure deficit in the crown of treated trees. We speculate that misting (crown cooling) is allowing normal resumption of pollen germination, fertilization and early embryo development of second year cones, which occurs in the month of June.

Pollen supply was high (Figures 1-2) and good synchrony between pollen shed and seed cone receptivity was evident (Figures 3-6 and Tables 2-3). We recorded lower levels of pollen cloud density in 2001 from those recorded in 2000 but still levels of pollen capture recorded were well beyond 100% pollen load for lodgepole pine. Both pollen clusters and first-year cone numbers at KAL (Table 4) were not affected by 2000 treatments and remained at about the same number as 2001. At PRT both pollen clusters and first-year cones increased (Table 5). Second-year cone mass increased slightly at both orchards.

For 2001, we sampled collections of SMP-cones, open-pollinated cones (both insect bagged in April 2001) and non-bagged open-pollinated cones from each block and found that filled seed per cone (see Table 6D) ranged from about 18 (control block, no insect bags) to about 30 (all KAL blocks with insect bagging). At PRT, open-pollinated cone yields (no bagging) remained low for the control block (9 FSPC) but were increased to about 15 FSPC in the mist block. However, for cones treated with SMP in 2000 (Table 7) and insect-bagged in April 2001, cone yields were similar to KAL (29.6 FSPC). This suggests that seed losses due to *Leptoglossus* are substantial. Seed Losses due to *Leptoglossus* may still occur at KAL but not to the same extent (Table 6D). For comparative purposes, a list of yields by tree (clone) for each year and orchard is provided in Appendix B-2 and B-3.

Conclusions

Data from two years of observation suggest that good seed yields can be obtained from north Okanagan lodgepole pine seed orchards if weather conditions (temperature and humidity) are "soft". We may be able to ameliorate weather conditions during hot/dry pollination periods (not tested) and certainly we can improve overall cultural conditions during hot/dry periods when fertilization and embryo development occur. Two years data can only give us clues about the manner in which significant factors result in seed loss. We can conclude that improved irrigation systems are needed and a more rigorous detection and control system for *Leptoglossus* may still be required. Whether we actually need a crown cooling system needs to be tested further, especially under more harsh weather conditions.

We cannot predict future weather conditions but we can estimate the likely hood of "soft" and "harsh" conditions during sensitive stages of reproductive development. Meteorological data for May/June from 1996 to 2001 (Appendix I) in the north Okanagan suggest weather patterns (temperature) vary considerably. When cool weather occurs during May and June, normal seed cone development and seed yields are possible. When hot, dry weather occurs during May/June, poor cone development and low seed yields occur. These observations are only generalizations because irrigation regimes and insect control also varied during these years. However, field trials that used insect protection (bagging) clearly show that orchard's exposed to cool weather during May/June can yield good seed yields.

Even with our stepped up control of *Leptoglossus* we are still seeing seed losses. Is there a limit to which we can control *Leptoglossus*? We certainly can improve cultural treatments (irrigation) but are there environmental conditions (temperature and humidity) beyond which even soil moisture at field capacity is not enough? For the two years we have studied this problem, we have not seen either pollen supply or pollen uptake to be a problem. However, if the pollination season is very hot and dry, will we see poor pollen uptake? The latter question still needs to be determined. We do have some evidence that specific cultural treatments (mist) can improve seed yields (2000 KAL) when applied during fertilization and early embryo development (June/July). But we can only speculate on what specific stage of reproductive development we are affecting and for what duration we need to apply treatments.

Appendix B-1 Environmental effects on seed yields at Kalamalka Seed Orchard (307), 1996 to 2001.

A. 1996 to 1999 Meteorological Data for Vernon. Data for 1996-1999 are only estimates based on meteorological data from the Environment Canada Met Station, ColdStream.

				Yields (FSPC)	
Year	General	May	June	OP	Bagged
1996	cool	cool	cool	6.7	15.5
1997	moderate	moderate	moderate	9.7	20.1
1998	hot	hot	hot	2.9	10.6
1999	moderate	moderate	hot	10	

note: meteorological data for 1997-1999 from ColdStream Environment Canada Station.

B. Meteorological data for 2000 and 2001 collected from meteorological stations at Kalamalka Seed Orchard 307.

		Heat and Vapour Pressure Deficit Sums (where T>24C)							
		HeatSumAir (T>24)		VPDSum (T>24)		VPDsum>2		Yields (FSPC)	
Year	Treatment	May	June	May	June	May	June	OP	Bagged
2000	Control	83.6	2291.2	9.8	225.7	25.4	224.8	9.7	
	hours	4	85	4	85	11	84		
	Mist	NA	706.6	NA	61.5	NA	63.8	15.9	
	hours		28		28		28		
2001	Control	1638.3	1495.1	169.6	145.3	180.9	160.5	18.8	25.5
	hours	59	56	59	56	64	63		
	Mist	1011.7	494.1	99.1	37.2	111.1	25.4	24.3	25.5
	hours	37	20	37	20	42	12		

Appendix B-2

Comparison of cones collected from KAL sample trees in 2000 and 2001. 2000 cones were OP'ed in 1999 and not insect bagged in 2000. 2001 cones were either OP'ed or SMP'ed in 2000 and insect bagged in 2001.

			OP-99 noBAG-00		OP-00 BAG-01		SMP-00 BAG-01	
TRT	TREE	PST	TSPC	FSPC	TSPC	FSPC	TSPC	FSPC
Con	933	FF35	21.9	9.0	37.7	27.3	44.8	34.5
	933	PP36	22.1	4.0	25.8	9.8	26.2	16.3
	1504	LL36	10.1	5.3	21.0	10.7	29.7	16.7
	1510	MM35	14.3	6.6	37.2	30.2	40.5	31.2
	1512	FF36	26.1	8.9	37.5	23.0	43.8	35.8
	1512	PP35	19.8	10.3	35.0	23.2	42.5	34.8
	1513	LL35	22.5	6.3	43.3	37.5	37.8	34.2
	1530	HH35	24.9	15.1	44.3	35.8	54.5	43.5
	1531	II35	27.6	23.4	32.3	27.5	29.3	25.7
	1531	NN35	17.9	11.6	38.7	21.3	32.8	21.3
	1532	OO35	33.1	16.5	57.0	45.8	54.3	35.5
	1534	EE36	12.5	2.3	29.3	22.5	22.3	16.5
	1539	HH36	13.8	3.0	20.5	3.3	20.3	10.0
	7714	OO36	48.3	18.5	50.0	28.5	56.5	41.5
	9949	KK35	22.4	5.0	45.2	36.5	56.0	41.2
Irr/Mist	933	PP12	27.9	21.0	44.3	33.0	49.3	39.7
	1504	QQ14	19.5	11.0	21.2	12.8	24.8	16.7
	1510	QQ5	18.8	10.8	42.5	33.0	45.5	36.5
	1511	QQ13	31.0	19.3	44.5	35.7	45.7	34.8
	1513	PP13	16.9	6.4	41.8	34.0	39.0	31.2
	1520	PP4	13.5	7.5	20.2	14.7	25.5	18.2
	1523	QQ4	20.8	13.3	27.7	20.5	38.0	26.3
	1527	PP11	23.6	6.9	M	M	47.7	21.2
	1531	QQ12	23.5	18.5	29.2	25.8	33.0	27.8
	1533	PP7	22.5	16.5	30.0	21.8	35.0	24.2
	1536	PP10	6.4	2.6	M	M	32.2	22.5
	1538	QQ11	28.9	17.6	M	M	47.3	38.3
	1540	QQ15	31.0	21.4	39.5	29.7	47.2	34.3
	1540	QQ8	22.9	13.4	40.8	28.7	47.2	32.7
	7714	QQ9	42.9	9.5	52.8	30.8	59.0	41.0
Irr	937	KK8	11.6	0.1	20.0	1.2	24.0	1.0
	939	LL4	14.1	4.4	30.3	20.8	42.0	32.0
	946	LL15	18.1	10.8	30.7	21.5	32.5	22.7
	1502	KK11	19.9	16.1	M	M	34.8	28.8
	1504	KK10	21.9	11.4	M	M	24.0	15.0
	1511	LL12	20.5	11.1	44.0	29.3	43.6	30.2
	1519	LL6	26.4	14.5	33.7	26.7	44.8	39.3
	1526	KK5	18.5	6.4	47.3	23.7	51.3	30.8
	1526	LL14	29.1	10.5	41.8	13.7	43.7	25.8
	1529	LL13	36.4	20.5	61.2	41.5	63.8	52.8
	1529	LL7	25.3	11.5	55.3	31.5	70.5	49.8
	1532	LL8	34.0	17.0	47.0	29.0	48.3	36.3
	1538	KK16	33.0	18.1	49.5	34.2	50.0	35.2
	7708	KK4	24.0	14.4	44.0	41.2	43.3	39.8
	9977	KK13	17.1	11.8	31.8	17.7	37.7	29.7
Mist	946	FF12	19.9	15.3	32.3	19.7	M	M
	962	FF5	37.8	26.6	43.0	34.8	50.2	39.0
	1502	GG14	13.0	10.5	25.8	22.2	29.2	25.3
	1506	FF11	13.3	6.1	M	M	34.8	25.3
	1507	GG9	16.9	11.1	31.7	25.5	28.5	21.8
	1511	GG7	25.8	17.5	45.7	35.2	49.7	33.8
	1523	GG15	19.3	7.4	30.3	22.0	35.2	22.5
	1529	GG11	47.9	26.6	M	M	72.2	54.8
	1532	GG4	21.8	14.6	45.3	30.8	46.2	33.7
	1533	GG16	27.1	9.5	25.6	7.6	24.8	9.3
	1533	GG5	27.0	11.9	22.8	13.2	28.7	9.3
	1540	FF14	32.5	26.5	41.5	31.7	51.5	32.2
	7708	FF9	16.1	11.3	27.2	15.0	29.7	23.3
	9949	FF8	34.5	25.8	49.0	39.0	55.2	42.8
	9949	GG13	24.5	18.4	41.7	38.0	48.7	42.0

Appendix B-3

Comparison of cones collected from PRT sample trees in 2000 and 2001. Year 2000 cones were OP'ed in 1999 and not insect bagged in 2000. Year 2001 cones were either OP'ed or SMP'ed in 2000 and insect bagged in 2001.

			OP-99 noBAG-00		SMP-00 BAG-01	
TRT	TREE	PST	TSPC	FSPC	TSPC	FSPC
CON	1541	S3	7.9	5.4	21.3	16.2
	1542	S4	12.5	6.1	37.8	25.0
	1544	S2	26.6	6.9	39.3	31.5
	1544	T9	20.3	6.9	46.0	38.3
	1545	T14	5.9	2.3	18.3	7.7
	1547	S14	38.3	17.0	45.7	33.5
	1554	T6	32.5	9.1	45.7	34.5
	1557	S9	18.3	11.3	37.3	28.5
	1559	T2	19.6	3.5	41.7	37.7
	1561	S6	27.8	12.5	47.5	32.7
	1565	S5	17.3	5.9	41.5	26.2
	1568	S12	27.3	13.4	31.5	20.7
	1574	T12	17.4	9.1	42.5	30.5
	1575	S13	21.8	8.9	46.3	34.8
	1578	T11	13.9	2.6	53.7	46.5
IRR/Mist	1542	D12	21.4	5.8	35.0	25.3
	1542	E5	15.1	6.3	39.2	21.8
	1545	E9	3.8	1.5	17.8	9.5
	1546	E12	9.0	3.3	23.2	11.7
	1548	D6	14.6	1.1	29.0	18.0
	1551	E7	21.0	6.3	32.8	22.3
	1552	D13	9.3	4.3	22.3	20.0
	1554	D9	38.0	4.1	37.5	24.5
	1558	D8	12.6	10.4	33.5	28.0
	1562	D5	13.5	5.3	28.5	20.0
	1563	E2	12.3	4.8	24.2	17.0
	1567	D10	20.5	8.0	30.7	24.3
	1571	D14	18.8	8.1	34.7	28.3
	1573	E4	19.3	6.3	32.2	27.0
	1580	E3	12.1	2.0	34.3	25.0
IRR	1545	I9	4.3	1.5	12.0	6.0
	1545	J3	13.4	6.9	6.8	2.3
	1547	J13	31.1	13.9	38.3	25.7
	1548	J7	10.6	0.4	M	M
	1551	J2	26.6	6.9	28.0	19.2
	1552	I12	13.0	7.8	23.7	18.3
	1553	J10	8.1	4.0	32.3	23.8
	1554	J6	21.3	7.5	29.2	19.7
	1556	I5	20.1	8.4	33.2	25.3
	1567	I10	14.4	6.4	34.3	26.3
	1567	J5	5.3	1.5	25.3	19.0
	1569	J9	12.9	5.0	32.5	28.3
	1574	I4	16.3	4.0	36.8	27.3
	1577	I14	28.1	14.9	42.7	36.8
	1580	I13	18.4	6.5	30.3	26.3
MIST	1542	N3	17.9	6.0	42.8	33.2
	1548	N13	14.5	1.1	31.4	14.8
	1549	N14	7.4	2.8	31.0	23.8
	1551	N2	25.3	10.3	32.5	25.7
	1552	O3	5.9	2.6	25.8	16.8
	1553	O9	21.1	13.4	30.0	25.5
	1557	O2	21.5	12.4	38.5	29.8
	1557	O8	17.4	7.0	38.7	29.0
	1558	N5	13.0	6.5	33.3	31.0
	1563	O7	28.4	12.6	34.2	28.0
	1566	N12	9.0	2.3	20.5	12.7
	1567	O10	14.9	8.6	24.5	19.3
	1571	N7	18.1	11.6	30.0	23.0
	1576	N10	23.9	10.3	48.5	39.3
	1576	O5	34.4	4.6	52.7	45.2

Appendix B-4 Fall 2001 Foliar Analyses - Kalamalka PI Seed Orchard 307.

A. Foliar Analyses

Treatment	Plot	B	Ca	Cu	Fe	K	Mg	Mn	N	P	S	SO4	Zn	n
Adequate*		**15 ppm**	**0.10%**	**3 ppm**	**30 ppm**	**0.40%**	**0.08%**	**25 ppm**	**1.35%**	**0.12%**	**0.10%**	**80 ppm**	**15 ppm**	
Control		24.3	0.22	2.50	47.30	0.42	0.15	486.70	1.44	0.13	0.10	167.50	40.90	15
Mist / Irr	1	26.3	0.32	2.20	48.00	0.43	0.14	360.20	1.58	0.14	0.10	157.20	41.80	12
	2	39.2	0.55	2.20	50.40	0.42	0.16	425.30	1.84	0.19	0.13	246.30	47.00	12
	3	43.6	0.66	2.80	54.10	0.50	0.17	628.40	1.84	0.21	0.16	478.10	49.20	12
Irrigation	1	27.1	0.24	2.00	59.10	0.41	0.14	345.70	1.56	0.14	0.10	130.00	35.50	12
	2	34.9	0.27	2.00	45.90	0.47	0.14	409.30	1.73	0.16	0.12	207.40	32.80	12
	3	39.5	0.26	3.00	47.30	0.49	0.16	630.10	1.83	0.16	0.13	321.90	47.00	12
Mist	1	21.1	0.25	2.10	62.60	0.46	0.14	516.70	1.58	0.13	0.11	206.60	32.30	12
	2	25.2	0.55	3.20	68.00	0.49	0.17	540.90	1.94	0.20	0.14	371.50	41.10	12
	3	35.6	0.63	3.40	61.70	0.56	0.22	716.90	1.81	0.22	0.21	1076.40	53.30	12

*Adequate levels taken from Brockley (2001):
Foliar Sampling quidelines and nutrient interpretative criteria for lodgepole pine
MoF Research Program Extension Note 52 (8pp).

B. N Ratios

Treatment	Plot	N:P	N:K	N:Mg	N:S
Adequate*		**9**	**2.5**	**15**	**14**
Control		11.08	3.43	9.60	14.40
Mist / Irr	1	11.29	3.67	11.29	15.80
	2	9.68	4.38	11.50	14.15
	3	8.76	3.68	10.82	11.50
Irrigation	1	11.14	3.80	11.14	15.60
	2	10.81	3.68	12.36	14.42
	3	11.44	3.73	11.44	14.08
Mist	1	12.15	3.43	11.29	14.36
	2	9.70	3.96	11.41	13.86
	3	8.23	3.23	8.23	8.62

*Adequate levels taken from Brockley (2001):
Foliar Sampling quidelines and nutrient interpretative criteria for lodgepole pine
MoF Research Program Extension Note 52 (8pp).

Appendix B-5 Fall 2001 Foliar Analyses - PRT Pl Seed Orchard 308

A. Foliar analyses

Treatment	B	Ca	Cu	Fe	K	Mg	Mn	N	P	S	SO4	Zn	n
Adequate*	**15 ppm**	**0.10%**	**3 ppm**	**30 ppm**	**0.40%**	**0.08%**	**25 ppm**	**1.35%**	**0.12%**	**0.10%**	**80 ppm**	**15 ppm**	
Control	23.3	0.26	3.30	48.20	0.43	0.15	152.0	1.55	0.14	0.11	185.5	36.40	15
Mist / Irr	30.9	0.84	3.20	64.00	0.42	0.20	181.9	1.79	0.15	0.13	344.9	49.10	15
Irrigation	28.9	0.34	3.20	50.30	0.42	0.17	174.3	2.05	0.15	0.12	323.9	44.90	15
Mist	32.2	0.82	3.30	64.80	0.43	0.20	171.4	2.02	0.16	0.13	326.6	46.90	15

*Adequate levels taken from Brockley (2001):
Foliar Sampling quidelines and nutrient interpretative criteria for lodgepole pine
MoF Research Program Extension Note 52 (8pp).

B. N Ratios

Treatment	N:P	N:K	N:Mg	N:S
Adequate*	**9**	**2.5**	**15**	**14**
Control	11.07	3.60	10.33	14.09
Mist / Irr	11.93	4.26	8.95	13.77
Irrigation	13.67	4.88	12.06	17.08
Mist	12.63	4.70	10.10	15.54

*Adequate levels taken from Brockley (2001):
Foliar Sampling quidelines and nutrient interpretative criteria for lodgepole pine
MoF Research Program Extension Note 52 (8pp).

Appendix C Research Report by Owens *et al*.

Summary of results available on the study of "Causes of low seed production and methods of increasing seed and cone production in lodgepole pine seed orchards.

Author: John N. Owens

Abstract

The following summary covers the studies carried out by my lab on Pli reproduction that started in 1980, resumed in 1987-1998 and again in 1999-2000. This has been followed by a formal OTIP study carried out at four seed orchards in 2000-2001 and at two orchards in 2001-2002. Final data will be collected in the spring and fall of 2002 for the experiments done in the spring 2001.

Basically, the problem was the low seed set per cone at KSO compared to PGSO. In a nutshell, our findings indicate that very early-on the trees were severely topped resulting in a complete intermingling of pollen cones and seed cone, which in turn resulted in very high levels of selfing. Our experiments and developmental studies show clearly that selfing in Pli, as in other pines, results in seed abortion at the early embryo stage soon after fertilization, about late-June to early-July in Pli. By the time of fertilization the seed coat and female gametophyte are fully developed. Embryo and female gametophyte abortion at that time results in an empty seed with a small shriveled female gametophyte and no embryo. In the recent years that my lab has done studies at KSO and PGSO, the filled seed/cone at KSO has increased from 8 in 1997, 12 in 1999, 15 in 2000 to 28 in 2001 and empty seed has decreased from 23 to 12 to 9 to 11 in the same four years, respectively. Filled seed at PGSO has remained in the low 20's and empty seed 6-8/cone.

So, while we have been busy studying many aspects of Pli reproduction, the KSO trees have out-grown their problem. Leader shoots are now 1-2 m long and female cones on these are separated from most male cones. These studies have provided a lot of useful information and solutions beyond the main selfing problem. These include the cause for cone drop, a problem at PGSO; misting or sprinkling to aid pollination and reduce protandry in very hot dry springs: methods of increasing the mix of pollen in all orchards, etc.

I predict that with proper mixing of pollen in the orchard (physically blowing it around every day during pollination with tractor-mounted blowers), sprinkling to aid pollination and reduce protandry, plus SMP of early and late clones and insect control that a goal of 30 filled seed/cone and a cone retention of 80% is possible at all orchards. But, read on, maybe you don't agree.

Introduction

The current Pli planting requirements from the existing nine seed orchards are 72 million seedlings per year. With a sowing factor of 1.3, there is a present need for 94 million seeds/year. The current supply of Class A seed is much lower than this. In order to meet the needs for 2007, it has been estimated that 55,000 additional ramets are required. Seed set varies among seed orchards. At Prince George 20 seeds/cone or more are commonly obtained, whereas at KSO before 2000, the average was commonly less than 10 seeds/cone.

The purpose of the 2000-2001 project was to determine why seed production per cone was high at PGSO (about 40% of potential) and low at KSO (about 10% of potential) and from this information develop seed orchard management practices for KSO that would increase the seed/cone thus reducing the need for as many new ramets as was predicted at that time. New ramets will have to be established but the question remains concerning where these should be established. If the seed/cone problem at KSO can be overcome using orchard management techniques, then the ramets could be most easily established at this site. However, if the problem cannot be easily, consistently and economically solved at KSO, then the additional orchards should be established elsewhere. The deadline for making these decisions is in the winter of 2001-2002. This is a difficult timeframe to meet because Pli, like all pines, requires about 15 months from pollination until cone and seed maturity.

In order to get as much information as possible in the shortest time possible, three main studies were designed to look at seed/cone and cone/tree production at the PGSO where high seed/cone but low cone/tree are commonly obtained, at KSO where low seed/cone but high cone/tree are generally obtained and an intermediate location at PRT Armstrong where seed/cone appeared to be intermediate. Wherever possible, the same clones were compared using the same trials at each site. The advantage of our developmental approach is that we evaluate our results at several stages:

- immediately after pollination to determine pollination success as measured by the amount of pollen entering the cone (pollen-on)
- soon after pollination, after pollen has been taken into the ovule (pollen-in)
- in mid- summer to determine cone survival
- the following spring to determine cone survival after winter dormancy
- in June, one year after pollination, to determine fertilization success
- at cone maturity to determine cone survival and seed set as a portion of seed potential.

This type of study was designed because several studies carried out by my lab over many years have identified the most common cause of poor seed set to be low pollination success. This is especially true for conifers having a pollination drop mechanism--one of several pollination mechanisms.

Our first hypothesis was that at KSO, where it is commonly warmer and much dryer than most Pli natural stands, the pollination drop mechanism does not function efficiently resulting in fewer ovules being pollinated and fewer pollen per ovule, resulting in higher cone drop during the first year and lower seed set in the second year.

Our second hypothesis was that low filled seed at KSO also results from the high level of self-pollination in the topped trees. Topping results in extreme intermingling of pollen cones and seed cones on the same branches, much more than in untopped Pli trees at PGSO. A high level of selfing may not cause cone abortion or seed abortion in the year of pollination but selfing results in ovule and/or seed abortion early in the second year (about June when fertilization occurs) causing high numbers of normal appearing (large, rounded with a well developed seed coat) but empty seed.

A study was set up using the same two clones at PGSO and at KSO. Cones on ramets of each clone were cross-pollinated or self-pollinated at the same stages and in the same manner and other cones were left for open pollination at both orchards. Cones were sampled at several stages following pollination until dormancy and again at fertilization and during seed development to determine the effect of selfing on ovule, embryo, seed and cone development. Specimens have been studied anatomically to determine the effect of selfing on pollen germination within the ovule, pollen-tube development, fertilization and embryo and seed development.

Below is a brief summary of our results to date followed by a plan to complete the third year observations in 2002-2003. That study will evaluate a 2001 operational trial on misting and vigorously blowing pollen around at KSO and blowing pollen around at PGSO in order to see if we can increase further the seed set at KSO and cone retention and seed set at PGSO by this simple and relatively inexpensive method.

Results from earlier background studies

Early studies of Pli reproduction at the PGSO and in coastal stands on Vancouver Island described the reproductive cycle, the pollination mechanism and embryo and seed development (Owens et al 1981, 1982). From those studies it was recognized that:

- although Pli cones at both sites had many scales only about 25 were fertile (bore fertile ovules) making the maximum seed potential per cone only about 50
- the reproductive cycle is typical of most pines with pollination occurring in May or June, fertilization about one year later and cone and seed maturity occurring about 15 months after pollination (Fig. 1)
- the pollination mechanism was typical of other pines studied, having a pollination drop mechanism, inverted ovules and winged pollen
- fertilization, embryo and seed development were similar to other Pinaceae
- pollination studies showed that the first pollen grains to arrive at the cone were preferentially taken into the ovules but the amount of pollen taken into each ovule was low (about 1.2 grains per ovule).

Studies carried out a KSO in 1997 and 1998 (unpublished) showed that in a dry spring there was extreme protandry (pollen shed before female receptivity), whereas in a wet spring protandry was less or did not occur. This results because pollen cone and seed cone development are strongly affected by temperature but pollen release is affected by both temperature and humidity--rainy spring weather delays pollen release and reduces protandry. KSO records show that the degree of protandry varies considerably from year to year, apparently depending on rainfall and humidity. Results indicated that low pollination success resulted in high early cone abortion and low seed set. Studies by Ministry staff also indicated the *Leptoglossus* was a factor in reducing filled seed production.

In a study carried out under contract with the B.C. Ministry of Forests in the summer 1999, two seed orchards were primarily investigated: one at PGSO that has a history of high filled seed per cone and one at KSO that generally has low filled seed per cone. Some observations were also made using trees in clone banks at KSO, PGSO and at Skimikan (SKIM). Several aspects were studied including post-pollination cone abortion, fertilization success (FS), total seed per cone by seed category, seed survival by developmental stage, seed efficiency (SEF), and, the relationship of cone weight to seed development.

From this study we found that early cone abortion was much higher at PGSO than at KSO or SKIM, fertilization success was higher at PGSO than at KSO with SKIM intermediate, total seed per cone was higher (>30) at PGSO than at KSO (~20) and by seed category PGSO had a much higher percent filled seed (>70%) than at KSO (~30%). At all of the sites, insect damage accounted for less than 10% of seed loss. Again, SKIM was intermediate between KSO and PGSO in these respects. Seed potential per cone was similar at all sites but pollination success, fertilization success and filled seed were higher at PGSO than at KSO. Seed efficiency was much higher at PGSO (56%) than at KSO (21%) and SKIM was intermediate (35%). Cone weight was somewhat higher at PGSO than at KSO as was filled seed. Cone weight and average number of cones scales were higher and closely related at PGSO but not at KSO.

The problem and the questions to be answered

The basic problem is that in order to meet the demand for Class A seed by the year 2007, about 60,000 new Pli ramets must be established in the interior if seed production can be increased in interior seed orchards. The first question was, if new orchard must be established, should they be established at KSO? The second question was, is there a problem with seed production at KSO that does not exist elsewhere? A third question was, can the problems with low seed production in Pli at KSO be solved using seed orchard management specifically designed for Pli at that site?

Our results from earlier studies, the 1999 evidence and the years of experience by the seed orchard staff at interior Pli orchards indicates that there is a major problem with Pli seed production at KSO. Seed production per cone is commonly about 20% of that at PGSO and about 50% in the best years. Our evidence indicated that the problems centered on the pollination period. In dry years there is extreme protandry. In 1997, about 80% of the pollen had been shed before most ramets became receptive. Even in years when there is less protandry and abundant pollen enters the cone, many pollinated ovules abort at the time of fertilization, suggesting that a high level of self-pollination has occurred. Trees at KSO were severely topped several years ago and pollen cones and seed cones occurred on the same branches in the "lower" remaining portion of the crown. This would increase selfing. High levels of selfing significantly reduce seed set but this may not be evident in any genetic analysis of mature seed because the vast majority of selfed seed abort just after fertilization and would be empty and not included in mature seed analyses.

Proposal for 2000-2001

Our proposal had five main objectives:

- Comparing the pollination process using the same clones at KSO, PGSO and at PRT Armstrong.
- Determining the precise times and causes of seed and cone loss at the three orchards using control and open pollinations.
- Determining the importance and the time and method by which seeds are lost as a result of self-pollination.
- Determining the phenology, size, time of production and proportion of ovules producing a pollination drop at the dry KSO site and the wet PGSO site.
- Setting up a preliminary misting trial to determine the effect of misting on pollination drop production and pollination success at KSO.

Results to date from 2000-2001 study

This work was ongoing and could not be completed until the fall of 2001 when the cones were mature. However, because our approach involved observations at many stages, some results were available in the spring 2001 and were used to guide our studies for 2001-2002. Briefly, the status at that time of each of these studies and the important results are given as follows:

The phenology at pollination varied among the different sites but the pollination mechanism was similar at all sites. The pollination mechanism at KSO was as described in earlier studies at PGSO and coastal B.C. (Owens et al. 1981, 1982). It involves a pollination-drop mechanism in which there are inverted ovules, saccate pollen, sticky micropylar arms that collect pollen and a pollination drop that scavenges pollen from the arms and, if large, from other cone surfaces. Protandry did not occur at any of the sites during the relatively cool spring of 2001. Pollination success was highest at Stage 4 and with multiple pollinations and these were similar to open pollination. Pollination success was highest at PRT and lower but similar at KSO and PGSO. Pollination success measured as pollen taken into the ovules was about 80% and cones having less than this amount aborted during the first year. This is a surprisingly high pollination success requirement for cone retention, much higher than the 20% stated for Scots pine in Europe (Sarvas 1978).

Cone survival was measured about 2 weeks after pollination. Cones abort and drop if few ovules are successfully pollinated. Cone survival was high (~80%) at KSO and PRT but lower at PGSO (~50% in open pollinated trees). See Figs. 13-15.

The time and causes of seed losses required anatomical study and observations during the first (2000) and second (2001) years. The time of highest seed abortion is soon after fertilization and appears to result from self-pollination. This means any live Pli pollen in the ovule will stimulate ovule development for one year but if the pollen is only self-pollen, the proembryos or early embryos abort and if all embryos abort the female gametophyte aborts forming an empty sac within a well-developed seed coat. This is what we have called an empty seed. Empty seeds, indicating pollination but no embryo development, occurred in a similar manner at KSO and PGSO but was much higher at KSO. Flat (early aborted) and insect damaged seed were few in both years at both orchards. Even in a good year at KSO (2000) filled seed was significantly lower than at PGSO and the difference was attributed to empty seeds caused by failure at fertilization or during early embryo development. See details in Appendix B-1.

The effect of self-pollinations in 2000 on ovule, embryo and seed development was continued by comparing anatomically, at the light and transmission electron microscope levels, ovules from self-pollinated, cross-pollinated and open pollinated cones from the same clones at KSO and PGSO. The results support the hypothesis that most of the empty seeds produced in the past years at KSO were the result of self-pollination. These results are briefly tabulated and discussed in Appendix B-2. A manuscript is near completion and will be submitted to a scientific journal

Cones were sampled, dissected and ovules observed from Stage 3 through Stages 5 or 6 at the dry KSO and the wet PGSO sites to determine the phenology, size and time of pollination drop secretion. Pollination drops were infrequent, small and found in very few ovules at KSO in 2000 and 2001unless it rained. In contrast they were frequent, large and found in a high proportion of ovules every day at Stages 4 - 6 at PGSO where it rained almost every morning and the humidity was usually high for most of the day.

A preliminary misting trial using PVC pipe and two misting heads per tree was done at KSO in 2000. Trees were misted for 1hour every morning from 6:00 to 7:00 am and cones were sampled from misted and unmisted trees before and after misting. Cones were dissected and the proportion of ovules with pollination drops or pollen taken in was counted. These preliminary results indicate that misting greatly increased the percentage of ovule having a pollination drop over cones on unmisted trees. At KSO in misted cones and at PGSO pollination drops were most frequent at Stages 4 and 5. Ground irrigation did not increase pollination drop frequency at KSO. Weather data for the pollination period in 2000 was has been obtained from the Coldstream Station near KSO and from on site at PGSO. In general the highest RH at KSO about equals the lowest RH at PGSO during pollination.

Proposal for 2001-2002

Because pines have a two-year reproductive cycle, the results from the 2000 pollinations could not be fully known until the fall of 2001. Therefore, the main objectives for 2001-2002 were to complete the collections and studies begun in 2000. These objectives included:

- Determining the effects of pollination at different times and the causes of cone and seed loss at different times using the same clones at KSO, PGSO and PRT Armstrong. Cone counts were made in April to determine cone loss over winter and in the fall to determine final cone survival and seed set by seed category
- Determining the importance and the time of cone and seed loss as a result of self-, cross- and open-pollination using the same clones at KSO and PGSO.
- Setting up an operational misting trial at KSO to increase the amount of water in the air at KSO to about the level at PGSO and determine the effects of this treatment on pollination drop formation, pollination success, cone survival and seed set.

Materials and methods and results for 2001-2002

Details of the results to date are given in Appendices B1- B3.

Cones from all of the pollination treatments made in 2000 were counted in the fall of 2000 and spring and fall of 2001. See Appendix 3, Figs. 13-15.

All remaining treated cones were collected at cone maturity in the fall 2001 to determine final cone survival, seed potential, seed efficiency and proportion of all categories of seed produced (filled, rudimentary, aborted and insect damaged). These results are summarized and briefly discussed in Appendix B-1.

These data will be combined with those taken in 2000 concerning pollination success (pollen-on and pollen-in) and cone survival to determine the times and causes of seed and cone loss for the two-year reproductive cycle.

In the spring and summer of 2001 cones that were self-, cross- and open- pollinated on the same clones at KSO and PGSO were sampled in April, after winter dormancy, in June at fertilization and during embryo development to determine the sequence of embryo development and the times of embryo, megagametophyte or seed abortion. Cones were dissected and ovules further dissected and fixed for light and electron microscopy as in 2000. Developing ovules and seeds were studied to determine the causes of ovule and embryo abnormalities and abortions. All remaining cones were collected at maturity and the final cone survival, seed potential, seed efficiency and proportion of all categories of seed produced is being determined. These results are summarized and discussed in Appendix B-2.

With the cooperation of the KSO staff, a misting trial was set up in a section of the orchard. Four adjacent rows consisting of 10 trees per row were selected and an inexpensive PVC misting system was placed in each tree in the four rows. Four misting heads were placed in the four quadrants in the top of each tree to insure that the entire crown was misted. Trials in 2000 demonstrated that, because of wind direction, one or two misting heads per tree were not enough. Misting was done in the morning from 0700 to 1100, before pollen began to be shed, in order to increase the water in the air to about the amount determined to be present at PGSO. A similar four rows (40 trees) served as controls and received no misting. The temperature and humidity were recorded at two locations in the misted and two in the unmisted areas then later downloaded onto a computer.

Cones were sampled during the pollination period to determine the frequency, size and duration of pollination drop formation before and after misting and the effect of misting on temperature, humidity and pollination success. Results from 2000 indicated that misting, although inadequate that year, increased the number of pollination drops observed per cone (Fig.10).

Trees in both plots were left for open pollination. During the pollination period, cones were sampled daily to determine the pollination success, pollen-on the micropylar arms. After the pollination period cones were sampled from trees in both plots to determine the pollination success--pollen-in the ovules. Cone survival was determined soon after pollination was completed and in the fall 2001 (Figs. 11, 13). The 2000 results indicated the first period of cone drop occurred very soon after pollination.

In 2002 cone survival will be measured after winter dormancy and at cone maturity and samples of mature cones will be dissected and analyzed for seed potential, seed efficiency and proportion of filled, empty, rudimentary, insect damaged and empty seeds.

Proposal for 2002-2003

No new studies are proposed for 2002-2003 but results from the 2001 pollination and misting treatments at KSO and pollen blowing treatments at PGSO will be determined. These include:

- At KSO and PGSO cone survival will be counted on flagged branches in the spring 2002, after pollination, to determine winter survival and in the fall to determine survival at cone maturity. At KSO survival will be compared on misted and unmisted trees and at both orchards on trees for which pollen was vigorously blown around using a tractor blower.
- In the fall of 2002, cones will be collected from the flagged branches of trees receiving the above treatments, dried, weighed and measured then all seed extracted and analyzed for full, empty, rudimentary and insect damaged seed.
- All data from the 3-years of the study will be tabulated and analyzed statistically and a final report and one paper will be written and submitted for publication. The manuscript will cover the effect of pollination time, misting and blowing of pollen on cone and seed production.

Appendix C-1 Filled seed production per cone from four seed orchards in 2001 and for two seed orchards from 1997-2001.

The filled seed production per cone at KSO has increased and empty seed generally decreased since 1997, the years for which I have data collected in the same manner by my lab. (Table 2, below). In 1997 there was an average of 8 filled and 23 empty seed per cone. If one looks at the tree form at that time, they had recently been topped and formed a rounded shrub 1-2 m tall with few seed cones and any seed cones present were intermingled with pollen cones. Over 3-4 years leader shoots have been re-established and a tree-form is again present. There is now 1-2 m of leader shoots that bear abundant seed cones but few pollen cones. These are more likely to be cross-pollinated than seed cones in the lower crown. Our data from 2000 shows that filled seed increased in cones from the lower to the upper regions of the crown.

These data indicate that over the past 5 years the trees at KSO have slowly grown out of their problem and if not drastically topped again will continue to produce 25+ seed per cone. If pollen is vigorously blown around using the tractor blower, I predict that 35+ seed per cone will be produced at KSO with no other treatments except sprinkling to reduce protandry and enhance pollen uptake but this I now considered a secondary rather than the primary cause.

Filled seed at the four orchards (Table 1, below) varies with KSO highest followed by PRT, PGSO and VSOC. VSOC is a very young orchard and seed/cone will increase as pollen cone production increases and if they continue to blow the limited amount of pollen around. PRT could also benefit from blowing pollen around as at the other orchards but it also has greater insect problems shown in mature cones. PGSO appears to have a pollen supply problem from within the orchard. I predict, and the 2002 data will determine, that PGSO will benefit from vigorously blowing pollen around. There, pollen is limiting and although blowing pollen around may not drastically affect seed/cone, it could increase cone survival that is now only about 60% compared to over 80% at KSO. The initial cone drop soon after pollination results from too few ovules being pollinated per cone, less than 80% whether selfed or crossed. Simply stirring-up the pollen will increase the number of ovules pollinated per cone thus increasing cone survival and total seed production.

It should be kept in mind that seed production per ramet involves more than just cones initiated and pollinated and seed per cone but is also the proportion of cones that survive in pine. In many other conifers (Douglas-fir and hemlock), cone survival is less dependent on the number of ovules that are pollinated than in pine. But in others (spruce and cedar), cone survival or at least cone vigor and final size are very dependent on the proportion of ovules pollinated.

Table 1 **The average number of filled, empty, flat and insect damaged seed in mature lodgepole pine cones analyzed by seed extraction and the cutting test for cones pollinated in 2000 and maturing in 2001 for four seed orchards.**

Orchard	Filled Seed	Empty Seed	Flat Seed	Insect-damaged Seed
KSO	28.3	10.8	0.8	0.2
PRT	24.1	10.0	1.3	0.1
VSOC	17.6	4.7	3.0	0.3
PGSO	21.7	6.3	0.2	0.3

Table 2 **The average number of filled, empty, flat and insect damaged seed/cone in mature lodgepole pine cones over several years at KSO and PGSO.**

Orchard	Year	Filled Seed	Empty Seed	Flat Seed	Insect-damaged Seed
KSO	1997	8	23	1.0	2.0
KSO	1999	12	12	1.0	3.0
KSO	1999	12	12	1.0	3.0
PGSO	1999	25	8	0.2	0.5
KSO	2000	15	9	0.5	1.0
PGSO	2000	27	6	0.2	0.0
KSO	2001	28	11	0.8	0.2
PGSO	2001	22	6	0.2	0.3

Appendix C-2 Results from the Spring 2000 self-, cross- and open pollination experiments at KSO and PGSO and the subsequent anatomical study and cone analysis.

In the spring of 2000, six to 10 branches bearing a total of about 30 cones were bagged on two ramets of the same two clones, 1538 and 1536, at KSO and at PGSO. Half of the bagged cones on each ramet were self-pollinated and half were cross-pollinated with the same polymix of pollen containing no pollen from either clone. A similar number of cones on each ramet were flagged and left for open pollination (control). After the pollination period, the isolation bags were removed and replaced with mesh insect bags. Seed cones receiving all three treatments (open, self, cross) were sampled weekly for 8wks following pollination by which time cones were dormant, then twice per week he following spring (2001), during the fertilization period as determined by anatomical studies done in 2000. All cones were dissected and isolated scales or ovules were fixed, sectioned and stained for light and transmission electron microscopy. Observations were made of pollen germination, pollen tube development, ovule development, gamete formation, fertilization and early- and mid-embryo development. The remaining unsampled cones were collected at maturity and seed analysis was done using a cutting test.

Briefly, the results, as shown in Tables 1 and 2 (below), indicate that in both clones at both orchards, selfed cones produced only 5-16% filled seeds and about 50% empty seeds with 34-42% aborting due to a lack of pollination or other pre-zygotic factors (inhabition, abortion or abnormal pollen tube growth or failure to release sperm from pollen tubes). In both clones at both orchards, cross-pollinated cones produced 36-60% filled seeds, 1-15%empty seeds with 32-50% of seeds aborting due to lack of pollination or other pre-zygotic factors. In both clones at both orchards, wind-pollinated cones produced 37-60% filled seeds, 19-30% empty seeds with 17-44% of seeds aborting due to lack of pollination or other pre-zygotic factors. The anatomical studies show that there was no difference in development in pollinated ovules, whether self-, cross- or open-pollinated, until the early to mid-embryo stages about one year after pollination (late-June to early-July) at which time the embryos aborted and the megagametophyte aborted and shriveled leaving a rounded normal-looking seed but containing only the degenerated megagametophyte. These are the empty seeds in our cone analyses.

These results support those by other researchers working on several species of conifers and indicate that selfing results in a high proportion of empty seed as we have seen in past years at KSO. Self pollen does not adversely affect cone or ovule development in the first year but only manifests itself during embryo development in June or July of the second year. This late-acting form of self incompatibility, called self-inviability is found in all conifers studied in a similar manner thus far and in many hardwood species (teak, *Acacia, Pterocarpus* and many fruit trees). It is a primitive form of self-incompatibility not usually found in herbaceous species. I believe our results at KSO and PGSO indicate that very high levels of selfing at KSO in past years, due to the extreme topping, has resulted in the low filled seed at that orchard and that the problem has largely solved itself as the trees have grown out of this condition.

Table 1 **Average percentages of seed that is filled, empty, rudimentary (aborted) due to lack of pollination or flattened (aborted) due to pre-zygotic abortion for mature lodgepole pine cones from two sites**

Clone	Pollination Treatment	Site	Average percentage of each seed type per cone			
			Filled Seed	Empty Seed	Ovule Abortion (due to lack of pollination)	Pre-zygotic Abortion
1538	Self	Kalamalka	16.4	49.7	33.9	0
		Prince George	5.3	40.6	52	2.1
	Cross	Kalamalka	60.2	7.3	32.1	0.4
		Prince George	36.0	6.5	57	0.5
	Wind	Kalamalka	47.3	29.3	23	0.4
		Prince George	37.3	18.6	44.1	0
1536	Self	Kalamalka	4.8	53.4	41.8	0
		Prince George[1]	-	-	-	-
	Cross	Kalamalka	48.6	1.2	50.2	0
		Prince George	42.8	14.6	41.5	1.1
	Wind	Kalamalka	53.9	29.1	17.0	0
		Prince George	60.0	20.0	18.0	2.0

1 There is no data for this site as all treated cones were collected for embedment or aborted during the overwintering period.

Table 2 **Times of pollination treatment, fertilization and abortion events in lodgepole pine cones at two field sites in 2000 and 2001.**

Field Site	Time Of Pollination	Abortion Following Lack Of Pollination	Time Of Fertilization	Abortion During Embryo Development
Kalamalka	Early May 2000	June 19-20, 2000	May 23-24, 2001	June 16-17, 2001
Prince George	Early June 2000	July 5-6, 2000	June 15-16, 2001	July 10-12, 2001

Appendix C-3 Lodgepole Pine Preliminary Summary Report For Data Collected In 2001.

The following summary and graphs are the preliminary results for the data collected during the 2001 field season. Most of these data are the results from the 2001 experiments carried out at KSO and PGSO. Results from the 2000 experiments were included in the 2001-2002 proposal and 2000-2001 report. Additional results will be provided from the cone analyses that have just been completed but the statistics have not been done. The cone analyses results are for the cones resulting from Stages 3, 4, 5 and multiple pollinations at all three stages and open pollinated cones where no misting or blowing of pollen was done. It is emphasized that these results are tentative because the data have not been analyzed statistically.

Some of the most important points are briefly outlined below. More detailed comments are on the attached pages with the graphics.

As in 2000, protandry was not a problem at KSO, PGSO and PRT in 2001. The amount of pollen available at KSO was again higher at KSO than at PGSO but the difference was less than in 2000 (Figs. 1-3).

Pollen per ovule (pollen-on) and successfully pollinated ovules (pollen-in) were counted on samples of misted and unmisted cones at KSO and on the unmisted cones at PGSO at various stages of cone receptivity. Misting appears to have had an adverse effect on pollination at Stage 3 but by Stage 5 there was 6 to 8 pollen-on each ovule in misted and non-misted cones, respectively (Figs. 4-6). Despite this difference, there was no adverse effect on pollen taken into the ovule (pollen-in) by the time cones ceased to be receptive at Stage 6 at KSO (Figs. 7, 8). Unfortunately for the experiment, it was a fairly wet year at KSO and the misting treatment had little effect. This experiment should be tried again in a very dry spring to see if misting will have a beneficial effect on pollen taken in to the ovules. The lack of any difference between KSO and PGSO in pollen-on (Fig. 6) is likely due to the fact that there was little difference in the amount of pollen available at the two orchards in 2001 (Figs. 1-3). The high level of ovules with pollen taken in (~80%) is likely due to the fact that Pli only takes in 1-4 (average 1.5) pollen per ovule so 6-8 on the arms is quite adequate if a pollination drop forms. Misting increased the frequency of pollination drops in cones at KSO to equal or more than that observed at PGSO (Fig. 10).

Cone survival measured about two weeks after pollination, the time when insufficiently pollinated cones first year cones abort, shows no difference between misted and unmisted cones at KSO (Fig. 11). This is what would be expected because about 80% of the ovules in misted and unmisted cones had been successfully pollinated. At PGSO cone survival was low in 2000 when pollen abundance was lower but high in 2001 (Fig.11) when pollen abundance was almost equal to that at KSO (Fig. 1, 2). There is little difference in cone survival in the lower and upper crown at KSO and PGSO (Fig. 12). Again, this is a reflection of the similar abundance of pollen at both orchards in 2001. Cones are retained if ~80% of ovules are pollinated, whether it is self- or cross-pollen.

Cone survival was not a problem in open pollinations in 2000 and in 2001 at KSO. It appears that if cones are adequately pollinated (self or cross) most will survive the first year and there is little cone loss in the second year (Fig.13). However, at PGSO in 2000 but to a lesser extent in 2001 (Fig. 14) there was a large cone drop or loss following pollination and this was thought last year to be due too few ovules per cone being pollinated. However, the 2001 data indicate this may not be the case since the number of ovules pollinated was high. Other possibilities are the frequent night and morning frosts observed during pollination at (PGSO). Low temperature may also contribute to the higher second year cone loss in PGSO compared to KSO. Cone loss is a significant problem at PGSO but not at KSO or PRT (Fig. 15)

Temperature and humidity at pollination could be a potential problem at KSO but that did not appear to be the case in the two years of this study when temperatures were moderate and rains occurred. Even in cool wet years at KSO, as in 2001, misting had a beneficial effect in increasing the humidity during part of the day to levels similar to those measured at PGSO (Figs. 16-18). In a hot dry spring this could have a very beneficial effect on longevity of the pollination mechanism by delaying degeneration of the micropylar, thus enhancing pollination success. However, in cool wet springs there would be little or no benefit. Misting in the morning at KSO increased the humidity in the morning to mid-day hours (0700-1400) to levels similar or higher than at PGSO but the effect ended within about 2 hours of stopping the misting. Temperatures and humidity were similar at the three Okanagan orchards at pollination in 2001 (Fig.20). Misting had little effect on temperature during the pollination period in 2001 (Figs. 21, 22).

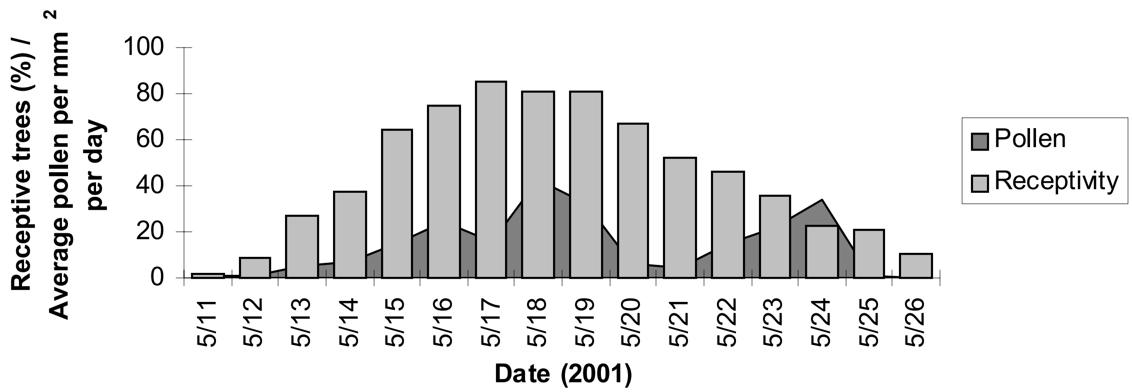


Figure 1. Female cone receptivity and relative pollen cloud density of *Pinus contorta* var. *latifolia* at KSO in 2001.

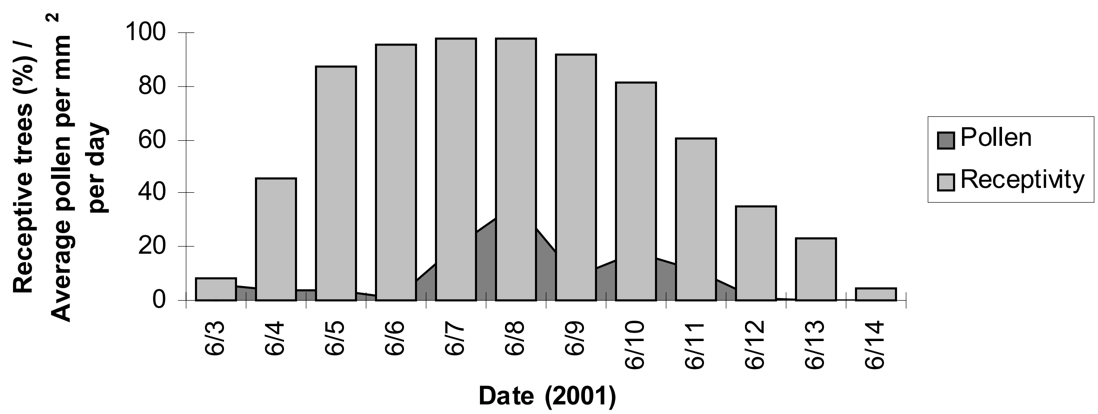


Figure 2. Female cone receptivity and relative pollen cloud density of *Pinus contorta* var. *latifolia* at PGSO in 2001.

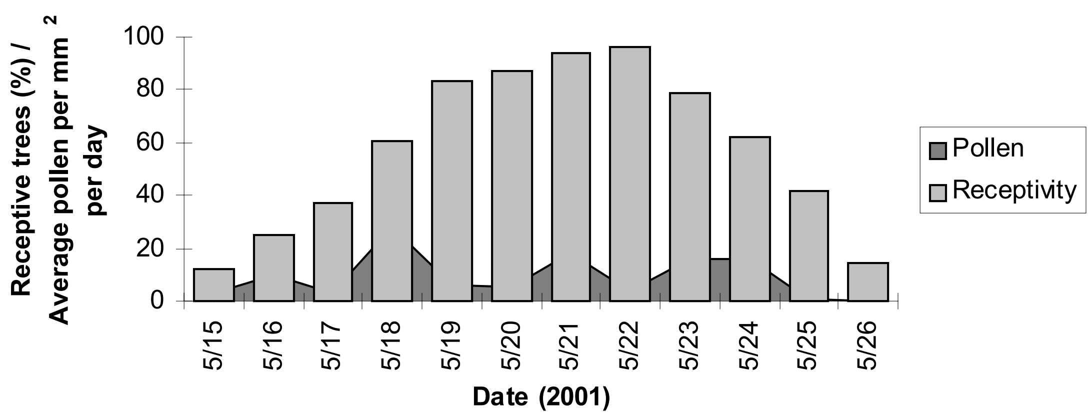


Figure 3. Female cone receptivity and relative pollen cloud density of *Pinus contorta* var. *latifolia* at PRT in 2001.

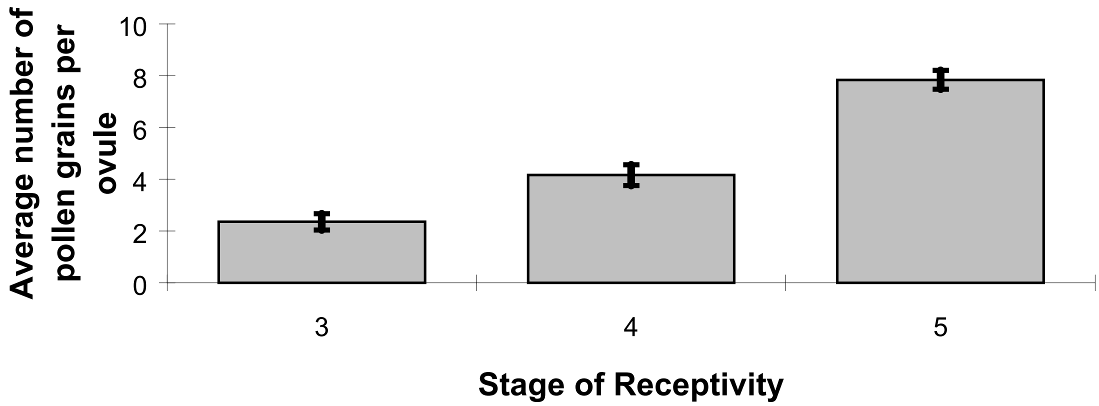


Figure 4. The average number of pollen grains per ovule at each stage of receptivity for the non-misted control treatment at KSO in 2001.

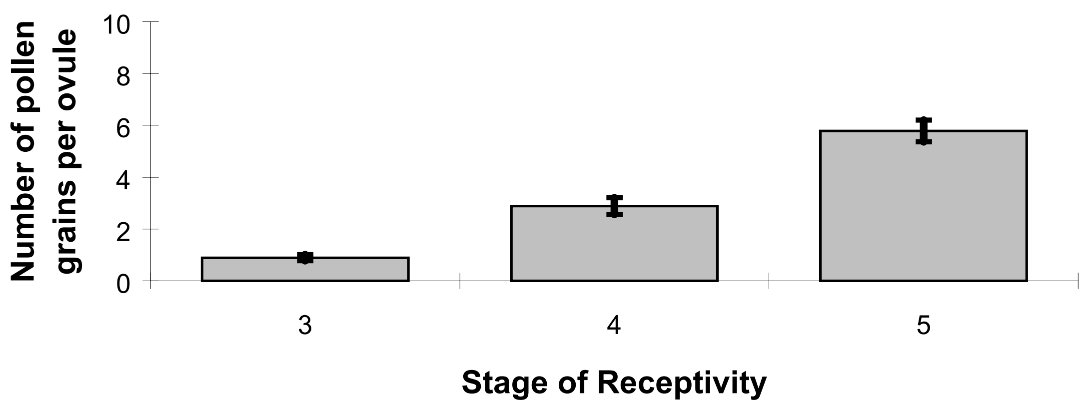


Figure 5. The average number of pollen grains per ovule at each stage of receptivity for the misted treatment at KSO in 2001.

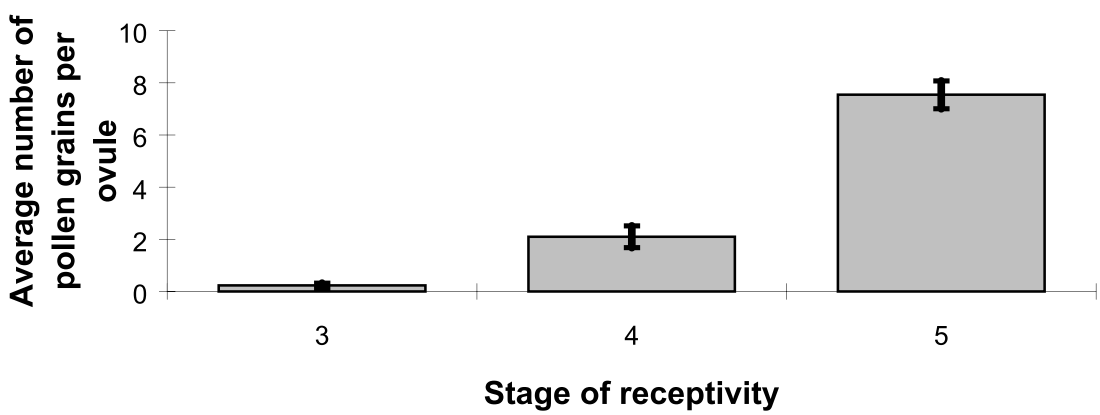


Figure 6. The average number of pollen grains per ovule at each stage of receptivity at PGSO in 2001 (equivalent treatment to non-misted control at KSO).

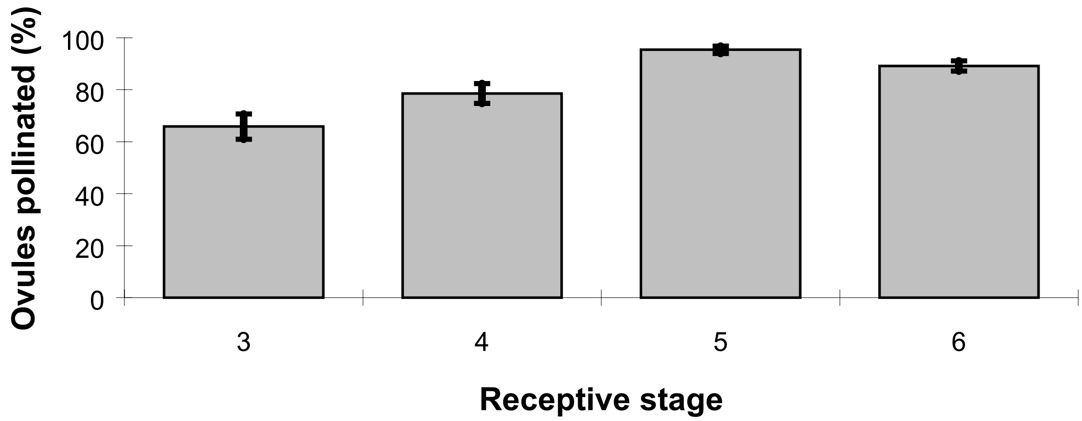


Figure 7. Percentage of ovules pollinated at each stage of receptivity for the non-misted control treatment at KSO in 2001.

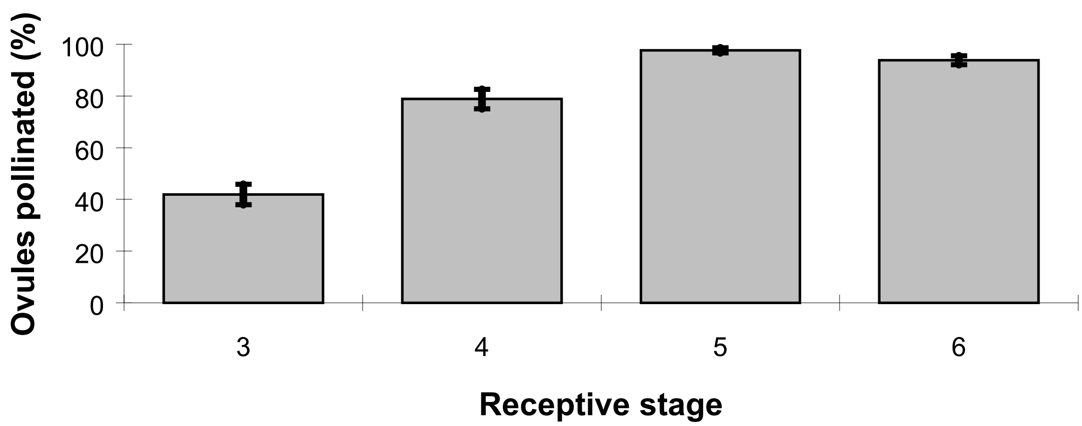


Figure 8. Percentage of ovules pollinated at each stage of receptivity for the misted treatment at KSO in 2001.

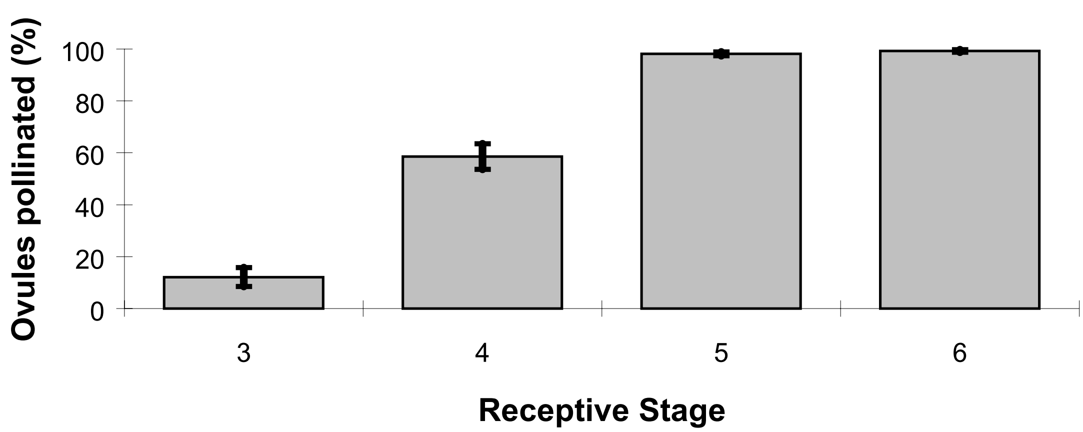


Figure 9. Percentage of ovules pollinated at each stage of receptivity at PGSO in 2001 (equivalent treatment to non-misted control at KSO).

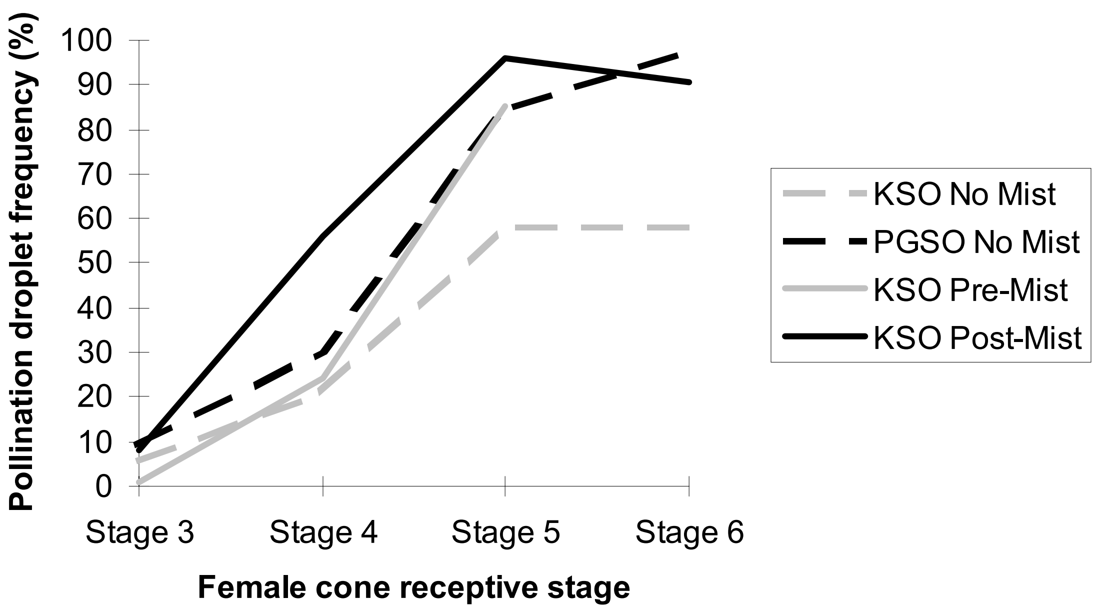


Figure 10. *Pinus contorta* var. *latifolia* pollination droplet frequency in receptive female cones by stage of cone development at KSO and PGSO in 2001.

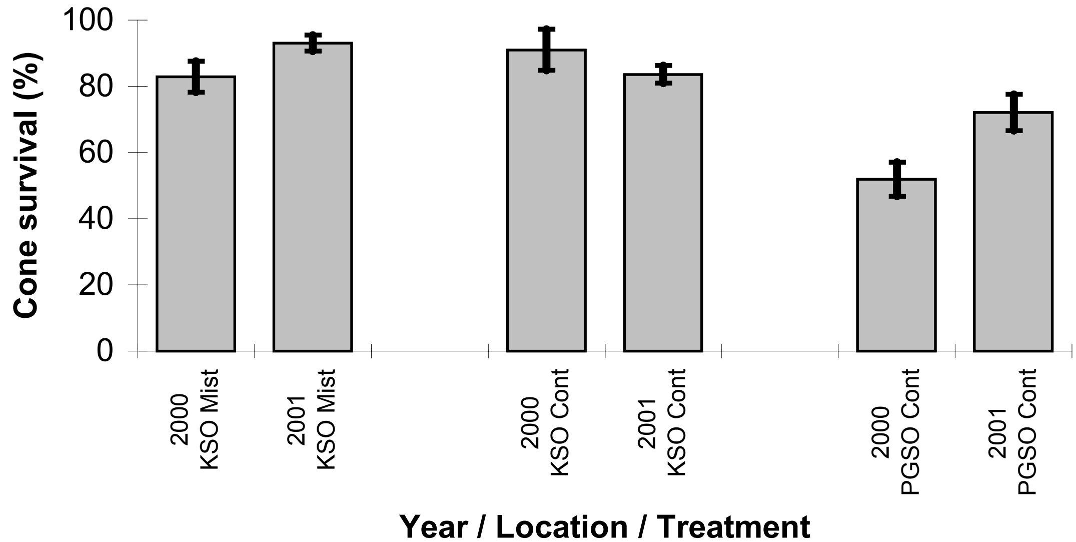


Figure 11. Post-pollination cone survival in 2000 and 2001 for the misted and control (open pollinated) treatments at KSO and the open pollinated treatment at PGSO.

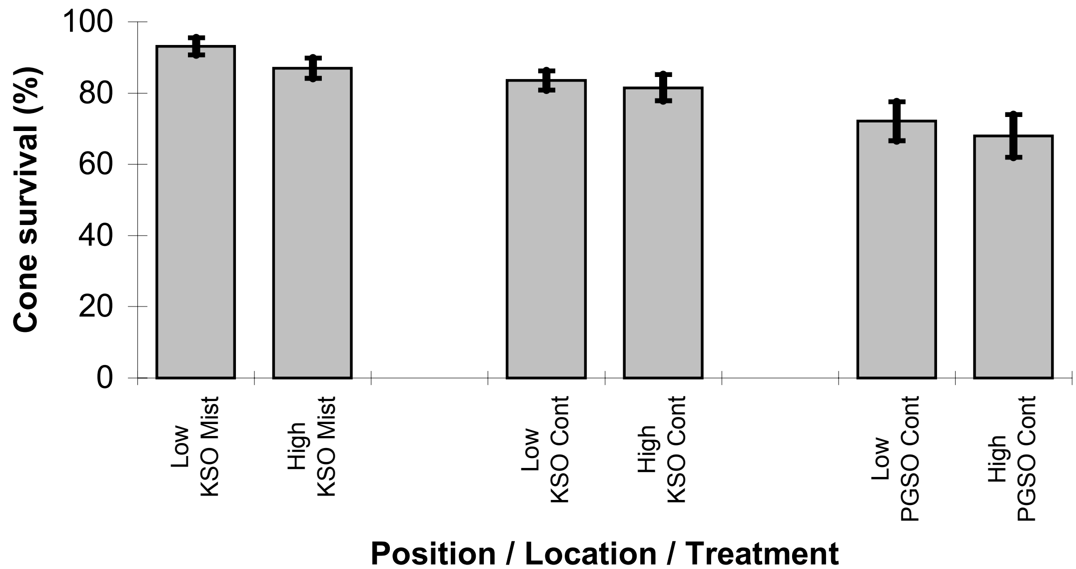


Figure 12. Post-pollination cone survival for the misted and control (open pollinated) treatments at KSO and the open pollinated treatment at PGSO based on cone position in 2001.

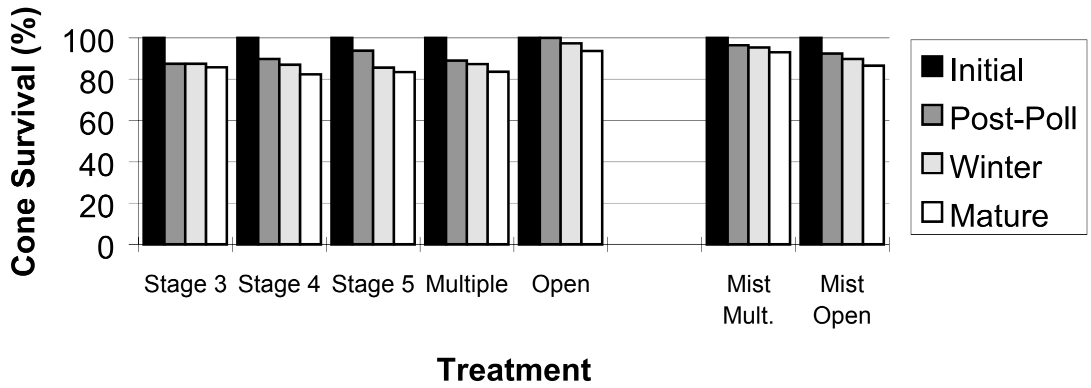


Figure 13. Cone survival by stage of development over the 2000 to 2001 reproductive cycle for each treatment at KSO.

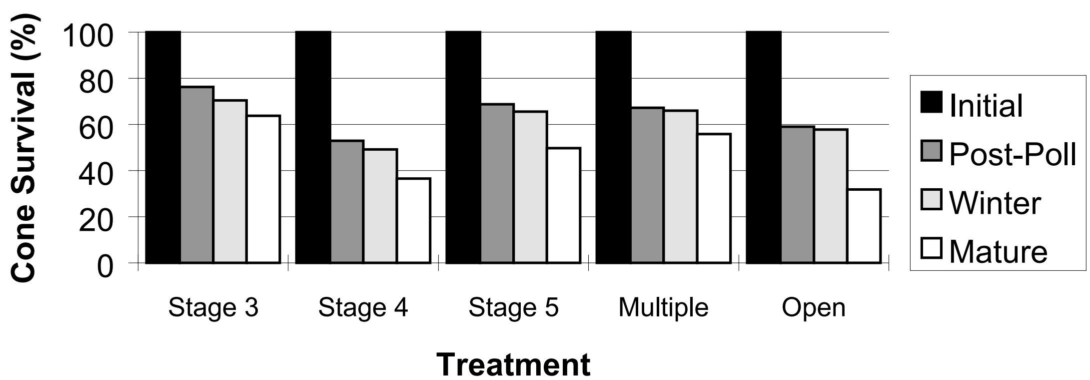


Figure 14. Cone survival by stage of development over the 2000 to 2001 reproductive cycle for each treatment at PGSO.

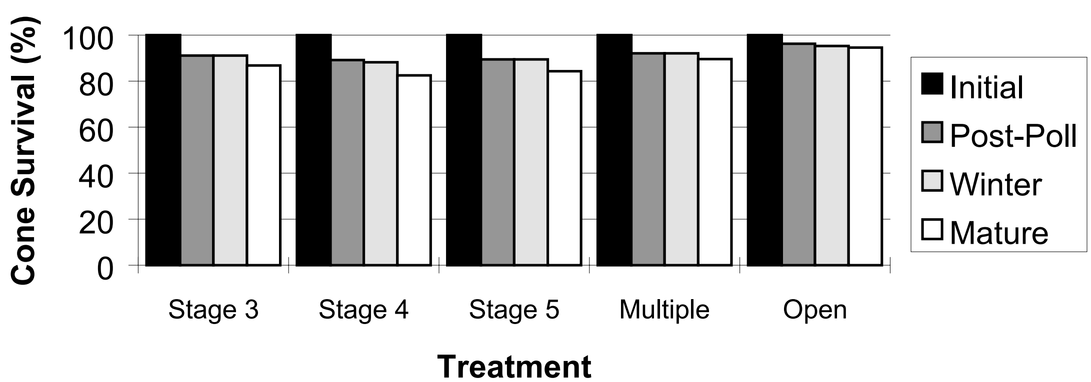


Figure 15. Cone survival by stage of development over the 2000 to 2001 reproductive cycle for each treatment at PRT.

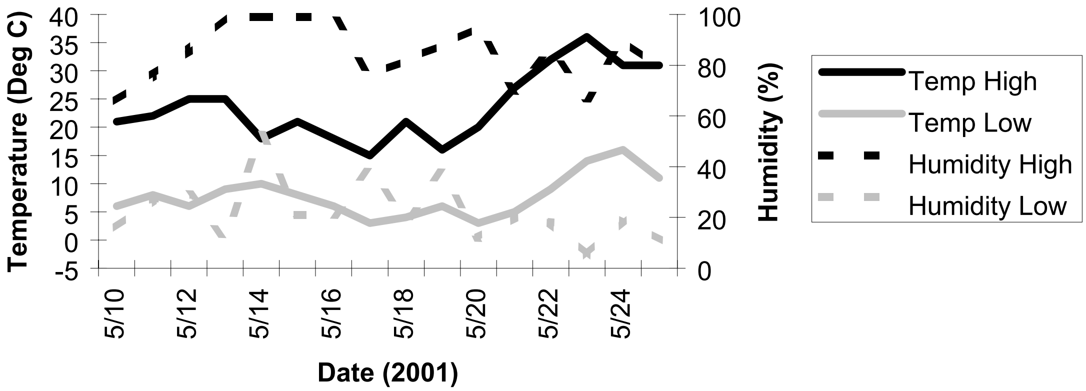


Figure 16. Temperature and humidity maximum and minimum values during female cone receptivity at KSO in 2001.

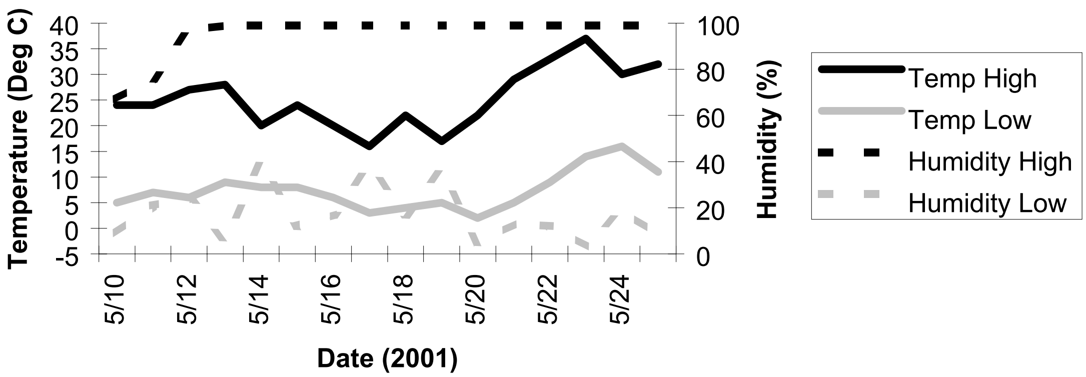


Figure 17. Misted treatment temperature and humidity maximum and minimum values during female cone receptivity at KSO in 2001.

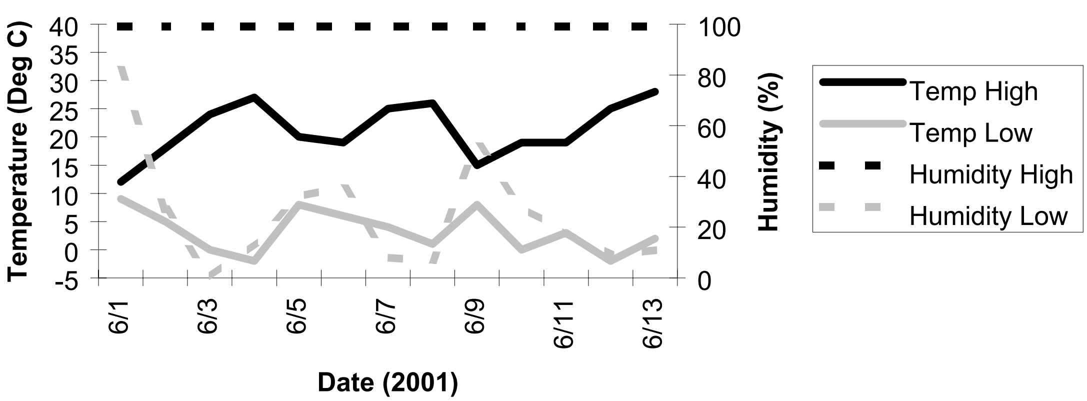


Figure 18. Temperature and humidity maximum and minimum values during female cone receptivity at PGSO in 2001.

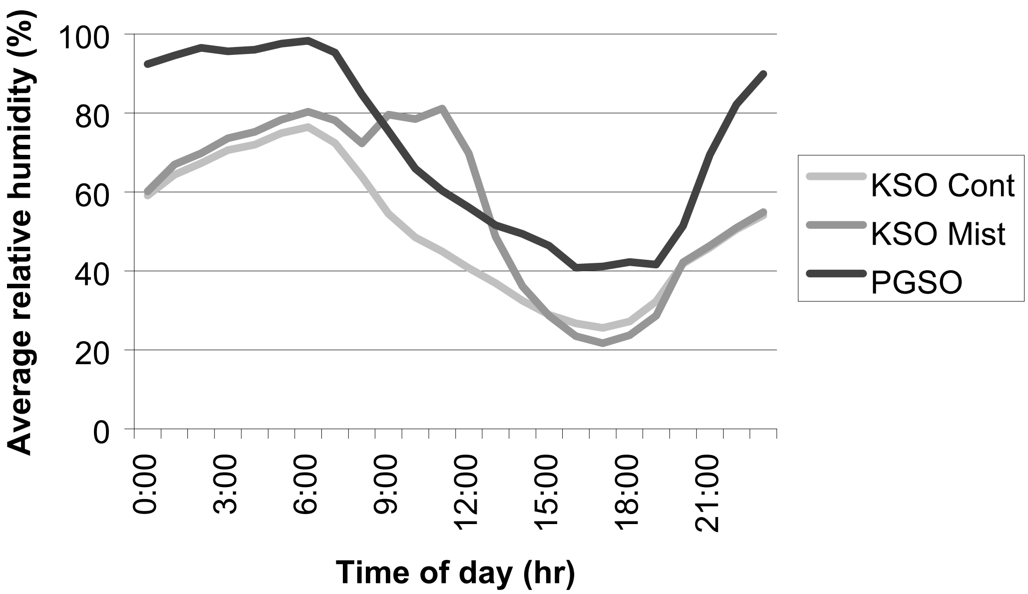


Figure 19. Comparison of average relative humidity per hour between ambient and misted treatments at KSO and ambient conditions at PGSO in 2001.

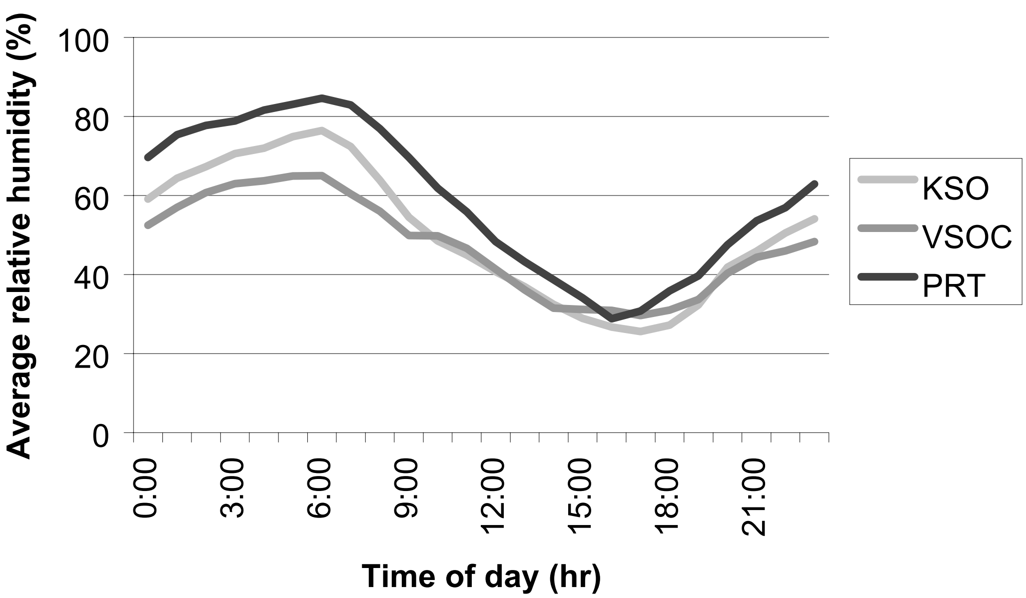


Figure 20. Comparison of average ambient relative humidity per hour during receptivity at the three Okanagan orchards, KSO, VSOC and PRT in 2001.

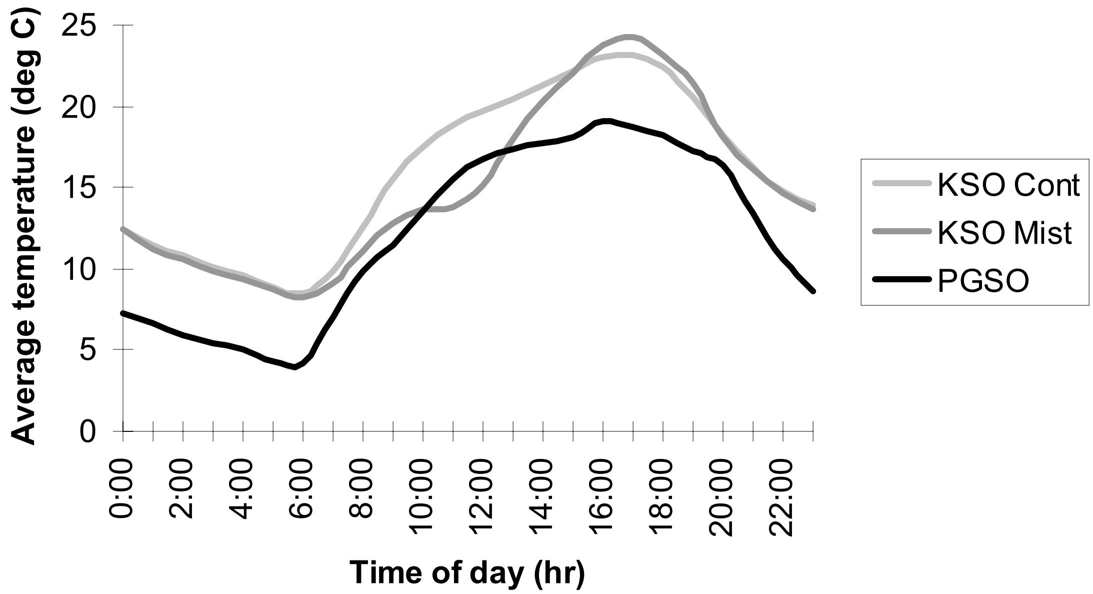


Figure 21. Comparison of average temperature per hour between ambient and misted treatments at KSO and ambient conditions at PGSO in 2001.

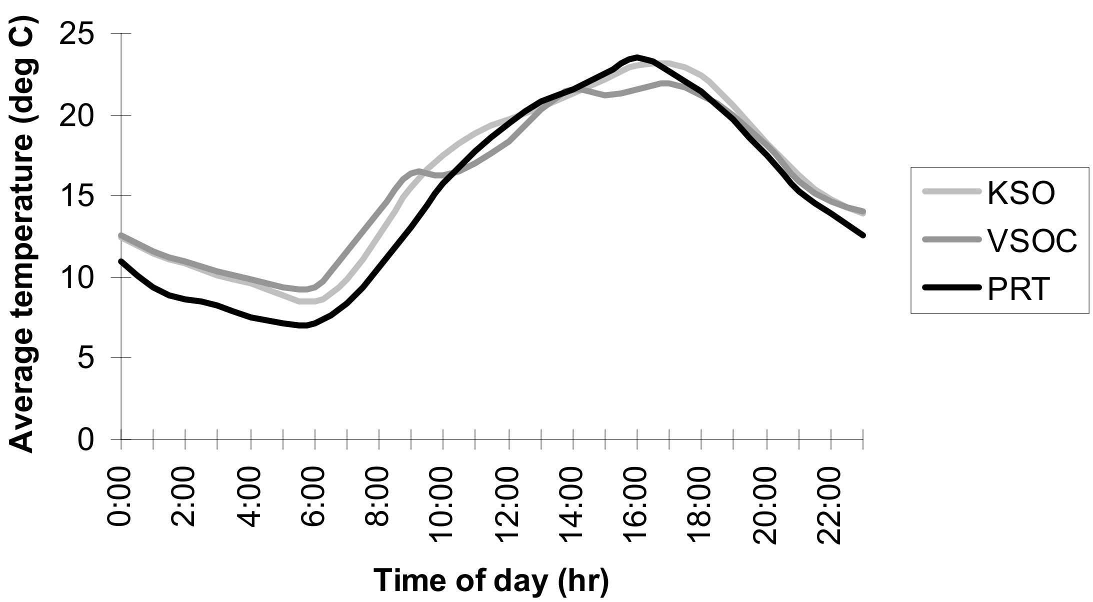


Figure 22. Comparison of average temperature per hour during receptivity at the three Okanagan orchards, KSO, VSOC and PRT in 2001.