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ANNA M. COLANGELI *
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ANNA M. COLANGELI*
JOHN N. OWENS*
SHEILA J. MORRIS*

* Department of Biology
University of Victoria
Victoria, B.C.
V8W 2Y2

Departmental Representative:

Jack Sutherland
Forestry Canada
Pacific Forestry Centre
506 W. Burnside Road
Victoria, B.C.
V8Z 1M5

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ABSTRACT

The causes of reduced seed yield in four Douglas fir (*Pseudotsuga menziesii* Mirb. Franco) trees at the Dewdney Seed Orchard in 1986 were found to be inadequate pollination (30%), low pollen vigor or viability (14%), embryo abortion (14%) and early ovule abortion (5%). The average seed efficiency (± s.e.) for the four trees was 40 (± 7). Conelet abortion in the four trees, averaged 43%, ranging from 20 to 64% per tree. Recommendations for increasing seed yield are discussed.

Total bacteria and fluorescent *Pseudomonas* populations were monitored in a Douglas fir Seed Orchard for one growing season and two pollination periods in 1986 and 1987. Seasonal variation in bacterial populations was observed, with the highest levels occurring in the late winter and early spring. Bacterial populations at pollination were higher in 1986 than in 1987. Conelet abortion at pollination was also higher in 1986 (55%) than in 1987 (11%). The results suggest that a causal relationship may exist between bacterial populations and conelet abortion at pollination. The potential role of ice nucleation-active bacteria in inciting frost damage in a Douglas fir orchard is discussed.

INTRODUCTION

Recent studies have shown that failure of ovules and seeds to develop, resulting in low seed set, can occur at several stages in the long reproductive cycles (Dogra 1967; Sweet 1973; Owens and Blake 1985; Kozinski 1987; Owens *et al.* 1989). The percentage of ovules or seeds lost at each stage varied with species and in some cases this has also been shown to vary with clones (Colangeli 1989; Owens *et al.* 1989). Consequently, each species must be studied to determine the stages in its reproductive cycle when losses occur and the severity of these losses with regard to potential seed set. This will be most important in seed orchards, especially containerized seed orchards (Ross *et al.* 1986), where some of the environmental and cultural conditions may be controlled in order to maximize seed production.

Conelet abortion about the time of pollination is a common occurrence in Douglas fir (*Pseudotsuga menziesii* Mirb. Franco) orchards. This is thought to result from low temperatures but disease and plant nutrition may also be factors. Recent literature has shown that some plant frost damage involves an interaction of certain surface bacteria and low temperatures. These bacteria make the plants on which they reside more susceptible to freezing damage by secreting proteins that act as ice-nucleating centers at temperatures not considered lethal. The bacteria that have been most frequently implicated with freezing injury are *Pseudomonas syringae* pv. *syringae*, *Erwinia herbicola* (reviewed by Lindow 1983) and *Xanthomonas campestris* pv. *translucens* (Kim *et al.* 1987).

Ice-nucleating bacteria are endemic epiphytes on over 90% of all plants analyzed ranging from grasses and small herbaceous plants to fruit orchards and forest trees such as *Eucaliptus* (Lindow *et al.* 1978; Gross *et al.* 1984; Canfield *et al* 1986; Kim *et al.*1987). A preliminary study by Lindow *et al.* (1978) failed to detect ice nucleation active (INA) bacteria on 8 out of 11 conifers. The bacterial population was very low in the 3 conifers where it was detected. One of these was Douglas fir, where INA bacteria were detected in a May collection, but not an October collection. *P. syringae* pv. *syringae* has been reported to cause terminal dieback in nursery grown *Pinus radiata* seedlings (Langridge and Dye 1982) and was isolated from stem cankers in outplanted one-year old *P. radiata* seedlings (Dick 1985). Due to the ubiquitous nature of INA bacteria it is possible that they may play a role in inducing frost sensitivity and frost damage in Douglas fir conelets at or near pollination. The presence of these bacteria on conifer cone buds and conelets has not been studied.

In order to identify the possible causes of cone abortion and low seed set in Douglas fir, two studies were conducted. The first, to determine the stages of ovule and cone development where seed efficiency decreased and the second study to determine if potential ice-nucleating bacteria are present on Douglas fir buds and conelets.

METHODS

Study 1: Four trees were selected in the Dewdney Seed Orchard (Ministry of Forests), 15 km north of Victoria, B.C. in early spring 1986. Approximately 50 cones were bagged before pollination and 0.9 ml of a polymix of fresh pollen from other sources was applied to each bag on two occasions during maximum receptivity. Two cones were randomly selected from each clone every 2 weeks from 25 April to 14 August. For each tree, twenty ovules per cone (40 per collection) were fixed, embedded and sectioned for microscopic examination to determine at which stage development stops, thus reducing seed production. The number of ovules with pollen in the micropyle, or pollination efficiency (PEF) was determined from the sectioned material.

All remaining bagged cones were collected before seed shed, dried, seed extracted from each cone separately and seed x-rayed to determine filled seed. The number of fertile scales and filled seed was determined per cone. Seed potential (SP) per cone was determined by multiplying the number of fertile scales by two because each scale contained 2 ovules, each with the potential of developing into a seed. Seed efficiency (SEF) was calculated by dividing the number of filled seed by SP and multiplied by 100%. In early June 1986, 10 branches from lower and mid-regions of the crown on the above five trees were randomly selected and the number of aborted and developing cones counted.

Study 2: A preliminary study using different selective media and techniques was conducted in spring of 1986. Douglas fir conelets were collected from five trees at the Dewdney Seed Orchard during pollination, first at bud burst (April 17) and one week later (April 24). Trees were sampled biweekly for total bacteria and fluorescent *Pseudomonas* from October 1986 to May 1987. In February 1987, the orchard was divided into four quadrants and sampling was intensified. Two twigs, about 4 cm long with potential seed-cone buds and later conelets were picked from 20 trees per quadrant to get an approximation of the overall bacterial concentration.

In the spring of 1986, 10 grams each of foliage and conelets from each of five trees were placed in 500-ml Florence flasks with 100 ml of 0.1 M phosphate washing buffer (Lindow *et al.* 1978). Flasks were shaken on a Multi-wrist shaker for 2 hours. Dilution platings of the conelet washings were made on two selective agar media, PSM medium (Burr and Katz 1981) and King's Medium B (KB) (King *et al.* 1954) as well as nutrient agar (NA). The plates were incubated at 27°C for 72 hours. Fluorescent colonies were identified by observing the PSM and KB plates under ultraviolet light (350nm). Total bacteria was determined from the nutrient agar plates. Four 10-gram replicates per quadrant were washed and plated as described above for the material collected in October 1986 to May 1987. The dilutions were made in sterile phosphate buffered saline.

All fluorescent colonies were tested for oxidase reaction (Kovacs 1956). Several strains of the oxidase negative *Pseudomonas syringae* were usually found in each of the samples where fluorescent

colonies were detected.

Conelet abortion at pollination was assessed for the five trees in 1986 and 40 trees in 1987 (10 trees per quadrant).

RESULTS

Study 1: Microscopic examination of 75 to 83 ovules per tree, collected shortly after pollination revealed PEFs of 81, 74, 43 and 74% for the four trees and averaged 68% (Table 1). Fifty-six, sectioned ovules per tree were examined shortly after fertilization. The fertilization rate in these ovules averaged 73, 68, 21 and 55%, respectively (Table 1). The average fertilization rate was 54%, 14% lower than the PEF, implying that 14% of the pollinated ovules were not fertilized.

SP varied among the trees, with average values of 73, 84, 64 and 78 per cone in trees 1 through 4 (Table 2). Of these small, flat empty seeds at maturity averaged 4, 9, 5 and 4 seeds per cone, respectively (Table 2) and the filled seed rate averaged 43, 39, 11 and 32 seeds per cone, respectively. SEF averaged 58, 46, 17 and 41% per cone for the four trees, respectively (Table 2). The average number of filled seed per cone was 31 seeds and SEF 40% for the four trees. The average SEF was about 14% lower than the average fertilization rate (54%), implying that about 14% of the fertilized ovules degenerated before seed maturity. This was verified by microscopic examination of sectioned material where a number of ovules contained degenerating or degenerated embryos.

The different factors affecting seed efficiency are summarized in Figure 1. About 5% of potential seeds were lost due to early embryo abortion, 30% of the seeds were empty because there was no pollen in the micropyles, 14% of the ovules had pollen but remained unfertilized, and 14% of the seeds were lost due to embryo degeneration.

The cone abortion rate in the four trees based on 10 branches per tree was 64, 30, 20 and 60%, respectively (Table 3). The average cone abortion rate was 43% (Table 3).

Study 2: The total population of bacteria sampled from the Dewdney Seed Orchard during pollination in 1986 averaged 1.3×10^7 colony forming units (c.f.u.) per gram of fresh tissue (Figure 2). The population of fluorescent *Pseudomonas* averaged 6.1×10^4 c.f.u./gram of fresh weight (Figure 2). Ovule and conelet abortion was extremely high in the five Douglas fir trees, ranging from 20% to 100% with an average of 55%.

The total bacterial population during pollination in 1987 was lower, averaging 1.2×10^4 c.f.u./gram. The population of fluorescent *Pseudomonas* at pollination avereaged 8×10^2 c.f.u./gram (Figure 2). Cone abortion rates in 1987 were lower than 1986, averaging 11% with a range of 0 to 90%. Conelet abortion did not vary among the four quadrants but did vary among the trees within a quadrant on any given sample date. The population of total and fluoresent *Pseudomonas* varied among the trees and the four quadrants on any given date.

Seasonal changes were found in the bacterial population between October 1986 and May 1987 at the Dewdney Seed Orchard (Figure 3). Total bacteria was just above the detectable level (1×10^2 to 10^3 c.f.u./gram) between October and early January 1987. The population increased to an average of 8.4×10^3 c.f.u./gram by the end of January. The total bacterial population reached a maximum of 6.1×10^5 c.f.u./gram in late February, followed by a steady decrease to 3.1×10^2 c.f.u./gram in early May. Fluorescent *Pseudomonas* were not detected in the samples until late February when the population increased to 1.3×10^3 c.f.u./gram. This level was maintained until mid-August when the population decreased to undetectable levels. The decrease in population occurred near the end of pollination. When aliquots of the conelet washings were incubated in selective media and the bacterial population grown up, fluorescent *Pseudomonas* were isolated. These bacteria were still present in the population, but at a low level.

DISCUSSION

In this study, the causes of reduced SEF in four Douglas fir trees in order of importance was inadequate pollination, pollen inviablity or low vigor, embryo abortion and early ovule abortion. These are the same factors implicated in reduced seed yield in other species (Kozinski, 1987; Colangeli 1989; Owens *et al.* 1989). The major reduction in seed yield was due to inadequate pollination where about 30% of micropyles contained no pollen even after controlled pollinations. This varied from 43% pollinated in tree 3 to 81% in tree 1. Many of these ovules had pollen on the stigmatic hairs but not in the micropyle. Recent reports on the pollination mechanism in Douglas fir have shown that the optimal time for pollination is 3 to 4 days after the conelets were 50% beyond the bud scales (Ho 1980; Owens *et al.* 1981; Owens and Simpson 1982). In the present study all conelets were pollinated on the same two days. The differences in pollination efficiency may be reflected in the variation in conelet phenology at the time of pollination.

About 14% of the ovules were pollinated but not fertilized. From microscopic examinations swollen and elongated pollen were found in the micropyle, but they never reached the nucellus. Also about 14% of the ovules were fertilized but failed to produce filled seed due to embryo degeneration. Embryo degeneration has been well documented in Douglas fir after inbreeding and selfing and in these cases is believed to be due to the accumulation of homozygous recessive lethals (Orr-Ewing 1957; Sorensen and Miles 1974; Shaw and Allard 1982). The pollen used in this study was unrelated, pollen so that embryo abortion cannot be attributed to selfing. A low percentage of embryo abortion has been found in other outcrossed studies (Kozinski 1987; Colangeli 1989; Owens *et al.* 1989).

No relationship was observed between cone abortion and SEF. Tree 3, which produced the fewest filled seed (SEF = 17 ± 2) had the lowest cone abortion rate (20%). This suggests that the factors resulting in cone abortion are not the same as those contributing to low SEF. Cone abortion may be

due to many factors. Temperature at pollination is probably the most significant. The results obtained thus far suggest that there may be a causal relationship between bacterial population levels and conelet abortion in Douglas fir. The population of fluorescent *Pseudomonas* and total bacteria was considerably lower in 1987 than it was for the same time period in 1986. Conelet abortion rate was also lower in 1987 compared to 1986.

Lindow *et al.* (1978) found detectable levels (7.9×10^2 cells/g fresh wt) of INA bacteria on Douglas fir foliage from trees sampled in Wisconsin in May. The total bacteria averaged 1.3×10^5 cells/g fresh wt. When the trees were resampled in October INA bacteria were not detected and the total population had decreased to 1.3×10^3 cells/g fresh wt. These results are similar to those observed at the Dewdney Seed Orchard where both total and INA populations were highest in the spring, coinciding with pollination. Seasonal changes in bacterial populations have been found in other species. Increasing populations of INA bacteria coincided seasonally with fruit tree development (Gross *et al.* 1984). Bacterial populations are often highest during specific stages of flower phenology (Andrews and Kenerly 1980).

Control of INA bacteria have included bactericides, antagonistic bacteria and ice-nucleation inhibitors, such as heavy metal (eg. copper) solutions (reviewed by Lindow 1983). Low concentrations of copper solutions added to seed orchards, through overhead irrigation systems if present could possibly control the ice-nucleating activity of the bacteria.

This study dealt only with total and fluorescent *Pseudomonas*, but other potential INA-strains such as *Erwinia* and *Xanthomonas* were occasionally detected. While only one growing season and two pollination seasons were monitored in this study, and two sample periods by Lindow *et al.* (1978), there is evidence that potential INA bacteria inhabit Douglas fir cone buds, conelets and foliage. Whether they play a role in inciting frost damage and are responsible for at least part of the observed cone abortion and possible control measures requires further study.

CONCLUSIONS AND RECOMMENDATIONS

The major factors reducing seed yield in Douglas fir are inadequate pollination, low pollen vigor and embryo abortion. Ice-nucleating bacteria may be responsible for the high degree of conelet abortion observed in some years. Seed yield in Douglas fir can possibly be increased by supplementally applying two or three applications of high quality pollen just after reproductive bud burst. Ice-nucleating bacteria have been successfully controlled by applying low concentrations of ice-nucleating inhibitors, such as copper solutions to plants during potentially susceptible periods such as pollination.

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Table 1: Pollination efficiency (%) and fertilization success (%) as determined by the proportion of ovules with pollen and proportion of fertilized ovules four cross-pollinated Douglas fir trees at the Dewdney Seed Orchard in 1986.

Tree	Micropyles with pollen	Pollination Efficiency	Fertilized Ovules	Fertilization Success
1	63/78	81	41/56	73
2	58/78	74	38/56	68
3	32/75	43	12/56	21
4	61/83	74	31/56	55
Total	214/314		122/224	
Mean		68		54

Table 2: The mean (±SE) seed potential (SP), flat seed (FLAT), filled seed (FILL) and seed efficiency (SEF) for four Douglas fir trees from the Dewdney Seed Orchard in 1986. Means are based on a sample of 10 cones per tree.

Tree	SP	FLAT	FILL	SEF
1	73 ± 2	4 ± 1	43 ± 5	58 ± 6
2	84 ± 2	9 ± 2	39 ± 4	46 ± 3
3	64 ± 2	5 ± 1	11 ± 1	17 ± 2
4	78 ± 2	4 ± 1	32 ± 3	41 ± 4
Mean	75 ± 4	5 ± 1	31 ± 2	40 ± 7

Table 3: Cone abortion in four Douglas-fir trees at the Dewdney Seed Orchard, in 1986. Based on a sample of 10 branches per tree.

Tree	Aborted Cones/ Total Cones	Cone Abortion (%)
1	122/190	64
2	49/166	30
3	50/247	20
4	134/222	60
Total	355/825	Mean 43

Figure 1: Summary of average percentages of potential seed lost due to various causes and final seed efficiency for four cross-pollinated Douglas fir trees.

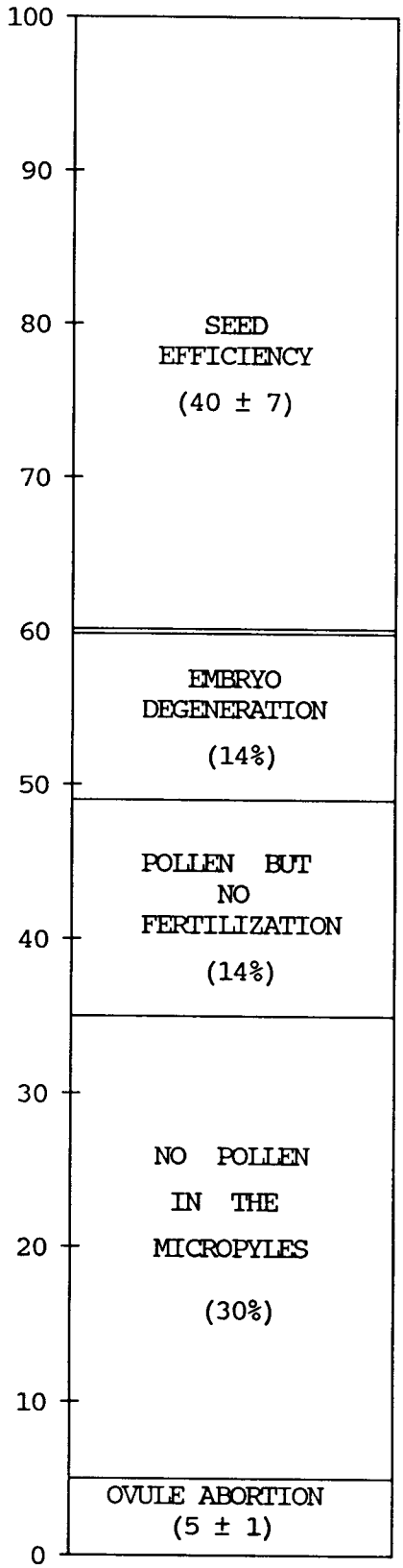

100
90
80
70
60
50
40
30
20
10
0
SEED
EFFICIENCY
(40 ± 7)
EMBRYO
DEGENERATION
(14%)
POLLEN BUT
NO
FERTILIZATION
(14%)
NO POLLEN
IN THE
MICROPYLES
(30%)
OVULE ABORTION
(5 ± 1)

Figure 2: Bacteria recovered from conelets collected at pollination in 1986 and 1987 at the Dewdney Seed Orchard. The populations are expressed as the log of the colony forming units (c.f.u.) per gram of fresh tissue. Vertical bars denote standard errors.

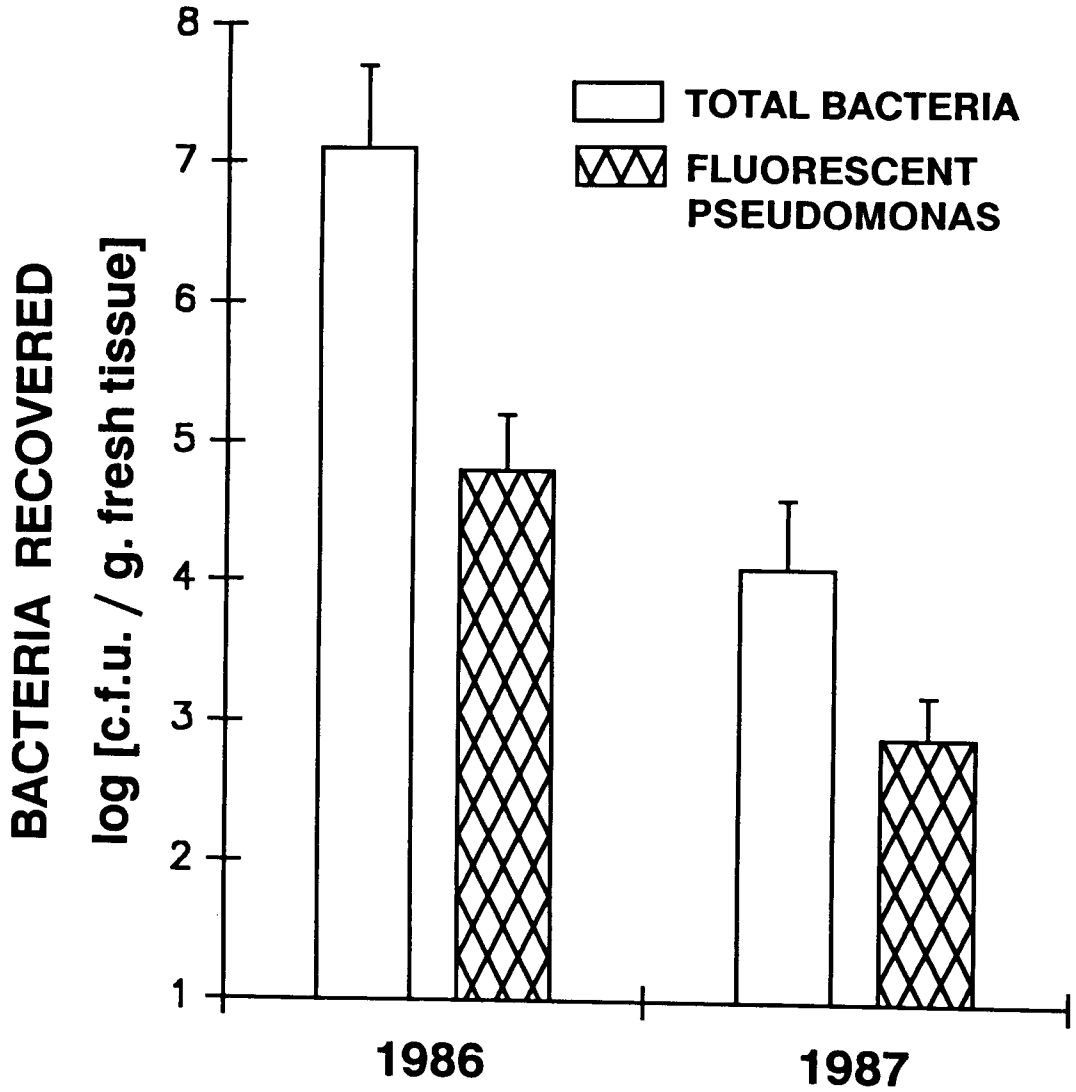
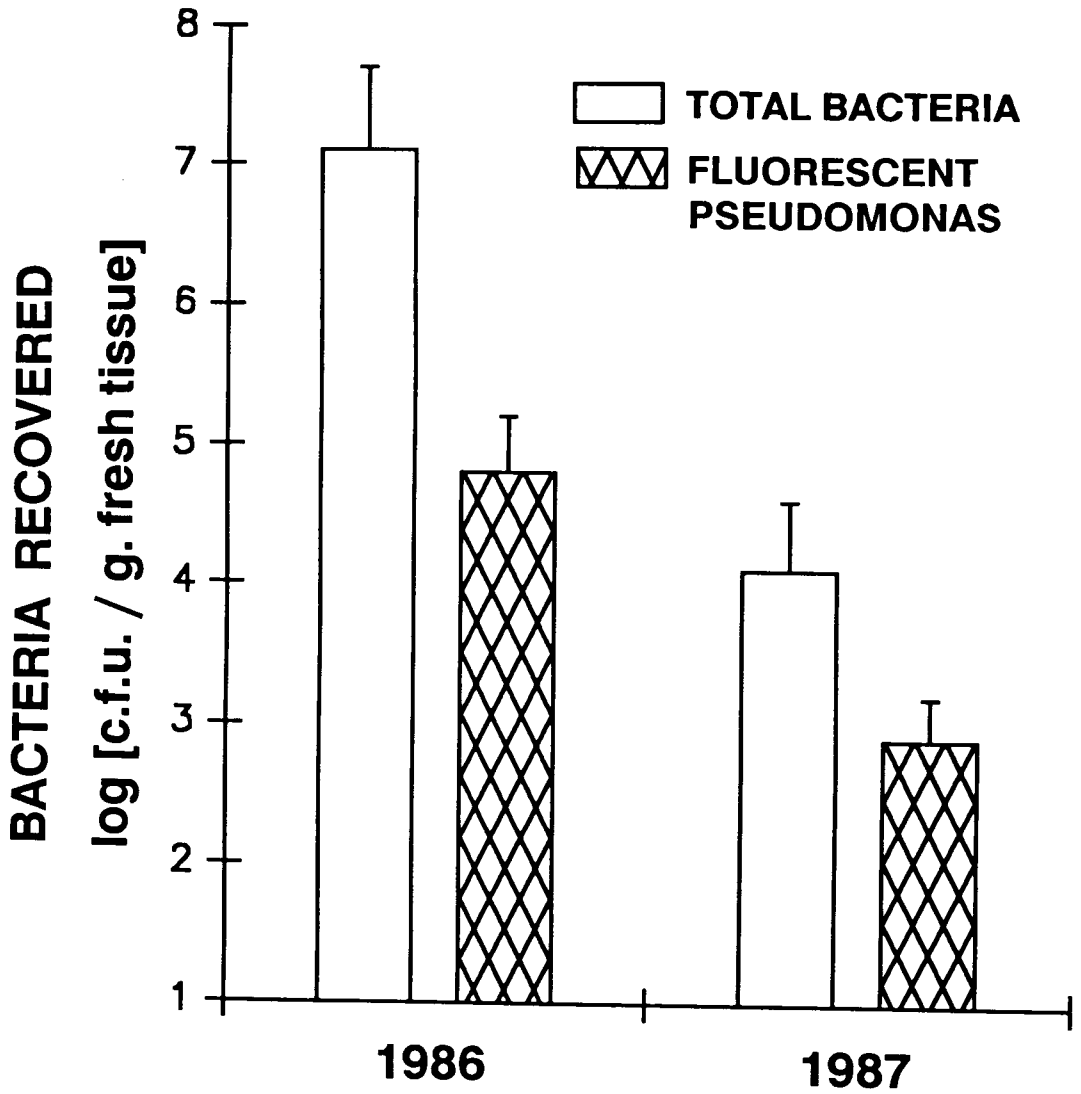

BACTERIA RECOVERED
log [c.f.u. / g. fresh tissue]
8
7
6
5
4
3
2
1
TOTAL BACTERIA
FLUORESCENT PSEUDOMONAS
1986
1987

Figure 3: Seasonal changes in the bacterial population in Douglas fir cone buds and conelets at the Dewdney Seed Orchard. The populations are expressed as colony forming units (c.f.u.) per gram of fresh tissue. Vertical bars denote standard errors.


BACTERIA RECOVERED
log [c.f.u. / g. fresh tissue]
8
7
6
5
4
3
2
1
TOTAL BACTERIA
FLUORESCENT PSEUDOMONAS
1986
1987

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