Biology, Sampling and Control of the Douglas-fir Cone Gall Midge,

Contarinia oregonensis Foote (Diptera: Cecidomyiidae), in Douglas-fir

Seed Orchards in British Columbia

by

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of

Biological Sciences

Gordon Edward Miller © 1982

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Cecidomyiidae), in Douglas-fir Seed Orchards in

British Columbia

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Biology, Sampling and Control of the Douglas-fir Cone Gall Midge,

Contarinia oregonensis Foote (Diptera: Cecidomyiidae), in

Douglas-fir Seed Orchards in British Columbia

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#### **ABSTRACT**

Emergence of adult Douglas-fir cone gall midges, Contarinia oregonensis Foote, began in mid April in the field, and lasted about 2 weeks. Adult emergence began outdoors at 0600-0700 Pacific Standard Time, peaked at 1100 and continued until 1900. Mating occurred on or near the duff from which females emerged. Virgin females appeared to "call" males by extending their ovipositors and waving them back and forth. Field trapping with living insects as lures showed that virgin females produce a sex pheromone. Oviposition patterns on previously infested conelets differed from those on uninfested conelets and suggested the presence of an oviposition deterrent.

The number of midge larvae present at cone harvest was related to the number of eggs laid, but not to density or numbers of parasites, indicating that natality rather than mortality is the dominant factor in <u>C</u>. oregonensis population dynamics in seed orchards. Rates of parasitism were low (<14%) and there was no indication that the parasites affected seed production in a positive manner.

The pattern of occurrence of Douglas-fir conelets varied among years; the upper crown third produced the most conelets in 1978 and 1981 while the mid third produced the most in 1980. C. oregonensis oviposited most frequently in the upper crown third, followed by the mid third, except where infestations were heavy and eggs were evenly distributed throughout the crown. Sampling plans were developed for estimating numbers of conelets per tree and numbers of C. oregonensis eggs and infested scales per conelet.

C. oregonensis damage was related to both numbers of eggs and number of egg-infested scales per conelet, the latter being the better

variable on which to base predictions because less time was required for conelet analysis. Sequential sampling plans were developed on relations between number of egg-infested scales and damage.

Dimethoate was the most effective insecticide tested. Benefit: cost analysis of an operational application was carried out to show the circumstances (i.e. crop size and infestation levels of cone and seed insects) under which an insecticide application is justified.

Data on population dynamics, sampling and control were used to develop a proposed pest management system.

## DEDICATION

TO JOAN AND JOHN B.

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### 1. INTRODUCTION

#### 1.1 Seed Orchards

The importance of cone and seed insects has increased recently due to the development of tree improvement programs and seed orchards. Seed orchards are plantations of superior trees that are intensively managed to produce frequent, abundant and easily harvested crops of genetically superior seed (Zobel et al. 1958). The concept of seed orchards and considerations involved in their establishment and management have been summarized by Zobel (1971), Faulkner (1975) and Konishi (1975).

In British Columbia (B.C.) the first Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco, seed orchard was established in 1963. Currently, there are 14 Douglas-fir seed orchards on 44.6 ha. All orchards are located on the coast, and all but one are located on Vancouver Island. The amount of Douglas-fir seed sown annually in B.C. nurseries averaged over 400 kg (approximately 43 million seeds) between 1963 and 1979 (Miller 1980), and demands for similar quantities are expected to continue. The objective is that 90% or more the the Douglas-fir seedlings planted on the coast will be from orchard seed by the mid 1980's (J. Konishil, pers. comm.). The annual seed production in the orchards must increase dramatically for this objective to materialize, since orchard production is currently far below the necessary levels (Table I). Depredations by cone and seed insects, in particular the Douglas-fir cone gall midge, Contarinia oregonensis Foote (Diptera: Cecidomyiidae), have been a major impediment to seed production (Miller 1980). The Coastal Tree Improvement Council of British Columbia

Forester in Charge of Seed Production, Silviculture Branch, B.C. Ministry of Forests, Victoria.

Table I. Production of Douglas-fir seed in B.C. seed orchards, 1968-1981 (M. Crown<sup>2</sup>, per. commun.).

Year	No. orchards producing	Total amount of seed produced (kg)
1968	1	0.233
1969	0	0
1970	0	0
1971	2	0.586
1972	0	0
1973	1	0.210
1974	1	0.137
1975	6	1.290
1976	9	72.903
1977	7	6.137
1978	8	68.260
1979	11	101.600
1980	8	49.965
1981	8	19.736

Orchard Manager, Coastal Seed Orchards, Silviculture Branch, B.C. Ministry of Forests, Duncan, B.C.

currently considers cone and seed insect research to have top priority.

Other important Douglas-fir cone and seed insects include the Douglas-fir cone moth, <a href="Barbara colfaxiana">Barbara colfaxiana</a> (Kft.) (Lepidoptera: Olethreutidae), the coneworms, <a href="Dioryctria">Dioryctria</a> spp. (Lepidoptera: Pyralidae), the Douglas-fir seed chalcid, <a href="Megastigmus spermotrophus">Megastigmus spermotrophus</a> (Wachtl) (Hymenoptera: Torymidae), the western conifer seed bug <a href="Leptoglossus occidentalis">Leptoglossus occidentalis</a> Heidemann (Hemiptera: Coreidae), and the Douglas-fir cone scale midge, <a href="Contarinia washingtonensis">Contarinia washingtonensis</a> (Diptera: Cecidomyiidae).

### 1.2 Life History of Douglas-fir

Douglas-fir seed-cone buds are initiated and differentiated the year prior to pollination and maturation (Allen and Owen 1972). On Vancouver Island, seed-cone bud burst ("flowering") occurs in April; such factors as elevation and weather determine the actual date. The cones normally remain erect and open to receive pollen for a few days but may remain open for 2 weeks in cool weather. After the cones close, they turn downward, reaching the pendant position in a few days; most cones are pendant by early May. They resemble mature cones at this time. Cone enlargement is completed by early July, but maturation continues until late August when the cones begin to dry and turn brown. Seeds are released from opened cones in September.

### 1.3 Douglas-fir Cone Gall Midge.

<u>C. oregonensis</u> was recognized as a species relatively recently.

Foote (1956) described the adults from material collected in 1916 and Hedlin (1961) described the immature stages. This gall midge feeds only in the cones of Douglas-fir and probably occurs throughout the range of its

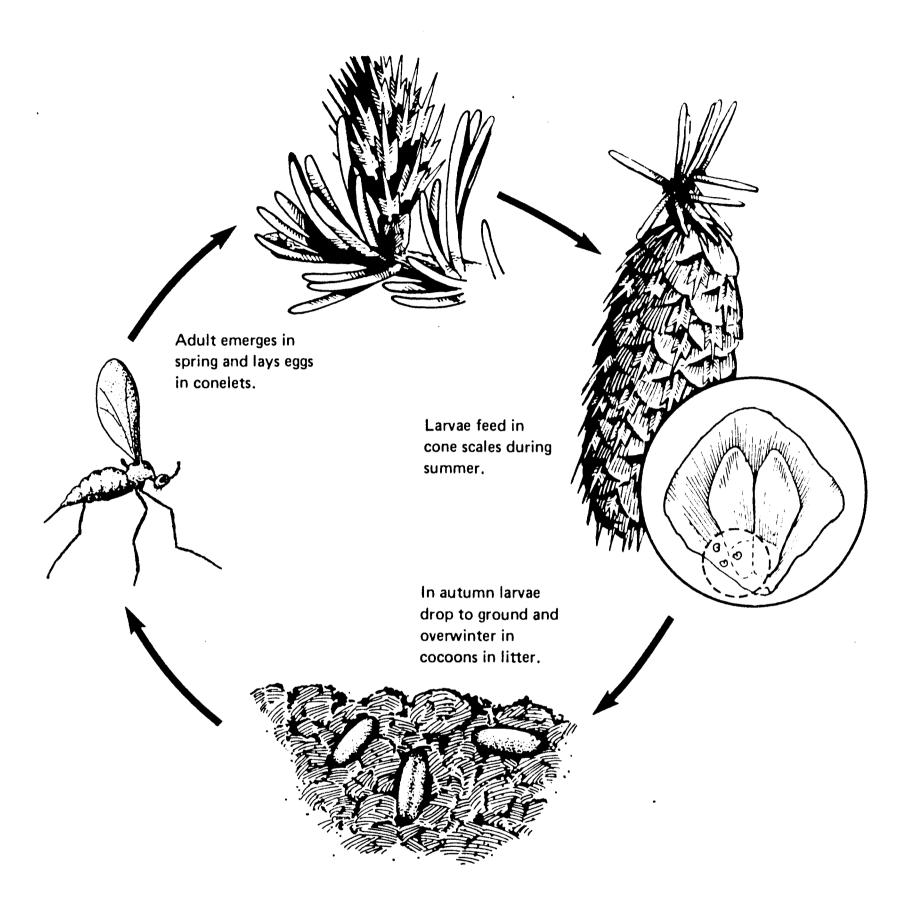
host, but is much more abundant in wet coastal areas than in drier interior regions (Hedlin 1974; Hedlin et al. 1980).

A gall-forming gnat or midge, probably <u>C. oregonensis</u>, was discovered in the cones of Douglas-fir in the early 1900's (Miller 1914), but the damage caused by this insect was considered insignificant, even into the 1950's (Keen 1952, 1958; Rudinsky 1955). <u>C. oregonensis</u> is now known to be a very important destroyer of Douglas-fir seed in coastal areas (Hedlin 1958, 1964a; Johnson and Heikkenen 1958; Koerber 1963; Kozak 1963; Johnson and Hedlin 1967). Seeds are damaged in two ways: 1) larval feeding inhibits seed development, and 2) gall formation causes seeds to become fused to the scale, making seed extraction impossible (Johnson and Heikkenen 1958; Johnson 1963b).

The life cycle of <u>C. oregonensis</u> in non-orchard situations is diagrammed in Fig. 1. The adult midges emerge in April and early May when Douglas-fir seed-cone buds burst (Johnson and Winjum 1960; Hedlin 1961; Johnson 1963a). Eggs are deposited in clusters near the bases of scales in cones open to receive pollen. Hatching takes place 2-3 weeks after oviposition. After hatching, larvae tunnel into scales and begin feeding around the conductile tissues. Early gall formation, which is visible in late May and early June, is indicated by the scale surface near the ovule becoming smooth, shiny and colorless. Galls are readily visible in late July. There are 3 larval instars, the first lasting 6 weeks, the second 2 weeks, and the third 6 or more months. The third-instar assumes a characteristic U-shape while in the gall. Fully developed larvae leave mature cones in autumn, usually after the dry cones become moistened by rain, and spin cocoons in the litter, where they overwinter. Prepupal development begins in December and takes about 2 months. Pupation takes about 6 weeks. A proportion

Figure 1. Diagram of the life cycle of

Contarinia oregonensis. Modified from Hedlin et al. (1980).



of the population will not emerge the year following larval feeding but remains in diapause as third-instar larvae for up to 3 years.

In properly managed seed orchards, the life cycle differs due to the removal of cones from the orchard prior to larval emergence. Each year the midge larvae are removed with the cones, preventing these larvae from overwintering on-site. Therefore, gall midge adults must migrate into orchards from nearby stands for orchard cones to be attacked.

### 1.4 Objectives

A recent problem analysis of the status of pest management in Douglas-fir seed orchards (Miller 1980) summarized the knowledge of Douglas-fir cone and seed insects, including C. oregonensis, and indicated areas where research was needed. Requisites for a pest management system include: i) knowledge of the species involved and their biologies, specifically their life cycles, habits, variations in population levels throughout the year, types of damage caused and population dynamics; ii) capability to evaluate and predict damage caused by the pests; and iii) available methodology for reducing pest populations or damage (Geier 1966). Prior to the studies reported herein, only 3 of the aspects of the biology of C. oregonensis were known, namely, the life cycle, variations in population levels throughout the year and the type of damage. No sampling techniques had been developed for predicting seed losses to the midge. Techniques for evaluating damage had been developed but not quantified fully. Several insecticides had been tested, but the use of many of the effective ones in B.C. seed orchards is unlikely because of their relatively high toxicities to nontarget organisms.

The overall objective of the following studies was to develop a pest management system for use against <u>C. oregonensis</u> in seed orchards. Specific areas of study included: i) reproductive behaviour of adults, ii) survival of immature cone gall midges in seed orchards from ovipostion to time of cone harvest, iii) development of sampling techniques for estimating populations of conelets and gall midge eggs, and for predicting damage, and iv) development of control techniques for use against <u>C. oregonensis</u>.

### 2.0 GENERAL METHODS

All sites, 7 seed orchards and 1 unmanaged stand, where cones were sampled at various times during this research are located on Vancouver Island, British Columbia (Fig. 2). Pacific Forest Products is referred to as PFP throughout the thesis.

Cones were collected at two stages of development: after the scales became appressed in the spring (early May) and at the time of cone harvest (August). Cones collected in the spring are referred to as conelets throughout this thesis to avoid confusion about season of collection. All cones were stored at 0° C until processed. Conelets were stored for a maximum of 2 months; thereafter mould became a problem. Storing for this length of time did not affect egg counts (Table II). Cones collected at harvest were sliced longitudinally along their axes (Winjum and Johnson 1960) and counts were made of galls exposed per axial slice as well as filled and damaged seeds.

Preliminary analysis of the data, using computer programs developed by C.S. Simmons for comparing data to the standard theoretical distribution models, indicated that conelet, seed and insect counts did not differ significantly from the negative binomial distribution in most instances. In the few instances where significant difference did occur, only in heavy infestations of C. oregonensis eggs and larvae, the log distribution fit best. As a result, unless otherwise stated, the data were transformed by  $\log_{10}$  (x+1), the standard transformation for the negative binomial distribution (Anscombe 1949), before analysis with parametric statistics. Percentages were transformed by  $\sqrt{x}$  before

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Figure 2. Location of study sites on Vancouver Island, B.C. All sites, except Metchosin (unmanaged stand), are seed orchards. BCMF refers to B.C. Ministry of Forests.

	SITE NUMBE	R NAME AND OWNERSHIP	LOCATION
MANGOLINER SIA	1 2 3 4 5 6 7 8	PACIFIC FOREST PRODUCTS Ltd. TAHSIS Ltd. DEWDNEY (BCMF) METCHOSIN KOKSILAH (BCMF) LAKE COWICHAN (BCMF) QUINSAM (BCMF) SNOWDON (BCMF)	SAANICH SAANICH METCHOSIN DUNCAN MESACHIE LAKE CAMPBELL RIVER CAMPBELL RIVER

Table II. Effect of storage duration at  $0^{\circ}$  C on counts of  $\underline{\text{C.}}$  oregonensis eggs. Ten conelets, collected at Koksilah from the same tree on 7 May, 1978, were dissected after each storage duration.

Storage duration (days)	Mean no. eggs/conelet <sup>a</sup>
1	189
15	212
30	194
45	177
60	196

<sup>&</sup>lt;sup>a</sup> Counts were not significantly different, ANOVA, P > 0.75.

analysis (Sokal and Rohlf 1969). In the analyses, several computer programs were used, namely: simple data descriptions (P1D), simple linear regression (P1R), stepwise regression (P2R) (Dixon and Brown 1979); analysis of variance and covariance with unequal (FLUND) and equal (ENB2) sample sizes, and multiple range tests (ENB2, MWLRAT) (C.S. Simmons<sup>3</sup>). Methods for other analyses were taken from Sokal and Rohlf (1969).

### 3.0 REPRODUCTIVE BEHAVIOUR

### 3.1 Introduction

Little is known about the reproductive behaviour of <u>C</u>.

oregonensis. The attack period has been delineated (Johnson and Winjum

1960; Johnson 1963a; Johnson and Hedlin 1967) but emergence periods have not
been quantified nor has mating and oviposition behaviour been described.

The reproductive behaviour of other cecidomyiids is not well known, although
aspects have been studied in detail in a few species (Barnes 1948, 1956;

Coutin 1964; Summers 1975).

Sex pheromones have not been identified for any species of cecidomyiid and evidence for a pheromone has been reported for only one gall midge, the Hessian fly, <u>Mayetiola destructor</u> (Say) (Cartwright 1922).

Observations of adult clover seed midges, <u>Dasyneura leguminicola</u> (Lint.)

(Guppy 1961), suggest the presence of a pheromone.

Many cecidomyiids attack at specific host developmental stages (Barnes 1948, 1956; Coutin 1964). <u>C. oregonensis</u> attacks open conelets (Johnson and Winjum 1960; Hedlin 1961; Johnson 1963a), but the exact timing of oviposition in relation to seed-cone bud flush has not been reported.

The objectives of these studies were: 1) to quantify adult emergence, mating behaviour and ovipositional activity, 2) to determine whether or not a sex pheromone was involved in the mating behaviour of <u>C</u>. oregonensis, and 3) to develop a laboratory bioassay for use in pheromone identification.

### 3.2 Materials and Methods

### 3.2.1 Method of obtaining adult midges

The <u>C. oregonensis</u> adults used during this research were obtained as follows: i) infested, dry, mature cones were soaked in water for 24-72 h so fully developed larvae would emerge from the cones, ii) these larvae were placed in a peat moss/sand mixture in jars and kept outside to overwinter at Victoria, iii) the jars were checked periodically and changed when necessary to ensure that they were free of mould and algae and that the peat moss was moist, and iv) the overwintered larvae and associated duff were placed in 30.5 x 30.5 x 61.0-cm insect cages when the cold treatment was terminated.

### 3.2.2 Adult emergence patterns and mating behaviour

To monitor adult emergence, 3 insect cages containing overwintering <u>C. oregonensis</u> larvae were set up as follows: 23 March 1979, in the field at Koksilah; 13 January and 30 March 1981, in the laboratory (21°C); and 30 March 1981, outdoors at Victoria. The field cages were placed under trees to prevent the insects from receiving direct sunlight.

Emerged adult midges were collected, sexed and counted daily.

During the peak periods of emergence, hourly collections were made on 5 days in the outdoors and on 3 days in the laboratory from the cages set up in .

March 1981. Mating behaviour was observed in these cages after adults had emerged.

On 25 April, 1981, 30 newly-emerged females were placed in each of 2 insect cages. Sixty newly-emerged males were added to one of these cages. The females in the cage without males were observed in the field to determine the diel periodicity of apparent calling behaviour (sex pheromone).

release) on 25 and 26 April. The length of time per call was measured at the ages of 1, 6, 12, 30 and  $36 \pm 1/2$  h. The diel periodicity of mating was determined in the cage containing both sexes by counting the number of matings that took place each hour on 25 April. The insects in the mating cage were replaced on 26 April and numbers of matings each hour were again counted. Observations of calling and mating behaviour were repeated in the laboratory on 27 and 28 April using the same procedures.

# 3.2.3 Evidence for a sex pheromone

#### 3.2.3.1 Field trapping

In the spring of 1979 and 1980, traps were set out when the Douglas-fir seed-cone buds had begun opening, the time when adult gall midges start to emerge (Hedlin 1961). Each trap consisted of a 20 x 20-cm piece of white cardboard coated on one side with a thin layer of Tree Tanglefoot. A small cage, consisting of a 3 x 1.5-cm piece of polyethylene pipe screened at both ends, was inserted into a hole in the centre of each trap. Living insects, used as test lures, were placed in the cages. The traps were checked daily to determine the condition of the lure insects.

In 1979, the traps were hung vertically in the bottom third of the tree crowns, 1.52 to 2.13 m above the ground, at Tahsis, where tree heights ranged from 4.57 to 7.62 m, and in the middle and top thirds of the tree crowns, 0.91 to 1.52 m above the ground, at Dewdney, where tree heights ranged from 1.22 to 2.13 m. Tahsis is located approximately 180 m from the nearest mature Douglas-fir trees, whereas Dewdney borders on a stand containing mature Douglas-fir trees.

In each orchard, 5 trap lines were set out in a randomized complete block design on 20 April 1979. Each block contained one replicate of each of 4 lures: 1) 3 virgin females, ii) 3 virgin males, iii) 3 virgin females plus 3 virgin males (on the assumption that mating would occur), and iv) no insects (control). The traps were collected after 4 days and 5 more randomized blocks were set out. The second set of traps was collected 3 days later because 2 virgin males at each orchard died the day before the first set of traps was collected. The traps were examined in the laboratory after collection and the sexes and number of <u>C. oregonensis</u> caught were noted.

Observations of the mating behaviour of <u>C. oregonensis</u> that emerged in the laboratory prior to field emergence in 1980 established that mating occurred on the duff and that female midges flew only occasionally before they mated. These findings suggested that the traps should be placed at ground level, rather than in trees, in an area where cones are not harvested. Cone harvest in orchards results in the removal of immature midges, leaving few on-site midges to mature and emerge the next year. Therefore, the traps were located just 2-3 cm above the ground in 1980 in a small plantation at the Pacific Forest Research Centre (PFRC), Victoria, B.C.

Five randomized block trap lines were set out 18 April 1980, each line consisting of 1 replicate of each of 5 lures: i) 3 virgin females, ii) 3 virgin males, iii) 3 mated females, iv) 3 mated males, and v) no insects. These traps were collected 3 days later and replaced by 5 more randomized-block trap lines which were also collected in 3 days. Some of the mated males, 1 in the first 5 replicates and 3 in the second 5, lived only 2 days in the trap cages.

The data were analyzed by analysis of variance and the Duncan's multiple range test for differences among means.

## 3.2.3.2 Laboratory bioassay

Dr. K.N. Slessor<sup>4</sup> and Mr. V. Salas-Reyes<sup>4</sup> provided female extracts for laboratory bioassay. The ovipositors of calling females were excised and rinsed in heptane. Each ul of rinse was equivalent to 2 females.

All bioassays were carried out in the same general manner. Male midges were placed in a cage and allowed to settle for at least 1 h. A watch glass containing rinse from which the heptane had been evaporated was placed on the floor of the cage and the behaviour of the males was observed. A control bioassay was run immediately prior to a rinse bioassay using the same insects and watch glass. A volume of heptane equivalent to the volume of rinse to be tested in the following bioassay was placed on and evaporated from the watch glass before it was placed in the cage. Unless otherwise stated, each test was replicated 5 times, 1 µl of rinse and 20 males were used in each test, and all tests were run before 1100 h. Each male midge was used in only one pair of bioassays (one extract and one control).

Bioassays were run to determine the effects of the following on male responsiveness: i) cage size, ii) number of males per test, iii) age of males, iv) strength of rinse (number of female equivalents), and v) time of day. Two cage sizes were tested, one was the 30.5 x 30.5 x 61.0-cm insect cage screened on all sides and with a wooden floor, and the other

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was 24.0 x 35.5 x 9.0 cm with a plexiglass front, screened back, and wood on the other sides and floor. All subsequent bioassays were run in the smaller cages. Tests were run with 5, 10 or 20 males to determine the effect of numbers on responsiveness. Males in 2 age groups, less than 3 h old and 24-27 h old, were tested. To determine the effect of stimulus concentration, bioassays were run with 0.25, 0.5, 1.0 and 5.0  $\mu$ l of rinse, corresponding to 0.5, 1.0, 2.0 and 10 female equivalents, repectively. To determine the effects of time of day, bioassays were run at hourly intervals during the day. These bioassays were replicated 3 times. Analysis of variance and Duncan's multiple range test for  $\geq$  3 means were used to analyze the data.

## 3.2.4 Oviposition patterns

The length and width of the abdomen and head capsule were measured for 25 newly-emerged female midges which had been preserved in 70% ethanol. These females were dissected and the number of eggs they contained counted to determine with regression analysis the relationship between the number of eggs and size of the female (data not transformed).

Oviposition behavior of females presented with uninfested and aborted conelets was observed. Ten mated females were placed in each of 3 30.5 x 30.5 x 61.0-cm insect cages along with a field-collected Douglas-fir branch supporting several uninfested, open conelets. These conelets had been bagged prior to bud flush to prevent infestation. At the same time, the females were presented with a branch bearing aborted conelets. After a female had completed oviposition in a conelet that conelet was removed, dissected and the eggs counted. The branches were replaced with fresh ones when fewer than 5 conelets were left. After these females were observed

for 2 days, they were left to oviposit until they died. The dead females were preserved in 70% ethanol until they were dissected and the number of eggs they contained counted.

Douglas-fir twigs bearing uninfested open conelets were presented for 1, 2 or 4 days to 30-40 caged, mated females. Ten conelets were dissected after each exposure period and the numbers of eggs counted. The relationship between length of time spent on a scale and mean number of eggs was examined with a  $\chi^2$  test. Ten to 15 conelets from each exposure period and a similar number of uninfested conelets were placed in cages containing 10 mated female midges. The oviposition behaviour of these females was observed. When a female visited a conelet scale, and extended her ovipositor, the length of time the visit took was measured and the scale marked. After the female left the conelet, the conelet was removed and dissected. Visits that lasted  $< 1 \min$ , 2 to 3 min or  $> 5 \min$  were used in the analysis.

To determine the developmental stages of cones attacked by <u>C</u>.

<u>oregonensis</u>, conelets at different stages of development were collected in
1981 at 4 sites (number of trees sampled in parentheses): Metchosin (4),
Quinsam (4), Snowdon (4) and Tahsis (6). Five conelets were collected from
each tree at the following stages of cone development: i) bud burst (sheath
split and separating from conelet), ii) conelets open (scales fully
reflexed) for 1 day, iii) conelets open for 3 days, iv) conelets open for 7
days, and v) conelets closed and turning downward. These conelets were
dissected and <u>C</u>. <u>oregonensis</u> eggs counted. Analysis of variance and
Duncan's multiple range test were used to analyze the data.

#### 3.3 Results and Discussion

## 3.3.1 Adult emergence and mating behaviour

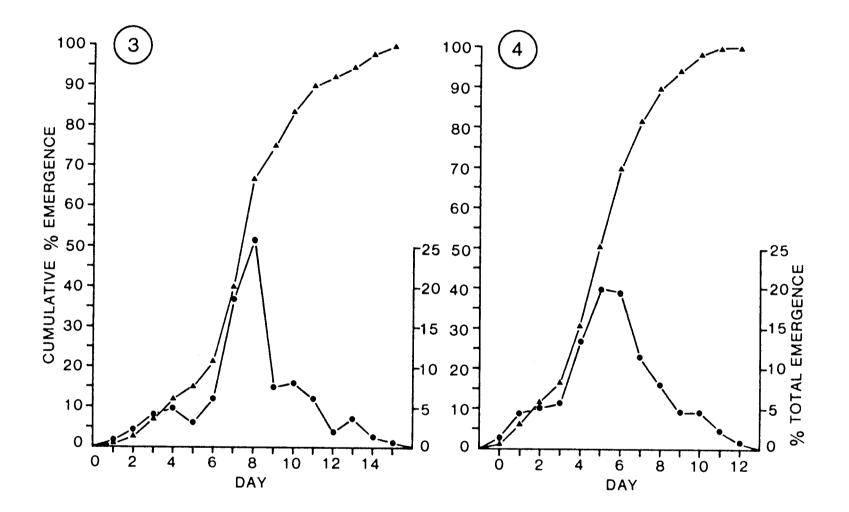
The emergence period of <u>C. oregonensis</u> in the field was short, lasting about 2 weeks and peaking after about 1 week (Figs. 3,4). In the laboratory, emergence that began on 25 February lasted 17 days, peaking on day 6 (Fig. 5) while emergence that began on 4 April lasted 11 days, peaking on day 4 (Fig. 6). Emergence periods of 2-3 weeks have been reported for several <u>Contarinia</u> spp. (Barnes 1948, 1956; Condrashoff 1963; Coutin 1964; Passlow 1965), including <u>C. oregonensis</u> (Hedlin 1961).

The amount of warming necessary for adult eclosion in the laboratory was related to the length of time the midges were chilled. Midges that were brought into the laboratory on 15 January, after 4 months of fall and winter exposure, began emerging after 41 days, while those brought into the laboratory on 30 March, after 6 1/2 months, began emerging after 4 days. Similar relationships between period of chilling and time of emergence have been noted in other cone and seed insects (Bakke 1970; D.S. Ruth<sup>5</sup>, pers. comm.).

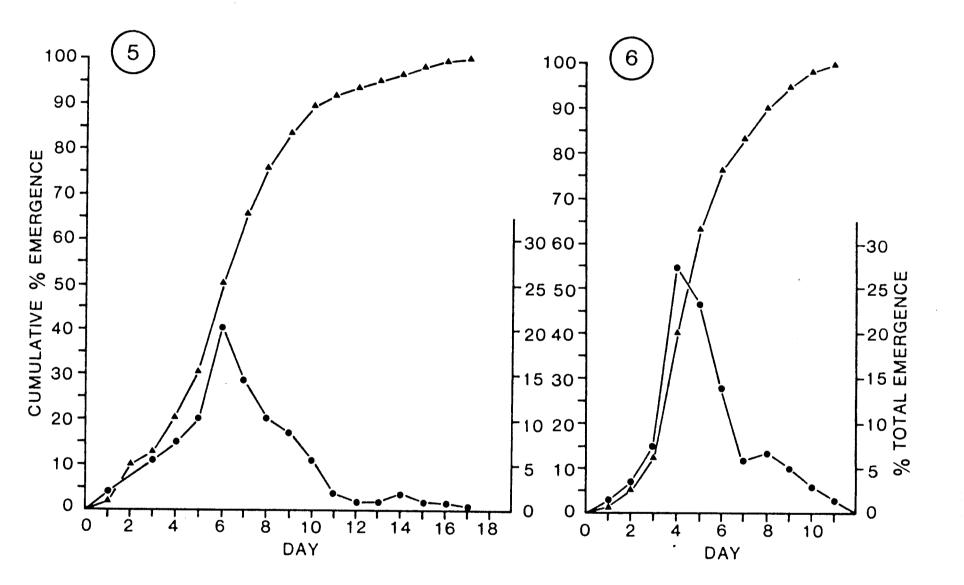
Daily emergence patterns for males and females did not differ in the field at Koksilah in 1979 (Fig. 7) or in the laboratory in 1981 (Fig. 9), but outdoors at Victoria in 1981 (Fig. 8), male emergence peaked on day 6, one day earlier than female emergence. Earlier peaking of male emergence has been reported for <u>Contarinia pseudotsugae</u> Condrashoff (Condrashoff 1963). Females constituted 53-58% of the total number emerged during all

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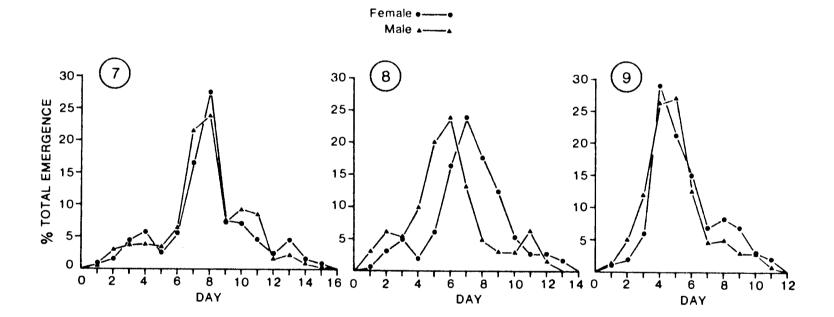
Figures 3,4. Daily emergence of <u>C. oregonensis</u> adults in the field: Fig. 3, Koksilah in 1979, day l = 18 April, N=176; Fig. 4, Victoria in 1981, day l = 20 April, N = 302.



Figures 5,6. Daily emergence of  $\underline{C}$ . oregonensis adults in the laboratory in 1981: Fig. 5, day l=25 February, N=118; Fig. 6, day l=4 April, N=174.



Figures 7-9. Daily emergence of adult male and female  $\underline{C}$ . oregonensis: Fig. 7, in the field at Koksilah in 1979, N=176; Fig. 8, outdoors at Victoria in 1981, N = 302; Fig. 9, in the laboratory in 1981 (4 April), N = 174.

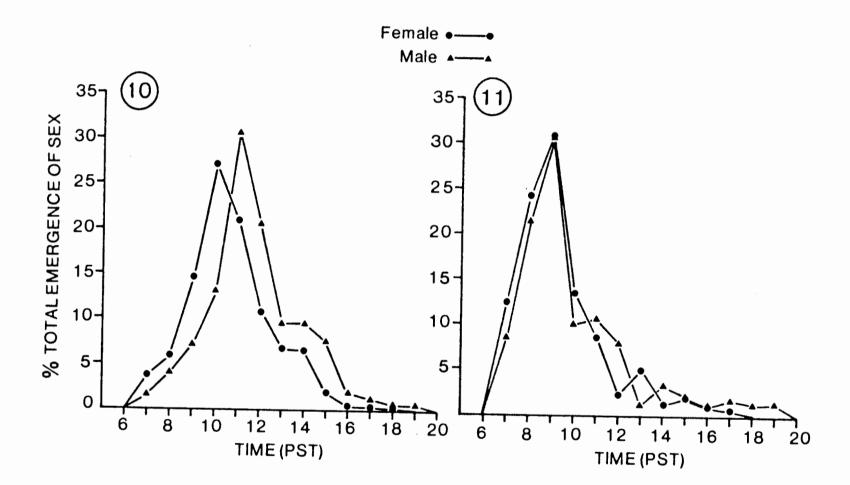


emergence periods. A predominance of females is common in Contarinia spp. (Barnes 1948, 1956; Condrashoff 1963; Coutin 1964; Summers 1975).

Adult eclosion began at dawn, between 0600 and 0700 h (PST), peaked at 1100 h, and continued until 1900 h outdoors (Fig. 10). Three-quarters of the adults had emerged by 1200 h. The pattern did not differ between sunny and cloudy days. In the laboratory, emergence began at dawn, peaked at 0900 h and was completed by 1900 h; and over 90% of the adults had emerged by 1200 h (Fig. 11). The difference between field and laboratory emergence patterns is probably due to the continuously warm temperatures in the laboratory compared to fluctuating temperatures out-Outdoors, female emergence peaked 1 h before male emergence (Fig. 10); females predominated between 0600 and 1000 h, after which males predom-In the laboratory, male and female emergence peaked at the same time (Fig. 11). The lack of difference in the emergence patterns of the sexes in the laboratory may be due to lack of variation in temperature. Summers (1975) found that emergence of the sexes in C. sorghicola was affected differentially by temperature and that differences between the sexes in time of emergence which occurred in the field did not occur in the laboratory.

The emergence patterns and male behaviour indicate that prevention of inbreeding is a dominant feature of  $\underline{C}$ . oregonensis adult behaviour. Male midges appear to require a premating flight before they will respond to females. Confinement in small vessels where flight was not possible immediately after emergence inhibited males, regardless of age, from responding to calling females in the same vessels. The amount of flight required was not great since males  $\leq 3$  h old, but which were held in cages where flight was possible, responded to female rinses (see following). The emergence

Figures 10,11. Diel periodicity of adult  $\underline{C}$ . oregonensis emergence: Fig. 10, outdoors, at Victoria, N = 193; Fig. 11, in the laboratory (4 April) in 1981 N = 108 (PST=Pacific Standard Time).



of males later in the day than females suggests that they have a premating flight immediately after emergence and respond to females sometime later, probably to those that emerge the next day. The 1-day earlier peak of male emergence would increase the probability of male-female contact after the male flight, especially when the short male life-span is considered. In these studies, males lived an average of 2.6 days; Hedlin (1961) reported 2.9 days. Female predominance of adult emergence early in the morning would promote immediate contact with the short-lived, but still vigorous males which emerged the preceeding day, and would also allow for the maximum amount of day-light hours for oviposition on the day of mating. Adult emergence and behaviour that optimizes the probability of male-female contact have been reported for other cecidomyiids (Barnes 1956; Harker 1961; Summers 1975).

Mating occurred on or near the duff. Prior to mating, females appeared to call (release sex pheromone) by fully extending their ovipositors and waving them back and forth (Fig. 12). Only virgin females exhibited this behaviour, and only they attracted males. Females called for the first time shortly after they emerged, sometimes before their wings were expanded. They flew only occasionally before mating but would climb up the side of the cage or other objects that contacted the duff. Males needed a period of activity before they would mate. The average time in copula was 11.3 sec (range = 3-21 sec). Short copulation periods have been reported for other cecidomylids (Reeher 1945; Guppy 1961).

Swarming is involved in the mating behaviour of some cecidomyiids (Downes 1969). However, in many species including <u>Contarinia spp.</u>, mating occurs on or near the duff or other source of emerging insects (Barnes 1948, 1956; Coutin 1964; Readshaw 1965) and not in swarms. Swarms of <u>C</u>.

Figure 12. C. oregonensis female (10 X actual size) exhibiting calling behaviour.



oregonensis females have been sighted on occasion, usually at the tops of trees or above branches bearing conelets. Swarming by females has also been reported in C. merceri Barnes, again in association with the host (Jones 1940). The reasons for such swarming are not known but they would appear to be associated with oviposition behaviour rather than mating.

The average time per call increased with the age of the females up to 30 h (Fig. 13). Females that reached 48 h of age appeared to call continuously; their ovipositors were fully extended until they died, but whether or not they were actually producing pheromone continuously is not known. Females that had not mated by 72-84 h of age lost their ability to retract their ovipositors and were not mated when males were present.

The number of calls per 30 caged females outdoors increased during the morning, peaked between 1100 and 1400 h, and then declined sharply until 1700 h after which there were no calls (Fig. 14). The number of calls peaked earlier in the laboratory than outdoors, between 0900 and 1000 h (Fig. 15). Photoperiod appears to determine the earliest time of day that mating can occur since no mating occurred before sunrise. The protracted time of mating in the laboratory is probably the result of the continuously warm temperature (21° C). Outdoors the numbers of calls and matings increased with temperature during the morning, suggesting a temperature effect. Photoperiod and temperature affect the time of day mating occurs in many insects (Sower et al. 1971; Sanders and Lucuik 1972; Carde et al. 1975; Baker and Carde 1979; Castrovillo and Carde 1979).

The number of matings was significantly (P < 0.05) correlated with the number of calls (r = 0.851 in the field; r = 0.956 in the laboratory) and followed the same diel pattern (Fig. 14, 15). The decline in numbers of calls and matings is probably the result of active females being mated.

Figure 13. Average time per mating call in relation to age of female. Females more than 48 h old appeared to call continuously.

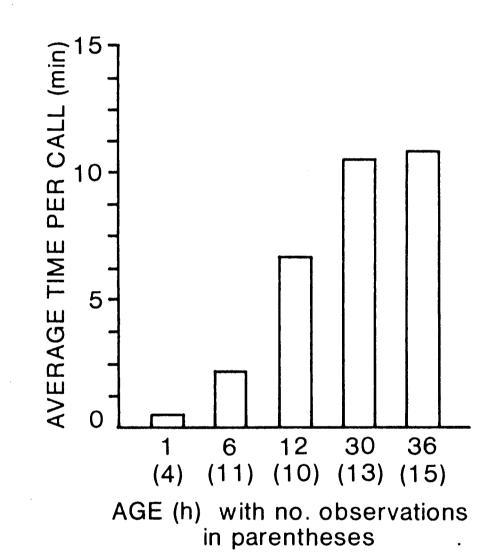
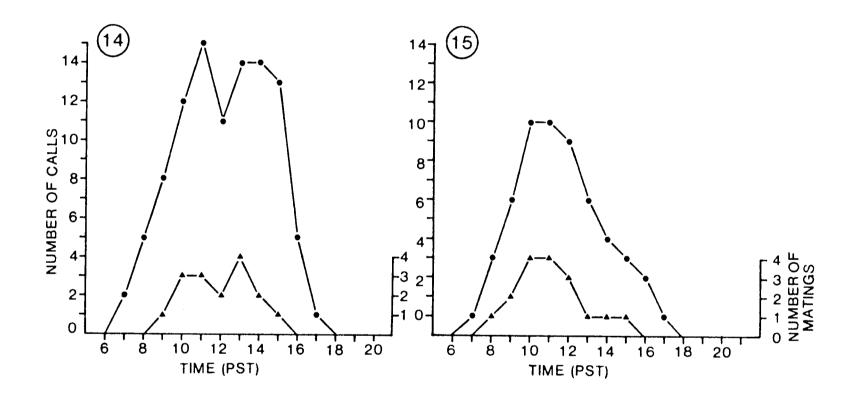


Figure 14,15. Diel periodicity of the number of mating calls and matings per 30 females: Fig. 14, outdoors at Victoria, Fig. 15, in the laboratory (4 April) in 1981 (PST = Pacific Standard Time).



Females mated only once whereas males mated several times, as reported in C. sorghicola (Walter 1941).

#### 3.3.2 Sex pheromone

#### 3.3.2.1 Field trapping

No midges were trapped at Tahsis in 1979. At Dewdney, significantly more male midges were caught on traps baited with virgin females or both sexes together than those baited with virgin males or on control traps (Table III). There was significant variation in catches between trap lines. The traps on the line nearest the edge of the adjacent natural stand caught several times more midges than traps on any other line. Two female midges were caught by the traps and there were no significant differences between lures. In 1980, traps baited with virgin females caught significantly more male midges than any other traps and there were no significant differences in mean trap catches among the other traps (Table III).

Observations of midge mating behaviour in the laboratory suggested possible reasons for differences between trap catches. Only virgin females exhibited calling behaviour and presumably released pheromone. Males appeared to need some period of flight before they would mate. The response in 1979 to traps containing both sexes would indicate that mating had not occurred in the cages, possibly because of the lack of a premating flight by the males.

Reasons for differences in trap catches between Tahsis and Dewdney are unknown but could be caused by the differences in locations of the traps relative to adjacent stands or to the differences in trap height relative to height of the tree crowns. The traps at Dewdney were considerably closer to a source of male midges than the traps at Tahsis. The importance of the

Table III. Numbers of  $\underline{C}$ . oregonensis males trapped in sticky traps using living insects as lures.

			No./caught/trap		
Year	Site	Lure	Mean <sup>a</sup>	Max	Min
1979	Dewdney	Virgin 🕈	6•9a	27	1
·		Virgin O	1.2b	8	0
		P + 0"	6.2a	28	0
		Control	0.4b	1	0
1980	PFRC	Virgin 9	13.2a	31	6
		Virgin of	0.6b	2	0
		Mated $Q$	0.5b	2	0
		Mated O	0.4b	2	0
		Control	0.1b	1	0

Means in the same year followed by same letter are not significantly different, Duncan's multiple range test,  $P \le 0.01$ .

proximity of the adjacent stand is supported by the larger trap catches on the trap line that was located nearest to the edge of the adjacent stand at Dewdney. This would also suggest that the pheromone has a short range. Cartwright (1922) found that the distance of attraction of virgin female Hessian flies was 3-5 m.

The need to place traps where females are emerging limits the potential usefulness of pheromones for monitoring <u>C. oregonensis</u> populations. Harvest of cones prior to the emergence of fully developed larvae removes potential overwintering midges from the orchards. Thus, traps should be placed in adjacent stands or under nearby mature trees to monitor the emergence of midges before they enter the orchard. Trapping outside orchards may not be feasible in all situations due to the presence of urban development around the orchards.

# 3.3.2.2 Laboratory bioassay

Rinses of ovipositors from virgin females were attractive to caged males in the laboratory (Table IV). Males did not respond at all to clean watch glasses or to those from which heptane had evaporated. Males responded to these rinses in several ways. They became generally agitated when the rinse was placed in the cage; locomotory activity (i.e. walking and flying) increased dramatically. Four specific responses were observed: i) flying back and forth or hovering in the column of air above the rinse, ii) landing on the watch glass, iii) searching the watch glass, and iv) wing buzzing on the spot of the rinse. Only the last 2 responses were recorded because they were the most positive responses and most males responded in one of these ways.

Table IV. Effects of various factors on responsiveness of male  $\underline{C}$ .

oregonensis to female rinse in the laboratory. N = 5 replicates except N = 3 for time of day bioassays. Duration of each replicate = 15 min. Unless otherwise stated, the small cages,  $1 \mu 1$  (2 female equivalents) and 20 males were used in each bioassay.

Mean % response	by males <sup>a</sup>	
Searching	Buzzing	
0 a	0 a	
10.5 b	5.5 b	
	18.0 cd	
	21.0 c	
17.0 d	13.5 d	
39.5	21.5	
41.5	25.5	
26.0	16.0	
	20.0	
30.0	21.0	
30.0	.21.0	
40.0	25.0	
40.0 a	27.5 a	
	21.0 a	
	21.5 a	
	3.8 b	
0 <b>c</b>	0 c	
	Searching  0 a 10.5 b 27.0 c 30.0 c 17.0 d  39.5 41.5  26.0 32.0 30.0  40.0  40.0  40.0  40.0  40.0  40.0  40.0  40.0  40.0  40.0	

If ANOVA significant, means for each factor followed by same letter are not significantly different, Duncan's multiple range test, when > 3 means, P  $\leq$  0.05.

Cage size, number of males and age of males had no significant effect on the proportion of males responding (Table IV). Cage size had a significant effect (t-test,  $P \le 0.01$ ) on the time needed to run bio-assay assays. The exposure time needed to elicit the maximum response of the males averaged 30 min (range = 23 - 38 min) in the large cage and 12 min (range = 8 - 15 minutes) in the small cage. Therefore, the smaller cages were chosen for standard bioassays.

The number of female equivalents and time of day had significant effects on male response (Table IV). One or 2 female equivalents illicited significantly more response than did 0.5 and 10 female equivalents. Nale response did not differ in bioassays run between 0800 and 1100 h but decreased significantly thereafter; no response occurred after 1200 h. The lack of difference in male responsiveness between 0800 and 1100 h indicates that the increase in numbers of matings in the laboratory during this period (Fig. 15) is determined by the increase in the numbers of females calling and not by an increase in male responsiveness. The decline in response after 1100 h corresponds with declines in the numbers of females calling and the numbers of matings (Fig. 15).

#### 3.3.2.3 Oviposition behaviour

Little is known about cecidomyiid host selection, including that of C. oregonensis. Chemoreception may be involved (Barnes 1932).

Once on the conelet, the female walks about with a stop and go movement characteristic of other cecidomyiids (Pitcher 1952; Coutin 1962, 1964; Parnell 1963), and periodically backs in between 2 scales. There was no obvious reason for selection of the scales. <u>C. oregonensis</u> females did not lower their heads to the surface of the conelets when selecting an

oviposition site, as do other cecidomyiids. <u>C. oregonensis</u> probably does not rely heavily on antennal chemoreception, as their oviposition sites are more easily located than are those of these other midges, which oviposit in injuries caused by other insects or other damaged areas of smooth-surfaced hosts.

Prior to egg deposition, the female extended her ovipositor and moved it back and forth across the surface of the scale. At this point the female either retracted her ovipositor and moved to another scale or conelet, or positioned the ovipositor and remained stationary for 1-27 min.

The number of eggs laid was significantly correlated (P < 0.05) with the length of time the female remained on the scale with her ovipositor extended (Fig. 16). Oviposition occurred during daylight hours, as reported by Hedlin (1961), the peak of activity occurring in the early afternoon. The average number of eggs per cluster laid in uninfested conelets was 5.4 (range = 1-38) (Fig. 17). Oviposition did not take place on aborted conelets even though these scales were visited, indicating that female midges can discriminate between healthy and dead conelets.

Numbers of eggs contained by females were significantly correlated with abdominal width and length (Fig. 18,19) but not with head capsule width (r = 0.294). The average number of eggs per female was 254, slightly more than the average of 238 reported by Hedlin (1961), and much higher than some other <u>Contarinia</u> spp. (Reeher 1945; Barnes 1956; Readshaw 1965). The average number of eggs remaining in dead females, which had oviposited at will over their life span, was 17.6, indicating that females laid 94% of their egg complement.

The oviposition behaviour of females was different on previously infested conelets than on fresh conelets. The numbers of visits to a scale

Figure 16. Number of eggs laid in relation to length of time the ovipositor was extended during a visit to a scale by a female.

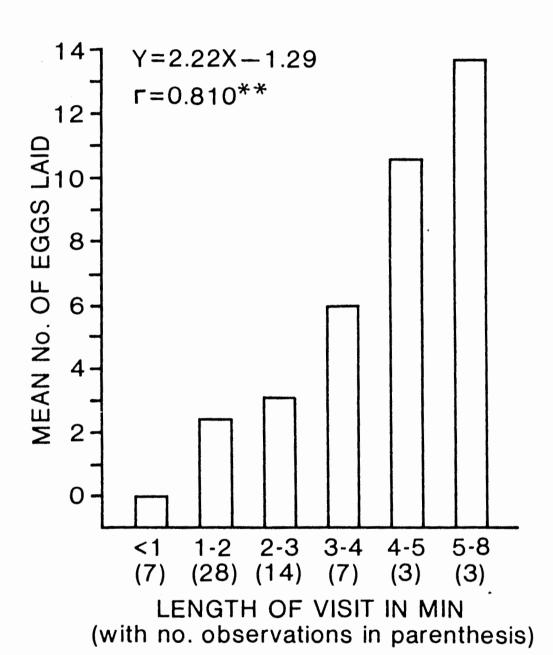


Figure 17. Frequency of eggs per scale laid by 10 females in uninfested conelets.

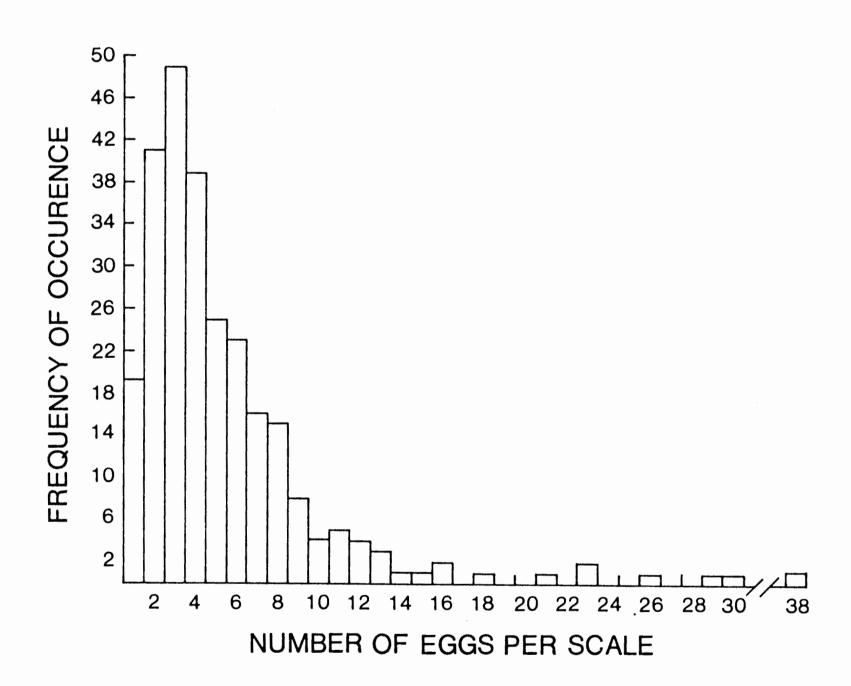
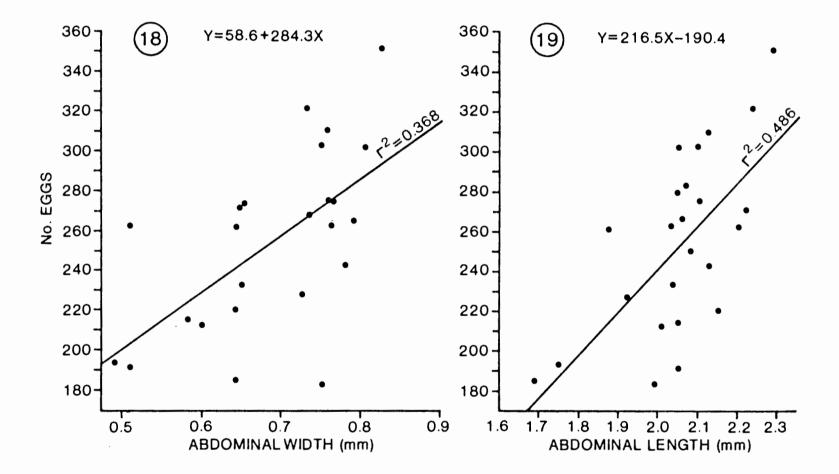


Figure 18,19. Relationship between number of eggs per female and her abdominal width (Fig. 18) or length (Fig. 19).



in which ovipositors were extended for < 1 min (exploratory visits), or > 5 min were positively correlated (P < 0.05) with the number of eggs previously laid, whereas the number of visits in which ovipositors were extended for 2-3 min was negatively correlated P (< 0.05) with the number of previously laid eggs (Fig. 20). The increase in exploratory visits suggests that oviposition was being deterred. Dissection of scales on which exploratory visits occurred showed that previously laid eggs were present in about 2/3 of the cases. Oviposition deterrence could be due to physical contact with a previously laid egg or to the presence of an oviposition-deterring or epideictic pheromone, as reported for numerous insects, including several dipterans (Prokopy 1981a, b), but not for any cecidomyiid. Epideictic pheromones have potential for reducing pest damage (Katsoyannos and Boller 1976). The decrease in visits in which the ovipositor was extended for 2-3 min and the increase in visits in which the ovipositor was extended for > 5 min suggests that females lay larger numbers of eggs when they do find suitable scales for oviposition.

The relationship between stage of cone development and <u>C</u>.

<u>oregonensis</u> infestation (Table V) suggests that young conelets are

preferred. At 2 1981 sites, Metchosin and Snowdon, the level of infestation
increased until the conelets had been open for 3 days but not thereafter,
even though adult cone gall midges were still active. However, infestations
did increase after this stage of development at the other 2 sites,
indicating that older conelets are acceptable for oviposition. Other
cecidomyiids, including other <u>Contarinia</u> spp., which attack reproductive
structures of plants are known to attack specific developmental stages
(Coutin 1964).

Figure 20. Relationship between length of previous exposure of a scale to ovipositing females and numbers of previously laid eggs, and duration of visits (ovipositor extended) to a scale by females; total number of visits was 386.

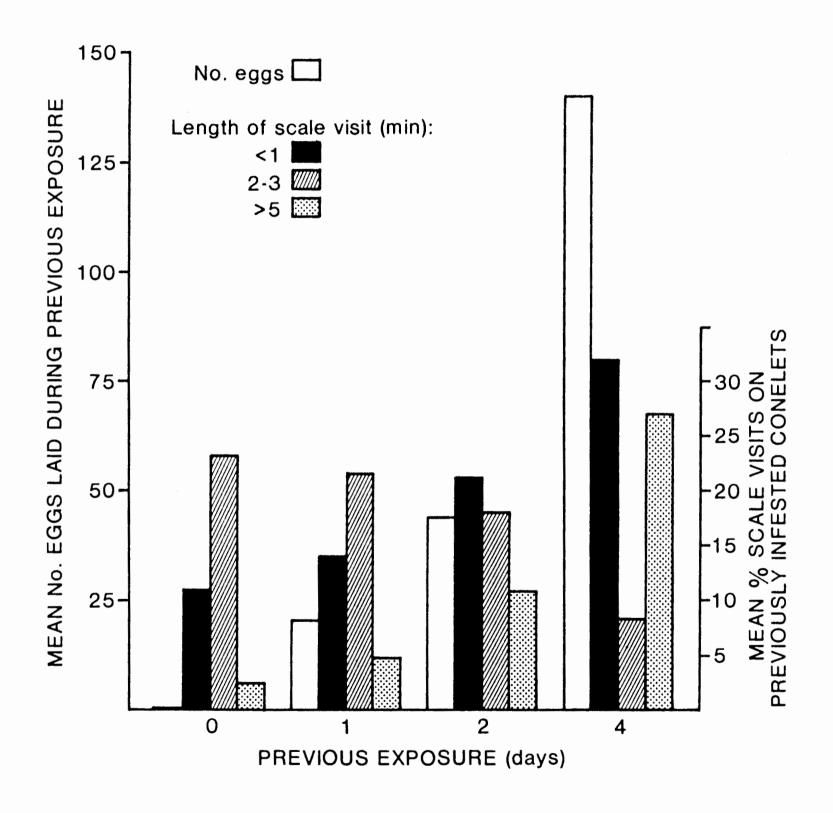


Table V. Infestation by  $\underline{C}$ .  $\underline{oregonensis}$  in relation to stage of cone development at 4 sites in B.C. in 1981.

Stage of cone		x no. eggs	/conelet	
development	Metchosin <sup>a</sup>	Quinsam <sup>a</sup>	Snowdon <sup>a</sup>	Tahsis
Bud burst	82.3 a	1.7 a	3.6 a	0.0 a
Open 1 day	163.7 ь	4.2 a	13.5 b	0.7 a
3 days	234.6 c	14.3 ь	29.3 c	12.8 ь
7 days	217.9 c		-	29.4 c
Closed and turning	228.2 c	23.7 с	27.6 c	31.8 c

Means for each site followed by the same letter are not significantly different, Duncan's multiple range test, P  $\leq$  0.05.

Production of unisexual families (insects in individual host organs are the same sex) have been reported for some cecidomyiids, including Contarinia spp. (Painter 1930; Barnes 1931; Baxendale and Teetes 1981). The genetic make up of the female has been suggested as the cause. In C. oregonensis, unisexual families occurred in 20 of 28 scales where midges from individual galls were reared separately. The presence of both sexes may indicate that > 1 female oviposited on that scale.

# 4.0 POPULATION SURVIVAL DURING THE SUMMER

# 4.1 Introduction

Very little is known about the population dynamics of C.

oregonensis. Fluctuations in cone crop size and the capacity for prolonged diapause (diapause lasting more than one winter) have been suggested as important factors determining population size (Hedlin 1964a). The following parasites have been reared from C. oregonensis: Torymus sp. (Hymenoptera: Eulophidae) and Platygaster sp. (Hymenoptera: Platygasteridae) in B.C. (Hedlin 1961); Torymus sp., Tetrastichus strobilus Burks (Hymenoptera: Eulophidae) and Zachalochlora milleri Crawford (Hymenoptera: Pteromalidae) in California (Bringuel 1968). All except Platygaster sp., are ectoparasites with one larva developing on each host. The endoparasite Platygaster sp., the only species not readily visible in the galls, kills the host only after it has left the gall and spun a cocoon in the duff. Parasitism by all species at Lake Cowichan in 1959 was 26% (Hedlin 1961).

Fluctuating cone crop size is an important factor determining population sizes of other cone and seed insects (Hussey 1956; Abrahamson and Kraft 1965; Kraft 1968; Mattson 1971, 1976, 1980; Forcella 1980).

Competition, a function of both crop size and insect population size, is also important (Hussey 1956; Kraft 1968). Natural enemies were minor factors in the regulation of populations of the Douglas-fir cone moth during a life table study (Nebeker 1977), although high rates of parasitism have been reported (Keen 1958; Hedlin 1960). The incidence of parasitism in other cone-infesting cecidomyiids has ranged from 20 to 37.5% (Hedlin and Johnson 1963; Hedlin 1964b).

The objective of this study was to determine factors that affect survival of  $\underline{C}$ . oregonensis between oviposition and cone harvest in seed orchards.

## 4.2 Materials and Methods

Cones were collected in the spring when conelets had closed and approached the pendant position and at cone harvest in August or early September. The sites and numbers of trees sampled were: Dewdney 10, Koksilah 6, Lake Cowichan 10, PFP 9, Quinsam 10, Snowdon 10, and Tahsis 9 in 1979; Koksilah 19 in 1980; Lake Cowichan 3, Quinsam 4, and Snowdon 4 in Ten conelets and cones were collected from each tree in 1979 and 1981. 1980, and 20 in 1981. The conelets were dissected and the numbers of eggs and egg infested scales counted. The cones collected at harvest were dissected and the numbers of galls, midge larvae, ectoparasites and filled seeds counted. To determine the rate of parasitism by endoparasites, larvae were manually removed from each gall and placed in jars, segregated by tree, with duff to spin cocoons and overwinter. Up to 500 larvae were reared from each tree. These cocoons were dissected 2 months later to determine the incidence of endoparasites. This proportion was multiplied by the original number of larvae in each cone to estimate the rate of parasitism.

Relations between numbers of midge larvae or galls and numbers of eggs and parasites per conelet or cone were examined with regression analysis. Tree averages were used in the analysis. The Y-intercept was set at 0, except when comparing number of galls to numbers of parasites. The comparative roles of natality and mortality can be determined by correlating the number of eggs with the size of the final population (Southwood 1978).

To determine the effects of intraspecific competition on insect size and associated fecundity, gall midge larvae were manually removed from galls which contained 1, 3, 6, 12-15, and > 25 larvae, and placed in jars with duff to overwinter in 1980. The larvae were segregated according to the number of larvae per gall, e.g., larvae from a gall containing 3 larvae were overwintered with larvae from other galls that contained 3 larvae. In the spring after adult eclosion, the abdominal lengths of 20 midges from each jar were measured and compared with analysis of variance and Duncan's multiple range test.

To determine the effects of interspecific competition (accidental predation), cones infested by the Douglas-fir cone moth and/or pyralid coneworms at Koksilah in 1978 were dissected and the numbers of gall midge larvae counted. Only trees bearing 5 or more moth-infested cones were used in the analysis. Counts in moth-infested cones and cones infested by C. oregonensis only from the same trees were compared by a t-test.

At Koksilah in 1978, some scales died and turned brown prematurely, a symptom of heavy gall midge attack (Johnson and Heikkenen 1958). Cones showing this symptom were collected in the first week of August and dissected. The number of midge larvae on each scale in these cones were counted and the condition of the scale noted. The differences among means of proportions of dead scales were tested with a  $\chi^2$  test.

The numbers of eggs in aborted and healthy conelets were counted at Koksilah in 1978 to determine whether or not abortion of conelets could be a significant midge mortality factor. Ten aborted and 10 healthy conelets were collected from each of 12 trees. Differences between means were compared with a t test.

## 4.3 Results and Discussion

Variations among trees in the number of eggs laid explained from 93 to almost 100% of the variation in the numbers of larvae present at cone harvest (Table VI). Mortality was not related to egg or larval density at any site. Thus, natality is more important than mortality in determining C. oregonensis population densities in seed orchards. A similar situation occurs in the olive fly, <a href="Dacus oleae">Dacus oleae</a> (Gmelin) (Diptera: Trypetidae), where numbers of eggs laid determined the subsequent number of pupae (Kapatos et al. 1977). In the yew gall midge, <a href="Taxomyia taxi">Taxomyia taxi</a> (Inchbald) (Diptera: Cecidomyiidae), the key factor in mortality was failure to achieve maximum fecundity (Redfern and Cameron 1978). The mortality rate was relatively constant, with respect to density, in Douglas-fir needle midges, <a href="Contarinia">Contarinia</a> spp. (Condrashoff 1963), which suggests that natality is more important than mortality in determining the numbers of surviving larvae.

Percent parasitism was low, < 1 - 12.4% for ectoparasites and < 1 - 6.5% for endoparasites, with the largest total parasitism rate at < 14% (Table VII). The relatively low rates of parasitism compared to those previously reported (Hedlin 1961; Kozak 1963) may indicate that parasites are not as common in seed orchards as in forest stands due to either the lack of movement of parasites into orchards or to the use of insecticides in orchards. The parasite species appeared to be <u>Torymus</u> sp. and <u>Platygaster</u> sp. The number of ectoparasite larvae increased with the number of host larvae at 6 of 8 sites examined individually while endoparasites showed this response at only 3 sites (Table VIII). The increase in parasite numbers with host numbers indicates that parasite densities are dependent on host density, as has been reported for the yew gall midge (Cameron and Redfern 1978), although such a relationship is obviously not consistent. Neither

Table VI. Relationship (log-log) between mean numbers of larvae per cone at harvest and numbers of  $\underline{C}$ . oregonensis eggs per conelet. All regressions comparing numbers of larvae to eggs were highly significant, P < 0.001.

			Mean no.		Regression parameters <sup>b</sup>			
Year	Orchard	Eggs/ conelet	Larvae/ cone	Survival (%)	Coefficients	r <sup>2</sup>		
1979	Dewdney	31.0	11.2	37.6	0.693 <u>+</u> 0.138	0.936		
	Koksilah	101.1	61.3	62.6	0.890 <u>+</u> 0.067	0.996		
	Lake Cowichan	40.0	17.4	34.9	$0.735 \pm 0.124$	0.951		
	PFP	45.8	28.3	63.0	0.873 <u>+</u> 0.039	0.997		
	Quinsam	64.2	43.5	72.3	0.915 ± 0.027	0.999		
	Snowdon	26.1	16.5	75.3	$0.851 \pm 0.061$	0.991		
	Tahsis	175.5	91.8	58.0	0.879 + 0.055	0.994		
1980	Koksilah	33.8	19.0	52.3	0.820 + 0.046	0.988		
-	All <sup>a</sup>	56.1	30.1	52.7	0.821 <u>+</u> 0.030	0.971		

Includes Lake Cowichan, Quinsam and Snowdon in 1981, these are not listed individually because of small sample size.

b Based on data transformed by  $log_{10}$  (x + 1).

Table VII. Levels of parasitism of  $\underline{C}$ . oregonensis by ectoparasites and endoparasites in B.C. seed orchards.

		Pa	arasitism (%)	
Year	Orchard	Ectoparasites	Endoparasites	Total
1979	Dewdney	6.2	2.6	8.8
	Koksilah	12.4	1.1	13.5
	Lake Cowichan	4.5	0.3	4.8
	PFP	9.4	1.8	11.2
	Quinsam	1.9	0.6	2.5
	Snowdon	1.0	1.2	2.2
	Tahsis	10.1	2.5	12.6
1980	Koksilah	0.6	6.5	7.2
1981	Lake Cowichan	9.3	4.1	13.4
	Quinsam	2.6	0	2.6
	Snowdon	0	0	0

Table VIII. Regression parameters comparing numbers of ectoparasites or endoparasites to numbers of host larvae.

			per cone '	Regression parame	Regression parameters <sup>a</sup>			
Year	Orchard	No. larvae	No. parasites	Coefficientb	r <sup>2</sup>			
Ectopa	arasites							
1979	Dewdney	11.2	0.8	0.236 <u>+</u> 0.111***	0.717			
	Koksilah	61.3	8.0	0.476 <u>+</u> 0.203**	0.879			
	Lake Cowichan	17.4	0.6	0.175 <u>+</u> 0.095**	0.659			
	PFP	28.3	2.1	0.339 <u>+</u> 0.081***	0.921			
	Quinsam	43.5	0.2	$0.079 \pm 0.045 \text{ ns}$	0.203			
	Snowdon	16.5	0.1	$0.081 \pm 0.048 \text{ ns}$	0.212			
	Tahsis	91.8	9.2	0.462 ± 0.115***	0.915			
1980	Koksilah	19.0	0.3	0.072 <u>+</u> 0.061*	0.258			
Endopa	arasites							
1979	Dewdney	11.2	0.5	$0.149 \pm 0.131*$	0.421			
	Koksilah	61.3	0.8	$0.079 \pm 0.056 \text{ ns}$	0.173			
	Lake Cowichan	17.4	0.2	$0.005 \pm 0.005 \text{ ns}$	0.013			
	PFP	28.3	0.5	0.101 <u>+</u> 0.092*	0.437			
	Quinsam	43.5	0.3	$0.005 \pm 0.005 \text{ ns}$	0.014			
	Snowdon	16.5	0.2	$0.027 \pm 0.018 \text{ ns}$	0.046			
	Tahsis	91.8	0.3	0.153 ± 0.143*	0.432			
1980	Koksilah	19.0	0.3	$0.102 \pm 0.098 \text{ ns}$	0.288			

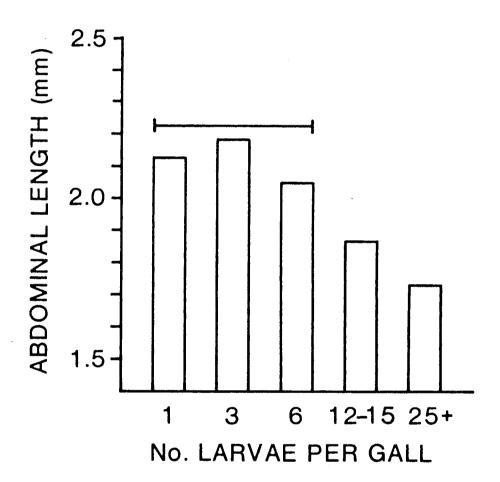
<sup>&</sup>lt;sup>a</sup> Based on data transformed by  $\log_{10}$  (x+1). Y-intercept set at 0.

b \*\*\* =  $P \le 0.001$ , \*\* =  $P \le 0.01$ , \* =  $P \le 0.05$ , ns = not significant.

numbers of galled scales nor numbers of filled seeds were related to numbers of parasites, indicating that parasites had no effect on amounts of damage caused by the midge, as shown in another cone and seed insect. In Great Britain, parasitism rates of >90% did not prevent seed losses of >90% to Douglas-fir seed chalcid (Hussey 1955, 1956). Parasites are not useful agents of gall midge control in seed orchards. They may be beneficial in nearby stands where they reduce the number of midges that could invade the orchard.

Intraspecific competition due to crowding can be a source of mortality in some cone and seed insects (Hussey 1956; Kraft 1968), fruit insects (Geier 1964) and gall midges (Redfern and Cameron 1978). As C. oregonensis larvae are isolated from each other in individual feeding chambers within the gall, canabalism would not occur, as it does in the Douglas-fir cone moth and seedworms (Cydia spp.). The oviposition behaviour of C. oregonensis, i.e. eggs are laid in clusters even when population densities are low, suggests that competition is not detrimental until a high critical density is reached. Crowding did not appear to result in outright mortality (unless the scale was killed) in that few dead larvae, other than those parasitized, occurred in galls, regardless of the number of larvae on each scale. However, crowding did affect the size of resultant adults, and presumably adult fecundity (Fig. 20), when the number of larvae per gall was large. Adults from galls containing 6 or fewer larvae were not significantly different in size but these were significantly larger than adults from galls containing 12 - 15 or > 25 larvae (Fig. 21). Reductions in size and fecundity occurred in the lucerne flower midge, Contarinia medicaginis Keiffer, when more than 6 larvae infested a flower (Strebler 1977).

Figure 21. Effects of larval crowding on size of resultant adults. Bars under common line are not significantly different, Duncan's multiple range test, P<0.05, n=20.



Larval competition for scales may be important, especially when many eggs occur on a scale. Up to 160 eggs have been found on a single scale. Dead first-instars were found on the outside of, or partially within, scales which contained healthy larvae. It was not possible to quantify this relationship due to problems involved in data collection. Redfern and Cameron (1978) found larval competition for buds to be an important mortality factor in the yew gall midge. The reasons for pre-establishment mortality are not known but could be due to physiological changes in infested scales which prevent establishment of further larvae, or to a build-up of toxins associated with egg hatch. Such mechanisms would prevent excessive numbers of larvae from penetrating scales and would conserve the larvae already inside.

The number of gall midge larvae per cone was reduced (t-test,  $p \le 0.01$ ) from a mean of 193.1 larvae in cones with <u>C. oregonensis</u> only to a mean of 99.7 in cones with 1 lepidopteran larva. In the 3 cones infested by 2 lepidopterans, the mean number of midges was reduced to 8.3. These data are consistent with the observation that competition with the Douglas-fir cone moth and coneworms is a significant mortality factor since these lepidopterans will eat gall midge larvae as well as cone tissues as they mine cones. The extent of interspecific competition between gall midges and cone moths would depend on size of the cone crop and population levels of the insects. Interspecific competition would be heaviest in years of light cone production at sites where all species were abundant. Heavy infestations of moths on the coast occur only in localized areas.

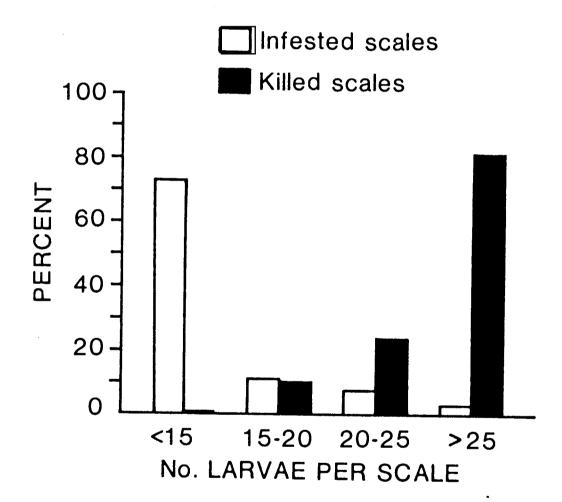
Abortion of conelets in seed orchards has ranged up to 80% with some trees completely aborting their crop (C. Bartram<sup>6</sup> pers. comm.). Therefore, a major influence on <u>C. oregonensis</u> population levels could be the aborting of conelets. Dissections of conelets showed means of 212.8 and 232.6 eggs in aborted and healthy conelets respectively (t-test,  $P \ge 0.40$ ). Presumably, the conelets aborted after gall midge oviposition since midges will not oviposit in aborted conelets.

Death of scales can be caused by larval feeding when the number of midge larvae exceeds 15-25, the carrying capacity of the scale (Fig. 22). Dead scales bore a mean load of 28.9 midge larvae. The incidence of dead scales due to gall midge infestation was much less than 1% during this study. However, during heavy infestation years, much higher proportions of scales can be killed (D.S. Ruth<sup>5</sup>, per. comm.).

In summary, studies of population survival and mortality factors over the maturation period of cones (1 summer) did not identify any agent that could be used in seed orchards for reducing damage by <u>C. oregonensis</u> since all mortality factors were associated with damage to seeds. For natural mortality agents to be useful as control agents, it must be possible to manipulate them outside of seed orchards in nearby stands in order to reduce gall midge populations that could invade seed orchards.

Forester, Silvicultural Branch, B.C. Ministry of Forests, Victoria, B.C.

Fig. 22. Percent of 1488 scales in 31 Douglas-fir cones infested and killed by  $\underline{\text{C.}}$  oregonensis at different densities.  $X^2$  test indicated significant differences in the proportions of dead scales among infestation levels.



## 5.0 SAMPLING PLANS

# 5.1 Estimating Numbers of Douglas-fir Cones

#### 5.1.1 Introduction

The size of a cone crop determines the management costs that can be economically justified. As a result, having a method for estimating the size of a crop is a necessary tool for efficient orchard management. The importance of estimating crop size in determining the need for insecticide applications has been pointed out by Yates (1977) and Miller (1982). Treating crops for insect control is not always justified, depending on several factors including crop size (section 6.2.1.3.3). The distribution of cones must be known if sampling techniques for estimating population sizes of cone and seed insects are to be developed since cones are not distributed evenly between trees or within crowns.

The pattern of occurrence of cones within tree crowns has been determined for slash pine (DeBarr et al. 1975), red pine (Mattson 1979) and Douglas-fir (Winjum and Johnson 1964). Aspect and verticle crown strata have been studied separately in some pines (Hard 1964; Smith and Stanley 1969). The study on Douglas-fir was carried out on unmanaged open-grown trees ranging from 9.6 to 22.4 m tall, and covered a wide range in numbers of cones per tree. The effects of cone density on cone distribution were not examined, a factor which appears to affect distribution (Kozak 1963), and a method for estimating size of cone crops was not proposed. To maintain and manage B.C. orchards as efficiently as possible, the desired tree height is 7.6-9.2 m (Konishi<sup>1</sup>, per. comm.). Trees are topped to maintain this height.

Sizes of cone crops on Douglas-fir can be estimated using binocular counts of cones on individual branches (Garman 1951; Winjum and Johnson

1962; Schenk et al. 1972), but the accuracy of such methods is less than desirable for use in orchards. Nebeker and Overton (1979) estimated the number of cones in a seed production area using an index- probability method which did not take into account within-crown variance, again without the accuracy needed for use in orchards.

The purpose of this study was to determine the distribution of conelets in the crowns of trees in Douglas-fir seed orchards and to develop a technique for estimating numbers.

#### 5.1.2 Materials and Methods

Tree crowns were divided evenly into 12 cells by aspect (north, east, south, west) and crown level (upper, mid and lower). The numbers of total branches, branches producing conelets and conelets were determined for each cell. These counts were segregated by branch type, i.e. whorl or internodal. In addition, 2 randomly-selected whorl branches in each cell were measured and the conelets on each counted. The counts were made in May or June, depending on orchard and year. Both healthy and aborted conelets were counted to determine the actual numbers present at the time of bud flush.

Conelet-bearing trees were randomly selected in 1978 and 1980 while in 1981, trees were visually classified as lightly, moderately or heavily producing and 8 to 10 trees were randomly selected in each category at each orchard. The orchards and numbers of trees sampled were: Koksilah 12 and Quinsam 12 in 1978; Koksilah 33, PFP 15, Quinsam 10 and Tahsis 15 in 1980; Koksilah 28, Quinsam 30 and Snowdon 30 in 1981.

Analysis of covariance, using total branches as the covariate, was used to analyze the conelet distribution data. Stepwise regression analysis

was used to relate total numbers of conelets to various sample cells. The relation between numbers of conelets and branch length on producing branches was examined for each level individually using simple regression with the intercept set at 0. Analysis of variance was used to examine the effects of crop size and tree topping on distribution of conelets. Duncan's multiple range test was used to test differences among means.

Formulae recommended for the calculation of sample sizes for finite populations via interation (Cochran 1963; Freese 1962) are:

$$n_0 = \frac{t^2 s^2}{p^2 \frac{1}{x}}$$
 (1)

$$n = \frac{n_0}{1 + \frac{n_0}{N}}$$
 (2)

where  $S^2$  is the population variance,  $\bar{x}$  is the population mean, P is the desired precision as a proportion, t is the Student t value which varies with sample size, and N is the population size. The population mean was known but the population variance was not in all cases. Population variance can be estimated from sample variance (Cochran 1963) with:

$$S^2 = \frac{s^2}{n} \left( 1 - \frac{n}{N} \right) \tag{3}$$

where  $s^2$  is the sample variance, n is the sample size and N is the population size. Stauffer (1982) points out that iteration using formula 1

does not always converge as would be expected; dividing both sides of the equation by  $t^2$ , so that  $\frac{n}{t^2}$  equals a fixed value, avoids the problem

of lack of convergence. Attempts to use formula I showed that convergence was not occurring; therefore, Stauffer's (1982) modification was used. Only significant factors in the distribution of conelets were considered in the development of the sampling technique. Data for insignificant factors were pooled prior to calculation of sample size.

Estimates of total numbers of conelets on whorl branches in the upper and mid levels using the branches that were measured in each sample cell were made to test the precision of the calculated sample sizes. The branches were randomly selected for the test and the conelet counts were transformed by  $\log_{10}$  (x+1) before estimating the accuracy achieved. These estimates were then inserted into the appropriate regression equation, depending on year, to estimate total numbers of conelets on each tree and these estimates were compared to actual counts to estimate the precision achieved.

#### 5.1.3 Results and Discussion

Crown level, tree and the interaction between level and tree were significant factors in the distribution of Douglas-fir conelets in all orchards (Table IX). Aspect was significant at Koksilah in 1980 but not in 1978 or 1981 or at any other orchard. In forest stands, aspect was significant in trees 9.6 to 22.4 m tall; the south side of the tree produced more cones than any other aspect (Winjum and Johnson 1964). A similar pattern has been reported for red pine in seed production areas (Mattson 1979). In the instance in my study where aspect was significant, the north and east

Table IX. Significance of factors in the distribution of conelets in crowns of trees 4.0 to 9.2 m tall in B.C. Douglas-fir seed orchards. Total branches was used as the covariate in the analysis of covariance. Conelets counts were transformed by  $\log_{10}(x + 1)$  before analysis,  $*=P \le 0.05$ ,  $**=P \le 0.01$ ,  $***=P \le 0.001$ , ns = not significant.

Year	Orchard	Aspect	Crown level	Tree	Aspect x level	Aspect x tree	Level x tree
1978	Koksilah	ns	***	***	ns	ns	***
	Quinsam	ns	***	***	ns	ns	***
1980	Koksilah	*	***	***	ns	*	***
	PFP	ns	***	***	ns	ns	***
	Quinsam	ns	**	***	ns	ns	ns
	Tahsis	ns	***	***	ns	ns	***
1981	Koksilah	ns	***	***	ns	ns	***
	Quinsam	ns	***	***	ns	ns	***
	Snowdon	ns	***	***	ns	ns	***

quadrants produced the largest numbers of conelets. A significant aspect x tree interaction was due to aspect being insignificant on some trees. The differences in distribution between my study and that of Winjum and Johnson (1964) could be due to differential survival among various parts of tree crowns as the cones mature, since I counted conelets while Winjum and Johnson (1964) counted mature cones. The differences may also be due to differences in tree height or stand density. Larger crowns associated with the taller trees in Winjum and Johnson's (1964) study may result in greater variations in the light intensity received by different parts of the crowns; cone production appears to be positively affected by greater light intensities (Puritch 1977). Stand density affects both the numbers of cones produced and the distribution of the cones (Matthews 1963; DeBarr et al. 1975; Mattson 1979).

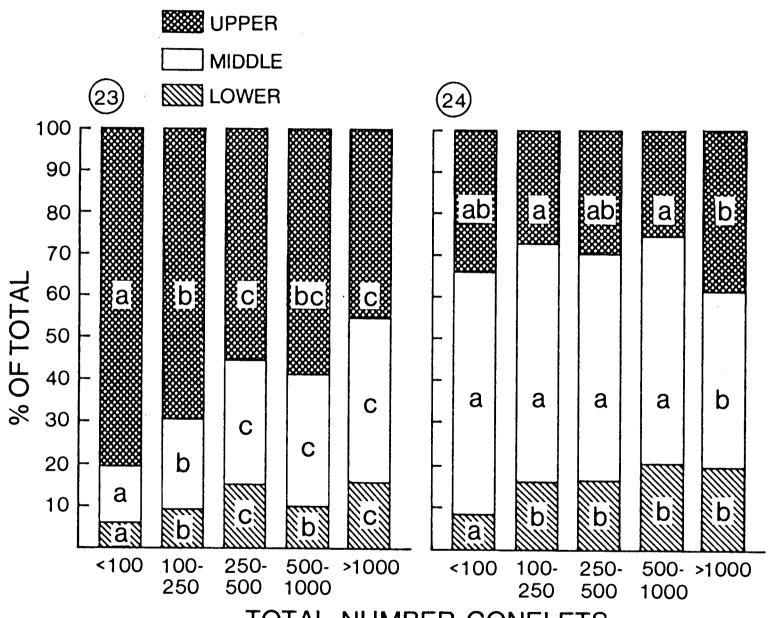
The relative importance of each crown level in cone production differed among years (Table X). In 1980, the mid crown was the most productive level at all 4 orchards, whereas in 1978 and 1981, the upper crown was the most productive. The reasons for the differences among years are not known, however, cone density was not the reason. Variations among sites in relative importance of each crown level have also been reported for red pine (Mattson 1979). In subsequent analyses, 1978 and 1981 data were analyzed together because of the similar conelet distributions. The relative importance of each level varied with the size of the total crop on each tree (Fig. 23,24). The proportion of the crop in the upper level increased as the total crop decreased while the proportion in the lower level increased with increasing total crops in 1978 and 1981. In 1980, distribution was not affected by total crop size to the same degree as in 1978 and 1981. The distribution did not differ significantly when trees

Table X. Mean numbers of conelets on whorl and internodal branches in each crown third of trees 4.0 to 9.2 m tall in B.C. Douglas-fir seed orchards, 1978-81.

Branch	Crown	1978	78		19	1980			1981	
type	third	Koksilah <sup>a</sup> Quinsam <sup>ë</sup>	Quinsam <sup>a</sup>	Koksilah <sup>a</sup>	pf.pa	<b>Quinsam<sup>a</sup></b> Tahsis <sup>a</sup>	Tahsis <sup>a</sup>	Koksilah <sup>a</sup>	Quinsam	Snowdon
Whorl	Upper	153.6 a	134.2 a	35.7 a	25.0 a	19.3 a	8.6 a	101.3 a	37.5 a	34.7 a
	Mid	108.8 b	92.3 b	75.8 b	32.7 b	29.2 b	36.0 b	67.1 b	24.2 b	15.5 b
	Lower	46.2 c	21.0 c	24.8 c	26.7 a	8.9 c	10.0 a	15.2 c	18.8 c	5.7 c
Internodal	Upper	14.4 a	13.1 a	9.8 a	10.7 a	7.3 a	3.5 a	9.8 a	1.6 a	3.5 а
	Mid	7.1 b	3.6 b	4.7 b	5.0 b	10.3 a	4.6 a	3•3 b	0.2 b	0.9 b
	Lower	1.1 c	0.8 c	0.5 c	1,3 c	1.0 b	0.2 b	o 6 ° 0	0.2 b	o 0

Means for each branch type in each orchard followed by the same letter are not significantly different, Duncan's multiple range test,  $P \le 0.05$ . ๗

Figures 23, 24. Proportional distribution of conelets in crown levels of Douglas-fir 4.0 to 9.2 m tall in B.C. seed orchards in relation to crop size: Fig. 23, 1978 and 1981; Fig. 24, 1980. Differences among crop sizes for each level are not significantly different (Duncan's multiple range test,  $P \le 0.05$ ) if noted with the same letter.



TOTAL NUMBER CONELETS

produced over 100 conelets. The reasons for the differences among years are not known. In 4 of 6 trees with over 2,500 conelets, there were no significant differences between levels, each level contributing about 1/3 of the total. Visual estimation suggested that topping of the trees stimulated more conelet production lower in the crown; however, there were no significant differences between topped and non-topped trees in the distribution of conelets.

Stepwise regression analysis showed that variations in total numbers of conelets on each tree were related to variations in numbers of conelets in the upper and mid crowns (Table XI). Inclusion of the lower crown added little to the overall relationship. However, the lower crown did add significantly to the relationship in trees with more than 2,500 Total numbers of conelets were strongly related to the number on whorl branches, whereas adding numbers on internodal branches added little to the relationship (Table XI). Internodal branches produced only 9.0% of the total conelets in my study and 9.1% of the cones in the study by Winjum and Johnson (1964). The relationship between total conelets and numbers of conelets on whorl branches within each level showed the same pattern as total conelets and numbers in each level. Regression parameters relating total numbers on trees to the sample cells that accounted for most of the variations in these totals are listed in Table XII. Tree totals would be estimated most efficiently by sampling whorl branches in the upper and mid crown levels. Both levels should be sampled because their relative productivities may vary among years or orchards, e.g., in 1980 the mid crown was the most productive while the upper crown was the most productive in 1978 and 1981.

Table XI. Increases in explained variation of total conelets when related to total conelets in each crown level, total conelets on whorl and internodal branches, and conelets on whorl branches in each crown level by stepwise regression analysis. All regression coefficients were highly significant, P<0.001.

Year	Factor	Step a no.	Variable	2 <sup>b</sup>	Increase 2 in r
1978	Crown level	1	Upper	0.9205	
and 1981		2	Mid	0.9711	0.0506
		3	Lower	0.9804	0.0097
	Branch type	1	Whorl	0.9979	
		2	Internodal	0.9994	0.0015
	Whorl branches	1	Upper	0.8979	
	in levels	2	Mid	0.9660	0.0681
		3	Lower	0.9770	0.0109
1980	Crown level	1	Mid	0.8667	
		2	Upper	0.9467	0.0801
		3	Lower	0.9736	0.0269
	Branch type	1	Whorl	0.9886	
		2	Internodal	0.9987	0.0101
	Whorl branches	1	Mid	0.8675	
	in levels	2	Upper	0.9381	0.0706
		3	Lower	0.9705	0.0324

<sup>&</sup>lt;sup>a</sup> The variable in step 1 is the variable that explains the largest proportion of the variation in total conelets.

b Based on data transformed by  $log_{10}(x + 1)$ .

Table XII. Regression parameters relating total numbers of conelets to numbers in sample cells which accounted for most of the variations in the total numbers as indicated in Table XI.

	Regression	parameters a	0 1	No. conelets in sample cell	Total no.
Year	Intercept	Coefficient	Sample cell	_a x	_a y 
1978	0.406 <u>+</u> 0.232	0.687 <u>+</u> 0.070	Total upper	2.1775	2.3975
and		0.285 <u>+</u> 0.046	Total mid	1.7390	
1981	0.003 <u>+</u> 0.025	1.009 <u>+</u> 0.010	Total whorl	2.3742	
	0.453 <u>+</u> 0.241	0.650 <u>+</u> 0.074	Upper whorl	2.1422	
		0.319 <u>+</u> 0.048	Mid whorl	1.7282	
1980	0.431 <u>+</u> 0.224	$0.737 \pm 0.066$	Total mid	2.2357	2.5166
		0.229 <u>+</u> 0.044	Total upper	1.8825	
	0.128 <u>+</u> 0.058	0.974 <u>+</u> 0.024	Total whorl	2.4513	
	0.617 <u>+</u> 0.237	$0.712 \pm 0.072$	Mid whorl	2.1805	
	•	0.202 <u>+</u> 0.046	Upper whorl	1.7207	

<sup>&</sup>lt;sup>a</sup> Based on data transformed by  $log_{10}(x+1)$  or  $log_{10}(y+1)$ .

The number of conelets per producing branch increased with branch length in all crown levels at all orchards (Table XIII). Productivity per unit of branch length was highest in the upper crown and least in the lower crown, as has been reported for red pine (Hard 1964; Mattson 1979).

The numbers of producing whorl branches necessary to estimate the total numbers of conelets on whorl branches in each crown level with 10% precision (% difference from observed population mean) varied between trees, ranging from 1 to 7 for both the upper and mid crown and 1 to 6 for the lower crown (Fig. 25). Sampling 6 branches in each of the upper and mid crown and 5 in the lower crown should result in 10% precision in 95% of the trees sampled. The precision achieved by randomly sampling up to 7 whorl branches in the upper and mid levels supported this finding. The achieved precisions did not vary significantly among orchards and were combined to determine overall means (Fig. 26). Using the estimated values determined in the trial of the technique and the appropriate parameters in Table XII, tree totals were estimated with 10% precision when 6 or more branches were sampled in both levels.

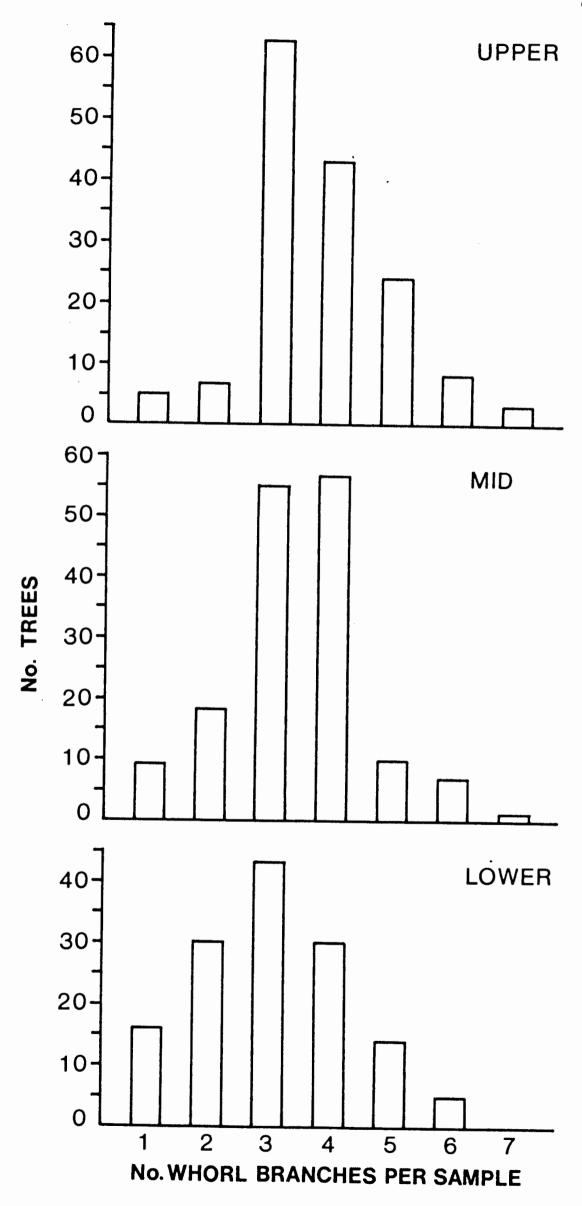
The lack of importance of the lower crown level in the regression analysis should be noted with caution. This level may be important in trees with more than 2,500 conelets, in which case it should be sampled if total crops on individual trees are to be estimated accurately. In B.C. Douglas-fir seed orchards, the effects of not estimating numbers of conelets in the lower level would be minimal with respect to management decisions since the critical crop size for deciding whether or not to manage a tree's crop is approximately 200, much lower than the crop size to which the lower level adds significantly to variation in total crop.

Table XIII. Regression parameters relating numbers of conelets to branch length. The Y-intercept was set at 0. All coefficients were highly significant,  $P \le 0.001$ .

			Regression para	ametersa	Mean	
Year	Orchard	Crown level	Coefficient	r <sup>2</sup>	branch length (cm)	Mean no. conelets
1980	Koksilah	Upper Mid Lower	0.036 ± 0.001 0.020 0.009	0.870 0.887 0.800	25.0 54.9 82.4	1.016 1.178 0.819
	PFP	Upper Mid Lower	$\begin{array}{c} 0.033 \pm 0.001 \\ 0.022 \pm 0.001 \\ 0.013 \pm 0.001 \end{array}$	0.876 0.863 0.814	26.6 45.0 63.7	0.934 1.002 0.864
	Quinsam	Upper Mid Lower	0.011 0.007 0.004	0.925 0.918 0.844	84.4 164.1 227.3	0.953 1.086 0.860
	Tahsis	Upper Mid Lower	$\begin{array}{c} 0.028 \pm 0.001 \\ 0.019 \pm 0.001 \\ 0.011 \pm 0.001 \end{array}$	0.904 0.916 0.875	26.3 50.9 69.0	0.782 1.023 0.777
1981	Koksilah	Upper Mid Lower	0.007 0.005 0.004	0.840 0.857 0.816	150.0 217.7 256.1	1.278 1.192 0.947
	Quinsam	Upper Mid Lower	0.006 0.004 0.004	0.799 0.778 0.806	142.6 202.0 247.2	0.920 0.875 0.913
	Snowdon	Upper Mid Lower	0.006 0.004 0.003	0.838 0.845 0.865	141.7 195.8 255.6	0.861 0.818 0.773

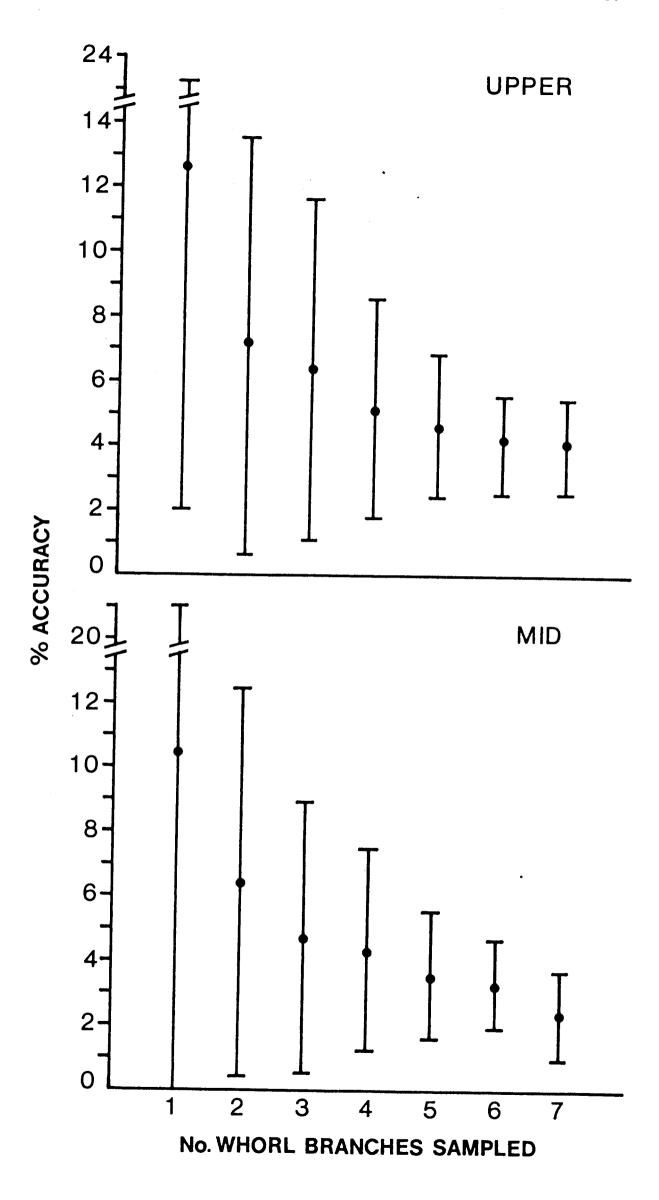
<sup>&</sup>lt;sup>a</sup> Based on data transformed by  $\log_{10}(x + 1)$ . Where coefficient not followed by standard error, standard error is <0.001.

Figure 25. Frequency distribution of the numbers of whorl branches per sample necessary for estimating mean total numbers of conelets on whorl branches in each crown level on individual trees with 10% precision.



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Figure 26. Mean % accuracy with associated standard deviations achieved by randomly sampling up to 7 whorl branches in the upper and mid crown levels.



The calculated number of sample trees required to estimate the total cone crop in an orchard with 10% precision ranged from 4 at Tahsis to 21 at Quinsam (1981). The high value should be used at orchards to ensure 10% precision. The mean number of cones on these trees would be multiplied by the number of producing trees to obtain an estimate of the orchard total. The number of trees required may change as more data are gathered.

Use of the foregoing technique allows an orchard manager to determine cone crop size on individual trees for deciding whether or not management techniques are economically justified for each tree.

## 5.2 Estimating size of C. oregonensis Egg Populations

## 5.2.1 Introduction

The within-crown distribution of cone and seed insects or their damage have been examined only in a few instances on pines (Coulson and Franklin 1968; DeBarr et al. 1975; Mattson 1976) and Douglas-fir (Kozak 1963). Kozak found no significant variations in percent of mature cones infested between different areas of Douglas-fir crowns for C.

washingtonensis, D. abietivorella and M. spermotrophus but found significant variations in the distribution of C. oregonensis. Cone gall midge infested proportionally more cones on the inner half of the branch than on the outer half, more in the upper third of the crown than in the middle and least in the lower third, and more on the south than on the north side of the tree. However, this uneven distribution in the crown did not occur in all instances. No sampling techniques were developed for estimating population size, although a technique was developed for classifying damage (Kozak 1964).

The purpose of this study was to examine the within crown distributions of  $\underline{C}$ . oregonensis eggs and galls, and to develop sampling techniques for estimating population size.

#### 5.2.2 Materials and Methods

Conelets were collected at the following orchards (number of trees indicated in parentheses): Koksilah (10) and Quinsam (10) in 1978; Koksilah (12) in 1980; Lake Cowichan (3), Quinsam (4) and Snowdon (4) in 1981. Each tree was divided into 12 cells: 4 aspects (north, south, east, west) by 3 crown levels (upper, mid, lower). Ten conelets were collected from each cell, 5 from each of 2 branches where possible, after the conelets had closed and approached the pendant position. The conelets on each sample branch were counted. The sample conelets were dissected and the numbers of eggs and egg infested scales were counted.

Analysis of covariance using number of conelets per branch as the covariate, and Duncan's multiple range test were used to analyze the within crown distribution of gall midge. The formulae to calculate sample size are those outlined in section 5.1.2.

#### 5.2.3 Results and Discussion

The only significant factor in the distribution of <u>C. oregonensis</u> eggs at all 6 orchard sites was the sample tree, while crown level and the interaction between crown level and tree were significant at 5 orchards (Table XIV). Branch, aspect and interactions involving either of these factors were not significant at any orchard site. The only orchard where crown level was not significant was Koksilah in 1978 where the highest infestation levels occurred (Table XV). Females oviposit more frequently in

Table XIV. Levels of significance of factors in the distribution of  $\underline{C}$ . oregonensis eggs and infested scales at 6 orchard sites in B.C.; ns = not significant, \* =  $P \le 0.05$ , \*\* =  $P \le 0.01$ , \*\*\* =  $P \le 0.001$ . Number of conelets per branch was used as the covariate in the analysis of covariance.

	19	78	1980		1981	
Factor	Koksilah	Quinsam	Koksilah	Lake Cowichan	Quinsam	Snowdon
Branch (B)	ns	ns	ns	ns	ns	ns
Aspect (A)	ns	ns	ns	ns	ns	ns
Crown level (CL)	ns	***	***	***	***	***
Tree (T)	***	***	***	***	***	***
ВхА	ns	ns	ns	ns	ns	ns
B x CL	ns	ns	ns	ns	ns	ns
A x CL	ns	ns	ns	ns	ns	ns
A x T	ns	ns	ns	ns	ns	ns
CL x T	ns	***	***	***	**	*

Table XV. Mean numbers of C. oregonensis eggs and infested scales per conelet in crown levels of Douglas-fir trees at 6 orchards in B.C., 1978-81.

Factor third Ko Eggs Upper 2	ah a	Quinsam <sup>a</sup> 99,4a	Koksilah <sup>a</sup> .	Lake		
Upper	39.5	99.4a		Cowichan	Quinsam	Snowdon
			7 <b>.</b> 4a	130 <b>.</b> 8a	34 <b>.</b> 8a	10.9a
	233.2	84.7a	6. la	95.6b	33.1a	9. la
Lower 2	223.0	61.7b	2.1b	60.7c	14.6b	5.0b
Infested Upper	25.0	17.la	2.3a	18.8a	6.la	3.0a
scales Mid	27.7	14.7b	1.9a	16.0a	5 <b>.</b> 6a	2.5ab
Lower	25.3	10.4c	0.7b	11.1b	3.2b	1.7b

If ANOVA significant, means for each factor at each orchard followed by the same are not significantly different, Duncan's multiple range test, P<0.05.

conelets in the upper crown at low population densities' but at high densities, females will distribute their eggs evenly throughout the crown. The crown level x tree interaction also support this finding in that the mean number of eggs per conelet in the mid and lower levels increased with increasing overall means per tree. The even distribution of eggs at high densities is probably due to an oviposition deterrent (section 3.3). Kozak (1963) found a similar stratification from the upper to lower crown levels in the proportion of cones infested by C. oregonensis in 1962 but not in 1961 when the proportion of cones infested was significantly higher. Kozak (1963) did not report numbers of midges so it is not known if the differences in distribution corresponded to differences in midge densities. However, the proportion of seeds that were galled was similar in both years (15.1 and 15.6% in 1961 and 1962, respectively) which suggests similar population densities. When sampling to estimate mean numbers of eggs per conelet, all crown levels producing conelets should be sampled individually. The infestation level of C. oregonensis on individual trees was not related to sizes of cone crops, as has been reported for the webbing coneworm, Dioryctria disclusa Heinrich (Lepidoptera: Pyralidae) (Mattson 1976).

Distance of the conelet from the branch tip did not affect the mean number of eggs per conelet at either Koksilah or Quinsam in 1978 (Table XVI). Numbers of <u>C. oregonensis</u> eggs were not significantly different in conelets of different length, numbers of scales or color (Tables XVII-XIX).

Eggs were not distributed evenly within conelets. The middle third of the conelet contained significantly more eggs than did the distal or proximal thirds which did not differ significantly (Table XX).

The number of conelets necessary to estimate the population means of eggs per conelet in each crown level with 10% precision varied among

Table XVI. Mean numbers of <u>C. oregonensis</u> eggs in conelets at varying distances from the branch tip at 2 B.C. orchards in 1978. The differences in each crown level at each orchard were not significant, Duncan's multiple range test.

Crown third	Distanc <25	e from 25-50	branch tip 50-100	(cm) >100
Upper	232.6	222.9	229.4	234.0
Lower	221.7	228.4	236.2	224.6
Upper	91.2	86.5	88.2	86.9
Lower	59.4	60.5	62.8	57.1
	Upper Lower Upper	Upper 232.6 Lower 221.7 Upper 91.2	Upper 232.6 222.9 Lower 221.7 228.4 Upper 91.2 86.5	Upper 232.6 222.9 229.4  Lower 221.7 228.4 236.2  Upper 91.2 86.5 88.2

Table XVII. Numbers of  $\underline{C}$ . oregonensis eggs in conelets of different lengths at 2 B.C. orchards in 1978. Differences among means at each orchard are not significant, Duncan's multiple range test.

	Conele	t length	(cm)
Orchard	<1.0	1.0-2.0	>2.0
Koksilah	234.3	247.8	240.1
Quinsam	74.2	68.3	78.4

different numbers of scales at 2 orchards in 1978. Differences among means for each Table XVIII. Numbers of C. oregonensis eggs and infested scales in conelets with factor at each orchard are not significant, Duncan's multiple range test.

Orchard Eg	64 Eggs	<40 Infested scales 24.0	40 Eggs 234.8	No. scales 40-49 Infested scales Egg 28.4 23	gs   1 · 3	50-59 Infested scales 27.7		>60 Infested scales 28.4
ω	83.4	11.0	9•62	9•6	91.2	13.1	0.8/	10.0

Table XIX. Numbers of <u>C. oregonensis</u> eggs in conelets of different colour at 2 B.C. orchards in 1978. Differences among means at each orchard were not significant, Duncan's multiple range test.

	Mean	no. eggs/com	nelet
Orchard	Red	Red/Green	
Koksilah	238.3	239.5	244.7
Quinsam	83.2	77.4	79.1

Table XX. Mean numbers of  $\underline{C}$ . oregonensis eggs and infested scales per conelet in the distal, middle and proximal thirds of the conelets at 2 B.C. orchards in 1978.

Orchard	Conelet third	Mean no.	per conelet Infested scales <sup>a</sup>
Koksilah	Distal	41.6a	6.9a
	Middle	128.2b	15.4b
	Proximal	53•9a	7.5a
Quinsam	Distal	9.3a	2.la
	Middle	35.5b	6.4b
	Proximal	11.5a	3.0a

Means for each factor at each orchard followed by the same letter are not significantly different, Duncan's multiple range test,  $P \le 0.001$ .

trees and ranged from 3-8 in the upper and mid crown and from 3 to 12 in the lower crown (Fig. 27). To estimate the population mean with 10% precision in 95% of the samples, 8 randomly selected conelets should be taken in each of the upper and mid crown levels and 10 from the lower crown level. The numbers of conelets necessary to estimate the mean number of infested scales per conelet also varied among trees and were similar to the numbers necessary for estimating mean numbers of eggs (Fig. 28). To estimate the populations means with 10% precision in 95% of the samples, 7 randomly selected conelets should be taken from the upper crown, 8 from the mid crown and 9 from the lower crown.

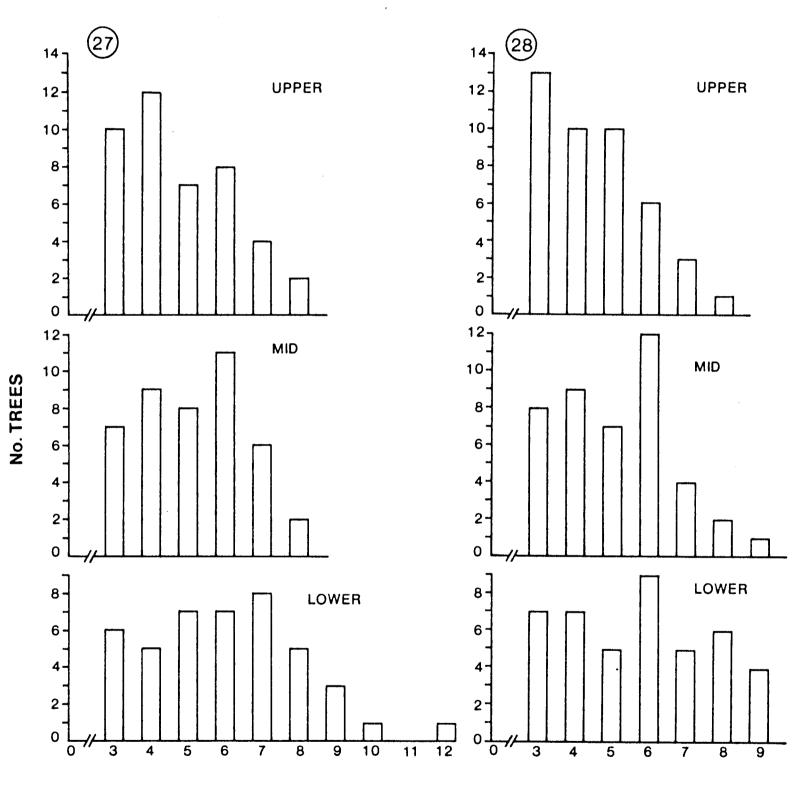
Stepwise regression analysis showed that mean numbers of eggs per conelet in the mid crown was more closely related to tree means than the other levels, accounting for over 98% of the total variation, as shown by the following (based on transformed egg counts):

Step no.	Crown level	$r^2$	Increase in r <sup>2</sup>
1	mid	0.9845	_
2	lower	0.9966	0.0122
3	upper	1.0000	0.0033

The regression equation relating mean number of eggs in the mid crown (X) to tree mean (Y) was log(Y) = 0.9794 log(X) - 0.0064. The relationship for infested scales was similar. Mid crown means were very similar to tree mean and can be used alone to estimate tree means.

The number of sample trees necessary to estimate the overall orchard mean number of eggs or infested scales per conelet with 10% precision ranged from 6 at Koksilah in 1978 (heaviest infestation) to 696

Figures 27, 28. Frequency distributions of the number of conelets per sample necessary to estimate the population mean of <u>C. oregonensis</u> eggs (Fig. 27) and infested scales (Fig. 28) per conelet with 10% precision in each crown level on individual trees.



No. CONELETS PER SAMPLE

(actual number of producing trees was <400) at Koksilah in 1980 (lightest infestation). Reducing the precision to 20% required that >400 trees be sampled at Koksilah in 1980. Because of the high tree to tree variation, it is not possible to determine mean numbers of <u>C. oregonensis</u> eggs or infested scales for orchards without sampling most, if not all, producing trees.

Calculation of number of sample scales to estimate the egg population within a conelet indicated that it is not feasible to select a few scales randomly. The variation between scales was so great that at least 75 to 80% of the scales in a conelet must be sampled.

This technique allows personnel to estimate populations of eggs or infested scales per conelet when necessary. Studies of population dynamics are an example of when such estimates are necessary. It is not a practical technique for orchard managers to use the technique when deciding whether or not to apply an insecticide because of the time needed for the conelet. By the analysis was completed the optimum time for spraying would be long past.

#### 5.3 Damage Prediction

## 5.3.1 Introduction

Damage prediction prior to the optimum time for application of a control action is a requisite for pest management (Geier 1966). The correlation between population levels and plant damage is frequently difficult to establish, since the amount of damage caused by a pest is a function of pest density, type of damage, and the biological characteristics of the host plant, which are differentially affected by biotic and abiotic factors. Methods of damage prediction have been developed only for a few forest insects (Mason 1969, 1978; Carolin and Coulter 1972; Raske and Bryant 1977) but not for cone and seed insects.

Losses to C. oregonensis have been predicted in forest stands by determining both the number of overwintering larvae in the litter below trees and the size of the current conelet crop (Johnson 1962b). This technique has short-comings for use in orchards, namely: i) overwintering larvae are counted but the proportion of the population that emerges, which will vary between years and sites due to prolonged diapause, is not taken into account, ii) factors affecting migration into orchards are not known, and iii) the technique could not be used directly in orchards because no overwintering cone gall midges occur in orchards due to cone harvest. Johnson and Hedlin (1967) suggest a method for determining the need for an insecticide application, based on egg counts, on 5 randomly selected scales per conelet, but the method has not been verified and the previous results (section 5.2.3) showed that subsampling within conelets gives unreliable estimates of the number of eggs within a conelet. Also, this method classifies infestation levels into categories much too broad for use in orchards.

An alternative to a fixed number of samples, such as shown previously (section 5.2), is sequential sampling. Sequential sampling classifies populations rather than providing estimates of population parameters and involves a flexible sample size (Ives 1954; Waters 1955; Onsager 1976). Samples are examined in sequence until the cumulative results fit one or more classes delineated by previously established limits. Usually sequential sampling is less time consuming than fixed sampling due to avoidance of oversampling (Knight 1967). Requirements for development of a sequential sampling technique are knowledge of the numerical distribution of populations, and a quantified relationship between insect numbers and damage so that meaningful class limits can be established. Kozak (1964)

developed a sequential technique for determining whether or not cone crops on individual trees in forest stands were worth collecting based on seed counts and damage by cone and seed insects, including <u>C</u>. <u>oregonensis</u>.

Not all scales in a cone are capable of producing viable seed, as pointed out for pines in the southern United States (Bramlett et al. 1979). Thus, to determine potential seed production and insect-caused damage per cone, it is necessary to know the number of scales in the productive zone of the cone and the location of apparent insect damage in relation to this zone.

The objectives of this study were to determine the number of scales in the productive zone of the cone and the incidence of galls in this zone, to determine how damage per cone at harvest is related to the number of eggs per conelet in the spring, and to develop a sequential sampling method for determining whether or not an insecticide application for control of C. oregonensis is warranted.

## 5.3.2 Materials and Methods

Conelets were collected after they had closed and approached the pendant position (early May) at the following orchards (numbers of trees indicated in parentheses): Dewdney (10), Koksilah (6), Lake Cowichan (10), PFP (9), Quinsam (10), Snowdon (10) and Tahsis (9) in 1979; Koksilah (19) in 1980; Lake Cowichan (3), Quinsam (4) and Snowdon (4) in 1981. Ten randomly-selected conelets were collected from each tree in 1979 and 1980, and 20 per tree in 1981. These conelets were dissected and the eggs and egg-infested scales counted.

At harvest, the same number of cones as conelets was collected from the same trees. These cones were sliced, slice counts taken and then

dissected so that total counts of filled seeds, galls and galled scales could be made. An additional 10 cones per tree, processed for seed extraction, were collected at Quinsam and Snowdon in 1980. The numbers of extracted filled seeds per cone was determined with x-rays and seed dissection.

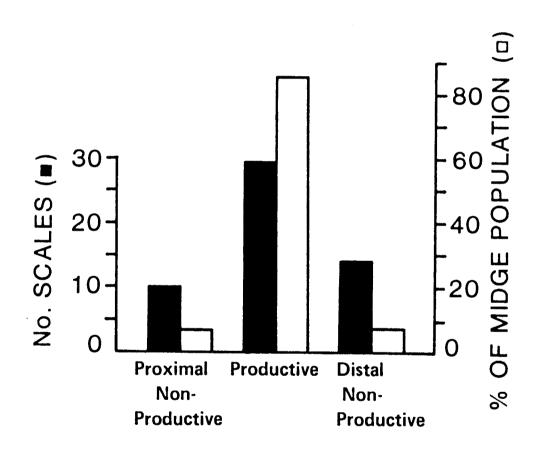
Regression analysis was used to analyze the data. Average count per conelet or per cone for each tree was used in the analysis. The procedure used for developing the sequential technique was that outlined by Iwao (1975). The egg counts obtained during the determination of distribution of  $\underline{C}$ . Oregonensis in tree crowns were used in the determination of  $\alpha$  and  $\beta$  for each crown level.

To determine the average potential number of seeds per cone, a sample of 100 cones from Koksilah and Quinsam, 5 cones from each of 10 trees at each orchard, was selected in 1979. The locations of filled seeds in the cones were noted as were the locations of galls in these same cones. The filled seeds were germinated according to the procedure outlined by Anonymous (1976) to determine seed viability and the actual productive zone of the cone.

#### 5.3.3 Results and Discussion

The mean number of scales in the productive zone (middle 2/3) of cones was 29, with a potential of 58 seeds; 85% of the <u>C. oregonensis</u> population also occurred in this zone (Fig. 29). Johnson and Winjum (1960) also found most of the midge larvae in the middle 2/3 of cones. Occasionally, some small seeds were produced outside of this zone, but the viability of these seeds was significantly ( $\chi^2$  test, P<0.05) lower, 38.6 vs 88.6%.

Figure 29. Distribution of scales and  $\underline{C}$ .  $\underline{oregonensis}$  larvae within cones in relation to the productive zone.



There were strong positive relationships between mean numbers of eggs per conelet in the spring and mean numbers of galls and galled scales per cone at harvest (Table XXI). Mean numbers of filled seeds per cone were negatively related to mean numbers of eggs per conelet but the relationship was much weaker (Table XXI), due to seed development being affected by several other factors including pollination, tree physiology, and infestation by other insects. It is impossible to know which galled potential seeds would have developed in the absence of galls. It is possible to estimate midge damage when numbers of seeds in insecticide—treated and untreated cones from the same orchard can be compared.

The relationships between mean numbers of infested scales per conelet and harvest counts were similar to those for mean numbers of eggs per conelet (Table XXI). This similarity is very significant because dissection of conelets to determine numbers of eggs can take up to 1 h per conelet in heavy infestations whereas determining numbers of infested scales takes only 1/4 of this time.

The number of larvae, and presumably eggs, on a scale affected the possibility of both potential seeds on a scale being damaged (Fig. 30). One or 2 larvae on a scale damaged 1 potential seed in 90% of the cases whereas, 3 larvae damaged both potential seeds in about 50% of the cases and  $\geq$  6 damaged both in all cases.

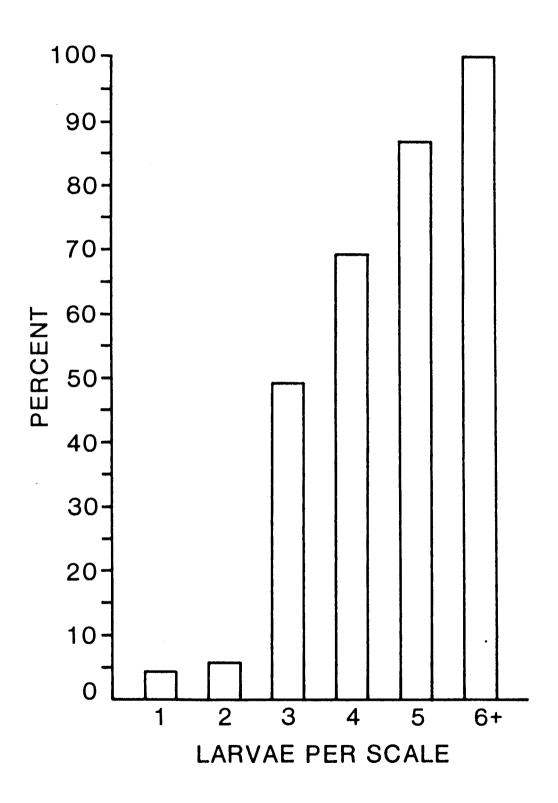
A sequential sampling plan was developed only for infested scales because time requirements of egg counts make damage predictions based on these counts impractical. Also, it is more difficult to estimate a critical egg density because of greater variation than for infested scales. The most accurate approach would probably be to determine the frequency of the numbers of eggs per scale in each conelet, taking into account the chance of

Table XXI. Relationship (log-log) between mean numbers of <u>C. oregonensis</u> eggs or infested scales per conelet and mean numbers of galls and filled seeds per axial slice, or galls, galled scales, filled seeds and extractible filled seeds per cone. All regression coefficients comparing numbers of eggs or infested scales to harvest counts were highly significant,  $P \le 0.001$ .

Variables	compared (mean no.)	Regress	lon parameters	
Spring	Harvest	Intercept	Coefficient	r <sup>2</sup>
Eggs per	Per axial slice:			
(56.1)	galls (2.9)	0	0.348 <u>+</u> 0.022	0.919
	filled seeds (2.2)	$0.774 \pm 0.147$	$-0.220 \pm 0.096$	0.184
	Per cone:			
	galls (12.5)	0	0.650 <u>+</u> 0.024	0.969
	galled scales (11.0)	0	0.622 + 0.024	0.968
	filled seeds (10.6)	1.502 <u>+</u> 0.225	$-0.370 \pm 0.147$	0.217
	extractable filled seeds (10.0)	1.566 <u>+</u> 0.228	$-0.434 \pm 0.149$	0.268
Infested	Per axial slice:			
scales per conelet	galls (2.9)	0	$0.573 \pm 0.032$	0.931
(9.7)	filled seeds (2.2)	0.699 <u>+</u> 0.118	$-0.290 \pm 0.131$	0.174
	Per cone:			
	galls (12.5)	0	1.080 ± 0.040	0.970
	galled scales (11.0)	0	1.033 ± 0.038	0.971
	filled seeds (10.6)	1.356 <u>+</u> 0.184	-0.466 <u>+</u> 0.203	0.186
	extractable filled seeds (10.0)	1.396 <u>+</u> 0.187	-0.548 <u>+</u> 0.207	0.232

<sup>&</sup>lt;sup>a</sup> Based on data transformed by  $log_{10}$  (x + 1).

Figure 30. Effect of number of <u>C. oregonensis</u> larvae per scale on probability that both potential seeds on a scale were damaged.



both potential seeds on each scale being damaged. However, such a technique would be even more time consuming than determining total eggs per conelet and thus, is impractical.

Attempts to use a procedure based on the type of theoretical distribution model best suited to the data (Waters 1955; Onsager 1976;

Southwood 1978) were not successful because k, a parameter indexing aggregation in negative binomial distributions, varied considerably between trees and a common k could not be estimated reliably. A similar problem occurred with other insects (Iwao and Kuno 1971; Burts and Brunner 1981). An estimate of common k is necessary for developing a sequential plan with this problem.

To make pest management decisions, in this case whether or not to apply an insecticide, it is necessary to know if the pest population exceeds a critical density. A critical-density threshold for <u>C. oregonensis</u> is a function of the number of eggs oviposited in orchard conelets (section 4.3) and the potential for seed development as affected by pollination, physiological factors, weather, etc. Based on increased seed production after insecticide applications (section 6.2.2), the critical densities were duplication estimated to be 2.0 and 4.0 infested scales per conelets for 10 and 20% seed loss, respectively.

Iwao (1975) lists a procedure for development of sequential plans relative to a critical density based on the "mean crowding" parameter (\*\*7) (Lloyd 1967) for measuring dispersion of populations. This procedure is valid for common theoretical distribution models (e.g. binomial, Poisson, negative binomial), assuming that the linear relationship

 $\dot{m} = \alpha + \beta m$  exists, where m is the population mean and  $\alpha$  and  $\beta$  are constant characteristics of the species concerned. Iwao (1968) shows the relationships between the theoretical distribution models and the  $\dot{m}$ -m relationship. The  $\dot{m}$ -m relationship

$$\dot{m} = 2.356 + 1.024 \text{ m} \text{ (r}^2 = 0.972)$$

was obtained from my data for individual trees. Differences between levels were not significant so data from all levels were used collectively in the determination of and .

To generate a sequential sampling plan for determining whether or not individual trees should be sprayed with an insecticide, 2.356 for  $\alpha$ , 1.024 for  $\beta$  and 2.0 or 4.0 for critical threshold density were substituted in Iwao's (1975) method. The derived equations for the upper and lower limits of sequential sampling stop lines at each of these critical densities were:

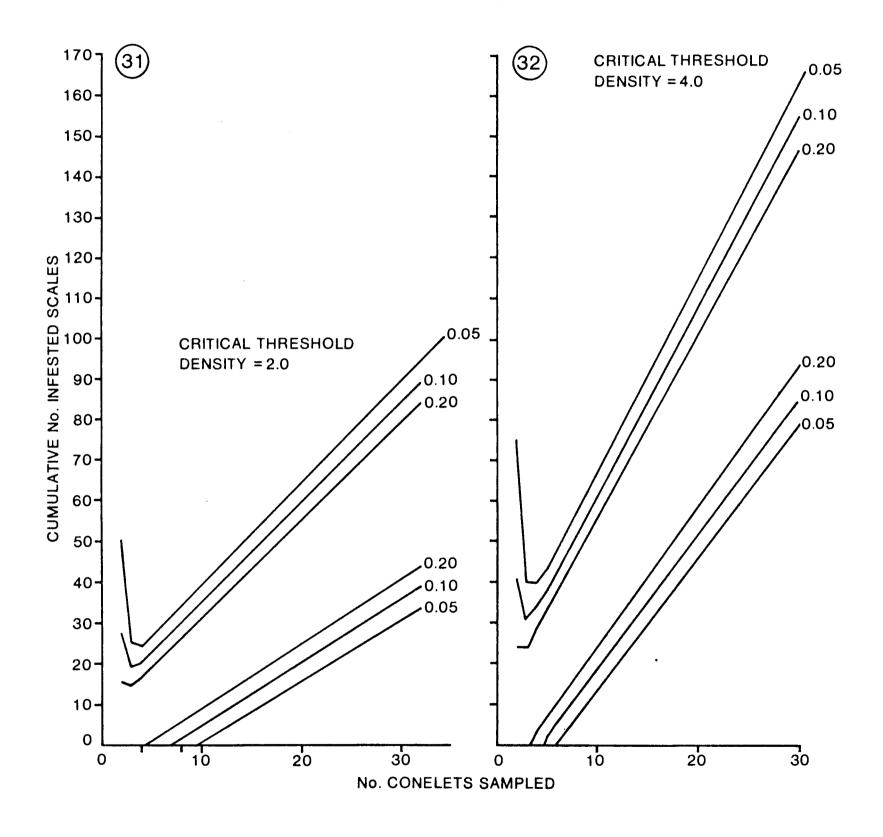
$$T_{0(n)} = 2.0 \text{ n} + t \sqrt{6.808 \text{ n}}$$
 critical density 2.0

and

$$T_{0(n)} = 4.0 \text{ n} + t \sqrt{13.808 \text{ n}}$$
 critical density 4.0.

Figs. 31 and 32 show curves generated from these equations using t values associated with error levels of 5, 10 and 20%. These curves can be used for all crown levels. As pointed out previously (section 5.2.3), means for trees bearing conelets in all 3 levels can be estimated accurately by sampling the mid crown only. When using this plan, one should stop sampling when the upper limit is exceeded, indicating that treatment was necessary or when the lower limit is exceeded indicating no treatment. A decision should be made by the time 24 conelets (7,8 and 9 from the upper, mid and lower crown levels, respectively) per tree have been dissected since it should be

Figures 31, 32. Sequential sampling graphs, using 3 error levels and critical threshold densities of 2.0 (10% seed loss) (Fig. 31) and 4.0 infested scales per conelet (20% seed loss) (Fig. 32), for determining the number of cones to be sampled from individual trees when deciding whether or not to apply an insecticide.



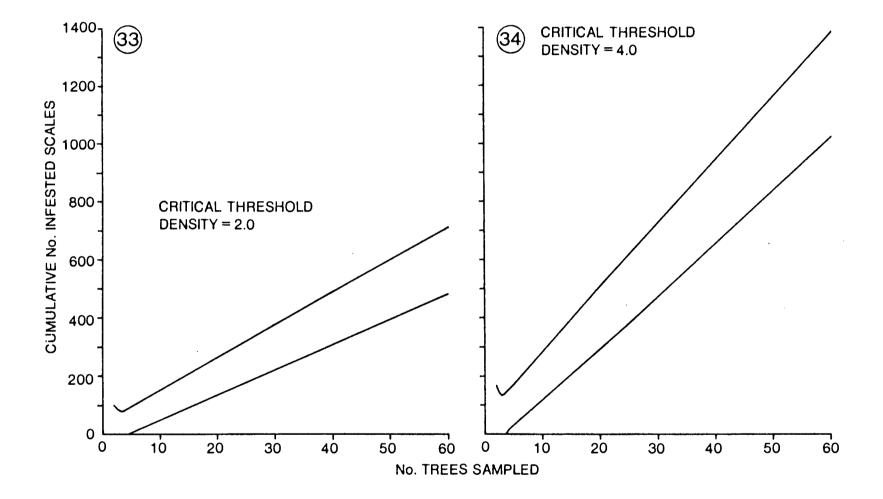
possible to estimate the population mean for each tree with 10% precision with this number (section 5.2.3).

The  $\alpha$  and  $\beta$  values calculated for two stage sampling, i.e. deciding whether or not to treat a whole orchard, were  $\alpha_1$  = 2.980,  $\alpha_2$  = 2.356,  $\beta_1$  = 0.990, and  $\beta_2$  = 1.024. The  $r^2$  value for the whole tree regression analysis was 0.949. Substituting these values, the above critical threshold densities, and 5 (the number experience has indicated as normally sufficient) or 9 (the maximum necessary for a crown level) for the number of conelets per tree (k), the following equations were derived for the sequential sampling stop lines:

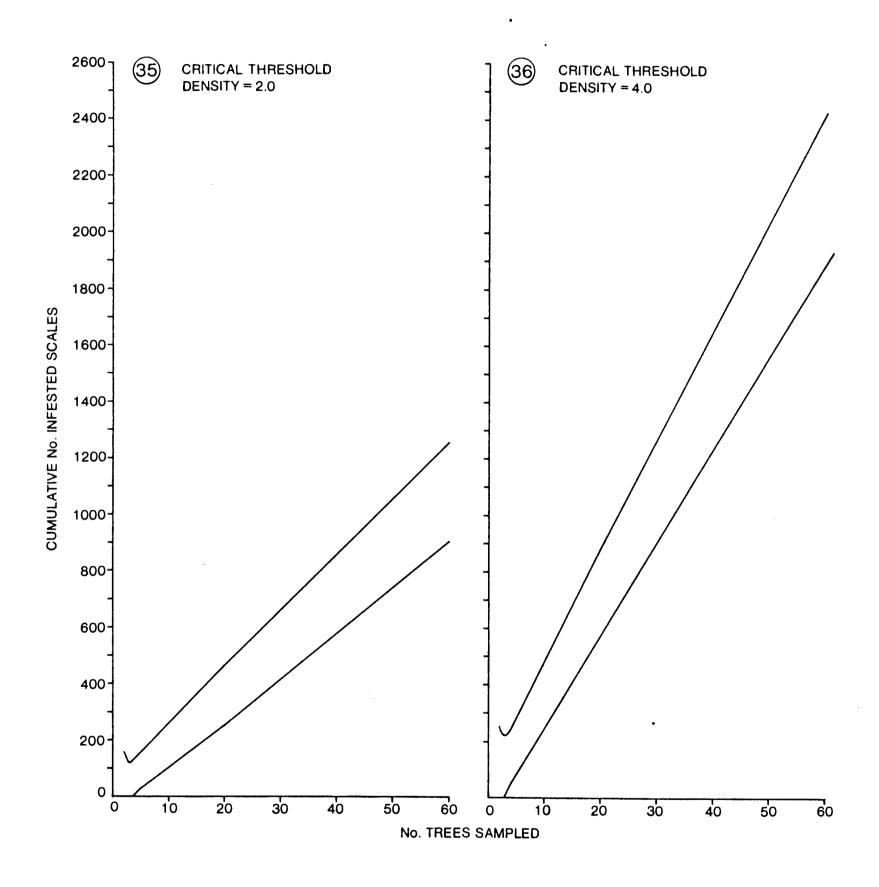
k	critical density	T0(kl)=
5	2	10ℓ <u>+</u> t √81ℓ
	4	$20l \pm t \sqrt{196l}$
9	2	$18l + t \sqrt{185l}$
	4	$36l \pm t \sqrt{419l}$

Figs. 33 to 36 show curves generated from these equations using t values associated with an error level of 10%. The number of sample trees necessary to make a decision with these graphs during the monitoring of 5 orchards in both 1981 and 1982 ranged from 8 to 30, a considerable reduction from the hundreds necessary for estimating the population mean for infested scales per conelet. The time required to process the samples from 8 to 30 trees was 1-2 days, an operationally feasible amount of time in relation to timing of systemic-insecticide application.

Figures 33, 34. Sequential sampling graphs, using a sample size of 5 cones per tree (k), 10% precision and critical threshold densities of 2.0 (10% seed loss) (Fig. 33) and 4.0 infested scales per conelet (20% seed loss) (Fig. 34), for determining the number of trees to be sampled in an orchard when deciding whether or not to apply an insecticide.



Figures 35, 36. Sequential sampling graphs, using a sample size of 9 conelets per tree (k), 10% precision and critical threshold densities of 2.0 (10% seed loss) (Fig. 35) and 4.0 infested scales per conelet (20% seed loss) (Fig. 36), for determining the number of trees to be sampled when deciding whether or not to apply an insecticide.



The calculated  $\beta$  values for both crown levels and whole trees were not significantly different from 1. Inserting 1 for  $\beta$  into the following relationship which relates the mean crowding variable to the negative bionomial index of aggregation variable, k (Iwao 1968):

$$\dot{m} = \alpha + \beta m = m (1 + 1/k)$$

results in

$$k = m/\alpha$$

As the population increases so will the index of aggregation. At large values of m, the distribution of egg-infested scales per conelet would follow a Poisson frequency distribution which applies random attack. Such a random attack pattern could be the result of oviposition deterence at high population densities.

# 5.4 Estimating Numbers of Filled and Damaged Seeds by Cone Slicing 5.4.1 Introduction

Axial cone slicing of Douglas-fir cones was first used in the 1950's (McWilliams 1950; Rudinsky 1955) as a method for exposing the inside of a cone so that the numbers of filled and damaged seeds could be estimated. The technique has been widely adopted because it is easier and quicker than completely dissecting cones. Various cone cutters have been developed for slicing cones longitudinally (Hopkins 1956; Winjum and Johnson 1960; DeBarr and Proveaux 1969; Fatzinger and Proveaux 1971).

In British Columbia, counts of filled seeds per axial slice are used as a guideline for deciding whether or not cone crops in forest stands are worth collecting (Dobbs et al. 1976). No significant differences were found between slice counts and total cone counts in percentage of Douglas-

fir seeds that were filled or damaged by cone and seed insects (Johnson and Heikkenen 1958; DeMars 1964). However, the exact relationships, in terms of actual counts, were not reported. Such relationships must be known if estimates of numbers of filled and insect-damaged seeds are to be made.

The purpose of this study was to examine the relationships between axial slice counts and total cone counts of filled and insect-damaged seeds.

#### 5.4.2 Materials and Methods

Mature cones were collected at the following orchards (number of trees in parentheses): Koksilah (10) in 1978; Dewdney (10), Koksilah (6), Lake Cowichan (10), PFP (9), Quinsam (10), Snowdon (10) and Tahsis (9) in 1979; Koksilah (19) in 1980; Lake Cowichan (3), Quinsam (4) and Snowdon (4) in 1981. Twenty cones were collected from each tree in 1978 and 1981, while 10 cones per tree were collected in 1979 and 1980. Each cone was sliced longitudinally (Winjum and Johnson 1960) and counts were made of filled seed and seed damaged by various species. The cone was then dissected and the seeds classified into the different types. Damage by <u>B. colfaxiana</u> and <u>Dioryctria</u> spp. were grouped because of difficulty in identifying damage done by each species in cones where both occurred. Regression analysis was used to analyze the data.

The relationship between total cone counts of extractible filled seeds and axial slice counts was examined in conjunction with some of the insecticide trials carried out during this research (section 6.2.2). Forty cones per tree were collected at each of 3 orchards (number of trees in parentheses): Koksilah (52) in 1978, and Quinsam (70) and Snowdon (71) in 1980. The number of cones per tree axially sliced was 10 in 1978 and 20 in

1981, with an equal number processed for seed extraction. Tree averages of numbers of extracted filled seeds and filled seeds per axial slice were compared via regression analysis. After seed extraction, processed cones were dissected and remaining filled seeds counted to determine extraction efficiency.

## 5.4.3 Results and discussion

Total cone counts were significantly related to axial slice counts for numbers of filled seeds,  $\underline{C}$ .  $\underline{oregonensis}$  galls and larvae, and seeds damaged by lepidopterous larvae and seed chalcids (Table XXII), showing that slicing cones can be used to estimate total cone counts, as reported previously (Johnson and Heikkenen 1958; DeMars 1964). The relationships when the data were not transformed were similar to the relationships with transformed data. Kozak (1964) reported the relationship (data not transformed) for total cone counts of filled seeds (Y) to axial slice counts (X) as Y = 3.04X - 0.33. The equation reported here is log(Y) = 2.65 +4.34 log(X). The differences may be due to sample size since Kozak's (1964) relationship was based on counts from 100 cones whereas the relationship reported here was based on 1438 cones. In spite of the high degree of significance of the relationship, the amount of unexplained variation was considerable: 26.9% for total filled seeds, 33.9% for extracted filled seeds, 21.4% for C. oregonensis galls, 26.1% for C. oregonensis larvae, 6.4% for moth damaged seed and 34.3% for seed chalcid. Total cone counts of filled seed of 12 to 51 were represented by an axial slice count of 5, the threshold number when deciding whether or not cones should be collected from a tree in a forest stand (Dobbs et al. 1976). The number of samples needed to estimate true mean slice counts for individual trees depends on the

(Douglas-fir cone moth and coneworms) and Douglas-fir seed chalcid. All relationships Table XXII. Regression parameters comparing total cone counts to axial slice counts of filled seeds, C. oregonensis galls and larvae, and seeds damaged by moths (log - log) were highly significant, P  $\leq$  0.001.

Characteristic		Axial	Total	Regression	ssion parameters <sup>a</sup>	Sa
Assessed	Z	Slice	Cone	Intercept	Coefficient	r <sup>2</sup>
Filled seeds	1438	2.5	13.5	2.65 ± 0.45	4.34 + 0.18	0.731
C. oregonensis galls	1438	2.4	11.5	$3.12 \pm 0.23$	3.49 + 0.09	0.786
C. oregonensis larvae	768	2.3	26.9	1.28 + 1.08	$11.14 \pm 0.47$	0.739
Moth damaged seeds	1238	0.7	2.7	$-1.08 \pm 0.04$	4.01 + 0.06	0.936
Seed chalcid	1238	9*0	3.3	$1.09 \pm 0.09$	$3.69 \pm 0.15$	0.657
Extracted filled seeds	194 <sub>b</sub>	3.5	15.8	$2.47 \pm 0.87$	3.83 + 0.25	0.721

a Based on data transformed by  $log_{10}$  (x + 1).

b Tree averages.

coefficient of variation but was estimated to be generally between  $20\ \mathrm{and}\ 30$  cones for 10% precision.

The relationship between the numbers of extracted filled seeds (Y) and axial slice counts (X) in this study was log(Y) = 2.47 + 3.83 log(X), similar to the equation Y = 4.61 + 3.73X (data not transformed) reported by Olson and Silen (1975). The average multipliers were 4.54 and 4.46 for my study and that of Olson and Silen (1975), respectively. The extraction efficiency was 87% in my study. Operational extractions at the Duncan Seed Centre using kilns for drying cones are generally 80 to 85% efficient (Bowden-Green<sup>8</sup>, pers. comm.). Extraction efficiency can vary substantially with cone quality which is affected by maturity when collected and the presence of mould.

Buncan Seed Centre, B.C. Ministry of Forests, Duncan, B.C.

- 6.0 CONTROL TECHNIQUES
- 6.1 Delaying Seed-Cone Bud Burst

### 6.1.1 Introduction

Reproductive bud burst can be delayed up to 14 days by spraying Douglas-fir trees with cold water when the air temperature is >11°C during the period of bud development in the spring (Silen and Keane 1969; Silen and Copes 1972). Currently, 3 Douglas-fir seed orchards in British Columbia have installed solid-set overhead irrigation systems for delaying reproductive bud development, primarily for prevention of pollen contamination (Fashler and Devitt 1980).

The effects of delaying reproductive bud burst on cone and seed insect infestations are not known. C. oregonensis appeared to be a suitable candidate for control by disruption of synchrony between presence of ovipositing females and susceptible host stage because of a restricted oviposition period; females oviposit in conelets which are open to receive pollen (Hedlin 1961; Johnson 1963a). Oviposition is not possible once the scales of the conelets become appressed. Disrupting the synchrony between insect ovipostion and susceptible host stage is used to control damage by cecidomyiids, such as the sorghum midge Contarinia sorghicola (Coquillet) (Wiseman and McMillan 1968; Huddleston et al. 1972) and the Hessian fly, Mayetiola destructor (Say) (Barnes 1956). Infestation levels of Douglas-fir needles midges, Contarinia spp., are affected by the degree of synchrony between adult emergence and presence of the susceptible host stage (Condrashoff 1963).

My objectives were: i) to assess cold water sprays as a potential control method for <u>C</u>. <u>oregonensis</u> and (ii) to determine the relationship between phenology of cone development and infestations of <u>C</u>. <u>oregonensis</u>.

### 6.1.2 Materials and Methods

Infestations of <u>C</u>. <u>oregonensis</u> were measured at Koksilah in 1978-1980, and at PFP in 1978 and 1980. Both orchards have irrigation systems for delaying reproductive bud burst. Koksilah was divided into water- treated (delayed) and untreated (control) blocks in all 3 years. PFP was divided into delayed and control blocks in 1980, but not in 1978 when all trees were treated. Tahsis was used as the control block in 1978. PFP was not sampled in 1979 because the cold-water treatment was not applied.

In 1978, 10 early- and 10 late-flowering trees were identified in each block at Koksilah, at PFP (delayed) and Tahsis (control). From each of these trees 25 cones were collected at Koksilah and 10 cones were collected at PFP and Tahsis at cone harvest (August 21-23). These cones were sliced axially (Winjum and Johnson 1960) and the numbers of <u>C</u>. <u>oregonensis</u> galls exposed were counted.

In 1979, the seed-cone buds and conelets were checked at Koksilah for stage of development 3 times per week from late March until the conelets became pendant (early May) on 55 trees in the delayed block and 29 trees in the control block. The trees in both blocks represented the same 11 families (half siblings). After the scales of the conelets became appressed (May 2-3), 10 conelets were collected from each tree. These conelets were dissected and C. oregonensis eggs and infested scales counted. At cone harvest (August 22-23), 10 cones per tree were collected and processed.

In 1980, the bud and conelet phenologies and levels of <u>C</u>.

<u>oregonensis</u> infestation at Koksilah were determined for 11 trees in the control block and 27 trees in the delayed block at Koksilah in the same manner as in 1979, with the exception that 20 conelets and 20 cones were collected from each tree. At PFP, 10 cones were collected from each of 10

trees in both the delayed and control blocks at cone harvest (August 24) and processed.

Analysis of variance and t-tests were used to analyze the data for each orchard and year. Differences among groups of  $\geq 3$  means were tested with Duncan's multiple range test. The date that each tree flowered was numbered, 1 April being day one, in order to calculate the average flowering times in treated and untreated blocks and thus determine the amount of delay in bud development. Infestation levels were grouped in several ways, depending on orchard and year, to determine the effects of the following factors on infestation levels of <u>C. oregonensis</u>: i) delaying seed-cone bud burst (all orchards and years) ii) time of bud burst (Koksilah 1979 and 1980), iii) length of time conelets were open (Koksilah 1979 and 1980), and iv) half-sibling family (Koksilah 1979).

### 6.1.3 Results and Discussion

Delaying seed-cone bud burst caused reductions of 87.6% and 99.2% in C. oregonensis infestations at Koksilah and PFP, respectively, in 1978 when 10 days delay was achieved and of 42.1% at PFP in 1980 with 6 days delay (Table XXIII). No reductions occurred at Koksilah in 1979 or 1980 when delays were only 2 and 5 days. Thus, amount of delay achieved is an important factor. The reduction at Koksilah in 1978 was similar to the best reduction achieved in an insecticide trial carried out there. Variations in the amount of delay achieved with cold-water treatment among years are the result of differences in weather and are beyond control. For example, untreated trees began bud development approximately 2 weeks before treated trees at Koksilah in 1979. However, just after untreated buds began developing, a 3-week series of cold lows moved through the region, slowing

Table XXIII. Effect of cold water sprays for delay of seed-cone bud burst on C. oregonensis infestations.

					Mean no./conelet	/conelet		Mean no.	Mean no./axial slice
Orchard	Year	Block	Delay (days)	Eggs	% Reduction	Infested scales <sup>a</sup>	% Reduction	Galls <sup>a</sup>	% Reduction
Koksilah	1978	Control		٩		1		1.5	
		Delayed	10	i	ı	ı	ı	0.2*	86.7
	1979	Control		62.9		10.8		6.3	
		Delayed	2	65.8	9*4-	12.4	-12.9	5.1	19.0
	1980	Control		24.3		6.7		1.8	
		Delayed	5	30.5	-25.5	7.9	-17.9	1.9	9*5
PFP	1978	Control		1		i		2.5	
		Delayed	10	t	i	ı	ı	<b>*</b> 0	99.2
	1980	Control.		í		ı		1.2	
		Delayed	9	ı	ı	I	ı	0.7*	41.7

\* indicates significant difference between paired means, t-test, P<0.05.

b - indicates no data collected.

development of the untreated buds, so that they were only 2 days ahead of the treated buds by the time warmer, drier weather returned.

Another important factor affecting the level of midge infestation is the synchrony between the presence of adult midges and susceptible stages of conelets. The greatest reductions in midge infestations (Table XXIII) occurred in years when early flowering trees were the most heavily attacked (Table XXIV). No reductions occurred when trees that flowered during the middle of the flowering period of an orchard were the most heavily attacked. For many cecidomyiids adult emergence and occurrence of susceptible host stage are well synchronized, especially when the host is susceptible for only a short time (Coutin 1964). Such is not always the case for C. The synchrony between adult cone gall midge emergence and oregonensis. occurrence of susceptible host stages appears to be affected by weather, as it is in other cone and seed insects and cecidomyiids (Hussey 1955; Condrashoff 1963). Kozak (1963) found that late flowering trees were more heavily infested than early flowering trees. Under such conditions delaying seed-cone bud development may result in increased rather than decreased infestations. The pattern of C. oregonensis infestation was similar when relating it to phenology of seed-cone bud burst or to the conelet scales becoming fully reflexed (Table XXIV).

There was no significant correlation between the length of time the conelets were exposed and cone gall midge infestation at Koksilah in 1979 or 1980 (Table XXV). Trees which flowered during 6-13 April 1979 were more lightly attacked than trees which flowered during 14-25 April, even though conelets on most of the earlier flowering trees were still open, and presumably available for attack coincidentally with the later flowering trees for several days. Such infestation patterns suggest that young

Table XXIV. Relationship between phenology of seed-cone bud burst and conelet opening (scales fully reflexed) and C. oregonensis infestation.

Orchard and			Infest	Infestation per	conelet	Infestation axial slice	ation per slice
sample class	Year	Date	No. trees	No. eggs <sup>a</sup>	Infested scales <sup>a</sup>	No. trees	No. galls <sup>a</sup>
Koksilah: trees							
Ъ	1979	Apr. 6-13	12	•	9.1	11	4.9a
according to			28	75.2b	13.4	28	6.7b
date of seed-cone		20-25	27	67.4b	•	<b>2</b> 6	5.5ab
bud burst		26-30	15	45.3a	10.2	13	<b>4.</b> 1a
	1980	Apr. 8-14	5	21.7	5.9	2	2.0
			7	17.8	5.3	7	
		19-24	20	35.3	9.1	20	3.2
		25–29	9	•	6.2	9	2.1
Koksilah: trees							
ק	1978	Apr. 16-18	<b>P</b>	1	ı	10	2.3a
according to		25-2				19	0.4b
date conelets		May 4-7	ı	l	l	10	0• 1b
rully open	1979	Apr. 9-19	11	29.7a	7.la	10	3.7a
			24	83.0b	14.2b	24	7.0b
	•	25-27	30	75.4b	12.9b	29	<b>9.</b> 0b
		Apr. 30-May 5	17	42.7a	9.6ab	15	3 <b>.</b> 6a
	1980	Apr. 15-17	ო	25.6	<b>8</b> •9	ო	2.7
		20-27	31	28.9	7.7	31	1.8
		Apr. 30-May 5	4	29.8	7.3	4	1.5
			4			(Cont'd)	( p

Table XXIV. (Cont'd)

Orchard and sample class	Year	Date	Infest No. trees	Infestation per conelet No. No. Infest rees eggs <sup>a</sup> scale	conelet Infested scales <sup>a</sup>	Infest axial No.	Infestation per axial slice No. No. rees galls <sup>a</sup>
PFP: trees segregated according to date conelets fully open	1978	Apr. 11-12 20-23 May 1-3	1 1 1	1 1 1	1 1	10 20 10	4.3a 0.4b 0.6b

If ANOVA  $P\leq 0.05$ , means for each orchard and year followed by the same letter are not significantly different, Duncan's multiple range test,  $P\leq 0.05$ . Ø

b No data collected.

Table XXV. Correlations at Koksilah between duration of conelet exposure (time of bud burst or time scales fully reflexed until conelets closed) and mean numbers of C. oregonensis eggs and infested scales per conelet.

			d	2	Duration (days)		Correlation	Correlation coefficient
	Year	na I	Dare	NO. trees	z X X	s exposed range	Eggs	Infested scales
Trees segregated according	1979	Apr.	6–13	12	15.2	10–19	0.403	0.507
to date of seed-cone bud		Apr.	14-25	55	9.3	4-17	0.063	0.065
burst		Apr.	26-30	15	5.2	3–7	-0-078	0.213
	1980	Apr.	8-27	38	8.3	3-14	-0.131	0.192
Trees segregated according	1979	Apr.	9–19	11	9.3	4-12	0.276	0.210
to date strobilus scales		Apr.	21-27	54	<b>4.</b> 8	2-11	0.186	0.033
fully reflexed		Apr.	30-May 2	17	2.5	2-4	-0.051	-0.160
	1980	Apr.	Apr. 15-May 4	38	4•5	2-10	0.007	0.085

a Phenological groups in Table XXIV were pooled if they were not significantly different.

 $<sup>^{\</sup>mathrm{b}}$  No correlation coefficient was significant.

conelets are more attractive for cone gall midge oviposition than are older conelets, as indicated in Table V.

Kozak (1963) found the converse of my results, indicating that phenologies of different cone developmental stages are important under differing circumstances. In my study, when the earliest flowering trees were the most heavily infested, infestation was related to phenology of seed-cone bud burst but not to the time conelets became pendant, whereas when trees flowered in the middle of the flowering period, infestation was not related to either of these factors. When the latest flowering trees were the most heavily infested, infestation was related to phenology of conelets becoming pendant but not to phenology of bud burst (Kozak 1963).

Tree family was a significant factor affecting cone gall midge infestation, but not when the effects of phenology of seed-cone bud burst were removed (Table XXVI). Hedlin and Ruth (1978) reported that clonal variation was not of practical significance in gall midge infestations.

Delayed seed-cone bud burst cannot be relied upon to control C. oregonensis under all circumstances. It reduced C. oregonensis infestations when seed-cone bud burst was delayed by about 10 days and adult midge emergence coincided with flowering on early trees, but was not effective when shorter periods of delay occurred or when midge emergence coincided with flowering on middle and late trees. Monitoring insect population levels is just as important in cold-water treated orchards as in untreated orchards because insecticide applications will be necessary on occasion, though hopefully less often. Fashler and Devitt (1980) note that further work is required on calibration of the system. Similarly, further study on the synchrony between insect attack and occurrence of susceptible host stage should be carried out to determine the interactions between these and

Table XXVI. Ranked relationship between half-sibling family and  $\underline{C}$ . oregonensis infestation, Koksilah (both blocks), 1979.

Spi	ring ass	essment	Fall	(harvest)	assessment
Family name	No. trees	Mean no. eggs/conelet <sup>a</sup>	Family name	No. trees	Mean no. galls/axial <sup>a</sup> slice
195	5	86.0 a	416	5	7•3 a
421	9	85.5 a	421	8	7.0 a
416	5	68.5 ab	196	12	6.4 ab
399	10	63.6 ab	195	5	6.3 ab
513	9	62.5 ab	577	5	5.5 bc
196	13	56.0 b	200	10	5.1 c
200	10	54.9 b	399	9	5.0 c
439	5	54.3 b	439	5	5.0 c
395	4	52.1 bc	513	10	4.9 c
577	.6	44.4 c	395	4	4.7 c
397	6	34.4 d	397	6	3.0 d

Means followed by the same letter are not significantly different, Duncan's multiple range test,  $P \leq 0.05$ . When date of seed-cone bud burst was used as the covariate in analysis of covariance, family was not significant.

weather to delineate the circumstances under which delaying seed-cone bud burst will effectively reduce C. oregonensis infestations.

### 6.2 Insecticides

### 6.2.1 Conventional Insectides

### 6.2.1.1 Introduction

Many contact and systemic insecticides have been tested against Douglas-fir cone and seed insects, especially <u>C. oregonensis</u> (Miller 1980). Contact insecticides can be used only as preventative sprays because they must be applied when adults are active. Systemic insecticides are used as larvicides because they are taken up by plant tissues. Unlike contact insecticides which must be applied without knowledge that significant damage will occur, systemic insecticides can be applied after cone gall midge oviposition is completed, and potential damage is predicted by means of egg counts. Currently, dimethoate is the only insecticide registered for use against Douglas-fir cone and seed insects, including <u>C. oregonensis</u> (Richmond et al. 1975).

Contact insecticides that have been effective against C.

oregonensis in at least one trial are azinphosmethyl, carbaryl, endosulfan,
and B.H.C. (Miller 1980). All systemic insecticides tested against C.

oregonensis as either foliar sprays or injections were effective in at least
one trial (Miller 1980).

Insecticides used in seed orchards should have low toxicity to nontarget organisms, especially mammals, because several orchards are located adjacent to residential areas. Dimethoate is the least toxic to mammals of the systemic insecticides tested; many of the others are

highly toxic and unsuitable. Acephate, a systemic insecticide low in mammalian toxicity, was effective against coneworms on Douglas-fir but has not been tested against <u>C. oregonensis</u>. Methoxychlor, another contact insecticide low in mammalian toxicity, has not been tested against any Douglas-fir cone and seed insect.

Johnson and Hedlin (1967) recommend that systemic insecticides be applied when conelets are closed and turning but before they reach the pendant position. Dimethoate was more effective when applied to conelets nearing the pendant position than when applied to open conelets (Buffam and Johnson 1965). However, applications after conelets have become pendant may also be effective. Dimethoate applied to cones that were pendant and 1.91-2.54 cm long (mid-May) resulted in double the number of filled seeds per cone (Dewey et al. 1975). When applications were made even later, early July, no control was achieved, even at concentrations up to 10% (McDermid 1965).

No studies of treating conelets at different stages during the recommended period have been reported. These effects of differences in timing should be known if insecticides are to be applied on an orchard basis, because variations in cone development among trees of up to 3 weeks occur in Douglas-fir seed orchards in B.C. In addition, considerable variation occurs among trees and even among different parts of a tree crown in phenology of cone development (Allen 1943; Orr-Ewing 1956). At any one time, conelets on some trees may be at the recommended stage of development for spraying, while others will not have reached this stage and others will have passed it. A single application to all orchard trees at one time may be desirable from the point of cost but multiple applications may be more effective.

Mistblowers, hydraulic sprayers and airblast sprayers are ground-based sprayers that can be used in seed orchards. Mistblowers and airblast sprayers are generally used to apply low volume, high concentration sprays whereas hydraulic sprayers apply high volume, low concentration sprays. Hydraulic sprayers appear to be more suitable than the others for applying systemic insecticides operationally because good coverage of the cone-bearing parts of the crown (foliage and cones) is essential for maximum effectiveness (Hedlin 1966; Johnson and Zingg 1967). Airblast sprayers are considered best in pine seed orchards in the southern United States (Merkel 1969) where contact insecticides are applied, but have not been tested in Douglas-fir orchards. Hydraulic sprays can be directed selectively to cone-bearing portions of crowns and are less susceptible to drift than airblast sprays because the droplets produced are larger. An airblast sprayer can apply a spray to an orchard faster than a hydraulic sprayer.

My objectives were: i) to test the efficacies of acephate, carbaryl (dust and emulsifiable concentrate) and methoxychlor for control of C. oregonensis, ii) to determine the effects of treating conelets at different stages of development, iii) to determine if 2 applications were more effective than single sprays, and iv) to compare the effectivenss of an airblast sprayer to that of a hydraulic sprayer.

- 6.2.1.2 Materials and Methods
- 6.2.1.2.1 Experimental trials
- 6.2.1.2.1.1 Efficacy Tests

The systemic insecticides dimethoate and acephate (only partially systemic) were screened for effectiveness at Koksilah in 1978. In the

undelayed (no cold-water treatment) block, the insecticides were applied at concentrations of 0.5 and 1.0% until the trees were soaked (until run-off occurred). The 0.5% - dimethoate treatment was applied to 20 trees; the other treatments were each applied to 10 trees. Twenty water-treated trees were used as controls. Originally 10 trees treated with 0.5% dimethoate were to be sprayed a second time, later in their cone development, but these later applications were not made because of equipment breakdown. In the delayed (cold-water treated) block each insecticides was applied at 0.5% only to 10 trees with another 10 as controls. The insecticides were applied with a motorized backpack sprayer on 1-6 May when the conelets were horizontal to nearly pendant. The irrigation system had been shut off by this time.

At cone harvest (21-22 August), 20 cones were collected from each treated tree where possible. Some trees could not be sampled adequately because heavy frosts during the pollination period killed nearly all the cones. Ten cones from each tree were sliced and seed counts made while the other 10 were dried and tumbled for seed extraction. The extracted seeds were dissected and the filled seeds counted. If a tree had only 10 cones, they were sliced.

Carbaryl dust (DU) was applied to open conelets in a mixture of pollen and talc with booster pollination equipment (Fig. 37) at Koksilah in 1979. The ratio of pollen: talc: carbaryl was 50:48:2. Ten trees were treated with carbaryl but all of the conelets on 2 of these trees aborted so only 8 were sampled at harvest. Eight trees pollinated in the same manner but without insecticide treatment were used as controls. At harvest (21 August) 10 cones were collected from each treated and control tree. These

Figure 37. Booster pollination equipment used to apply carbaryl dust at Koksilah in 1979.



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cones were sliced and the numbers of filled seeds, <u>C. oregonensis</u> galls and other insect-damaged seeds counted.

Carbaryl emulsifiable concentrate (EC) and methoxychlor EC were applied at different sites at Lake Cowichan in 1979. Both insecticides were applied at concentrations of 0.5 and 1.0%; eight trees were treated with each carbaryl concentration and 10 trees with each methoxychlor concentration. The same number of untreated, control trees was left at each site. Two methoxychlor-treated trees (1 at 0.5% and 1 at 1.0%) had all their conelets abort. The insecticides were applied to open flowers with a 10-L, motorized, backpack sprayer on 22-27 April. Twenty cones per tree were collected on 22 August, sliced and the filled and damaged seeds counted.

## 6.2.1.2.1.2 Timing applications of systemic insecticdes

At Dewdney, 75 trees with female buds that had flushed within a 2 day period (20-21 April 1979) were marked. Acephate and dimethoate were applied at 0.5% with a 3.75-L compression sprayer until the trees were soaked. Each insecticide was applied to 5 trees at each of the following stages of cone development: i) conelets open to receive pollen, ii) conelets closed and turning, iii) conelets horizontal, iv) conelets pendant, and v) conelets pendant for 5 days. In addition, 5 unsprayed trees were marked at the same time as the sprays were applied to serve as controls. Spraying began on 21 April and finished on 18 May.

Twenty cones per tree were collected on 10 August and sliced, and the numbers of exposed <u>C. orgonensis</u> galls and seed chalcid larva counted. The numbers of filled seed and seed damaged by lepidopterous larvae moths were also counted but were not analyzed because of the low numbers encountered.

# 6.2.1.2.1.3. Double vs. single applications

The effectiveness of double application of dimethoate was compared to that of a single application at Snowdon in 1980. The dimethoate was applied at 0.5% with a hydraulic sprayer until trees were soaked. Thirty trees were treated with 2 sprays, 30 with a single spray and 16 were untreated controls. The first treatment of the double application was made when 25% of the conelets were approaching the pendant position and the second when 75% were at this stage. The single-spray treatment was also applied when 75% of the conelets were nearing the pendant position.

At cone harvest (August 18), 40 cones were collected from each tree; 20 were sliced and 20 processed for seed extraction. The usual slice counts were made and the extracted seeds dissected to determine the number filled.

## 6.2.1.2.1.4 Airblast vs. hydraulic applications

The effectiveness of an airblast sprayer (Tecnoma No. PM90) was compared to hydraulic sprayer (Tecnoma No. 4506) at Quinsam in 1980. When the conelets were closed and turning down, dimethoate (active ingredient) was applied at rates of 0.56, 1.12 and 2.24 kg/ha with the airblast sprayer, and with the hydraulic sprayer at 0.5% until trees were soaked. The volume of spray per tree averaged 7 and 4 L for the airblast and hydraulic sprayers, respectively. The airblast spray wetted the trees but did not soak them. Ten trees were treated with each airblast rate, 20 were treated with the hydraulic sprayer and 20 were left as untreated controls.

At cone harvest (August 18), 40 cones were collected from each tree, of these 20 were sliced for seed and damage assessment, and 20 were processed for seed extraction.

## 6.2.1.2.1.5 Data analysis

Data for each experiment were subjected to analysis of variance and Duncan's multiple range test.

### 6.2.1.2.2 Operational trials

### 6.2.1.2.2.1 Efficacies

Two insecticides have been applied operationally in Douglas-fir seed orchards in B.C.: i) acephate at Tahsis in 1978 and 1979, and at PFP in 1979, and ii) dimethoate at Quinsam in 1979, 1980 and 1981, at Snowdon in 1979 and 1981, and at Tahsis in 1980. Orchard personnel applied both insecticides at 0.5% with hydraulic sprayers until run-off occurred. The sprays were applied in late April or early May, depending on the orchard year when the conelets had closed and were nearing the pendant position. Some of the conelets had already become pendant by the time the application was made at Tahsis in 1979.

Cone samples were collected at harvest in August. The numbers of treated and untreated trees sampled at each orchard, and the numbers of cones sampled per tree were:

no. of trees

orchard	year	treated	untreated	no. cones/tree
Tahsis	1978	10	10 .	10
	1979	17	17	10
	1980	10	10	10
PFP	1979	20	20	10
Quinsam	1979	15	15	20
	1980	20	20	20
	1981	10	10	10
Snowdon	1979	15	15	20
	1981	10	10	10

Ten cones per tree were sliced and processed in the usual manner. When 20 cones were sampled from each tree, seeds were extracted from the other 10 cones and the numbers of extracted filled seeds determined. The data were analyzed with t-tests.

## 6.2.1.2.2.2 Phytotoxicity

A few days after the operational applications of dimethoate,
Dimethoate 4E<sup>®</sup>, in 1979 and 1980, and the experimental trial at Snowdon in
1980, unexpected levels of phytotoxicity became apparent and the orchards
were surveyed for damage. The trees that were moderately or severely burnt
(approximately 50 and 100% of the needles turned red, respectively) were of
particular interest. Both grafted ramets and normally rooted trees were
treated at all orchards. The sprayers had been used previously only for

applications of insecticides and had been thoroughly rinsed before the applications were made to the orchards.

## 6.2.1.3 Results and Discussion

### 6.2.1.3.1 Experimental trials

Acephate and dimethoate significantly reduced the numbers of C. oregonensis galls and seeds damaged by all insects, while increasing the numbers of filled seeds in the undelayed block at Koksilah in 1978 (Table XXVII). The reductions in numbers of C. oregonensis galls per axial slice were 75 and 80% for the 0.5 and 1.0% acephate, respectively, and 89 and 98% for 0.5 and 1.0% dimethoate, respectively. There were no differences in efficacy between concentrations for either insecticide. Dimethoate at 1.0% gave significantly larger reductions of C. oregonensis than acephate at either concentration. There was no significant difference between insecticides in reduction of total insect damage, indicating that acephate may be more effective than dimethoate for controlling other Douglas-fir cone and seed insects. The number of filled seeds extracted per cone was doubled by all treatments; differences between insecticides and concentrations were not significant (Table XXVII). No significant reductions occurred with either insecticide in the delayed block where delaying seed-cone bud burst had reduced significantly the C. oregonensis infestation.

At Koksilah, 2.0% carbaryl dust applied with booster pollination equipment had no significant effect on the numbers of seeds damaged by cone and seed insects (Table XXVIII). Johnson and Winjum (1960) obtained more than 80% control with 3.0% carbaryl dust, so the concentration used in my experiment may have been too low.

Table XXVII. Numbers of filled seeds, C. oregonensis galls and total insect damage in cones and numbers of filled seeds extracted from cones sprayed with acephate, dimethoate and water at Koksilah in 1978.

				Mean no.		exposed/axial slice	Ğ	Extracted seed
Block	Treatment	Concn (%)	No. trees	Filled seeds <sup>a</sup>	Galls <sup>a</sup>	Total insect damage <sup>a</sup>	No. trees	Mean no. filled seeds/cone
Undelayed	Water		14	3.88a	1.48a	2.36a	12	12.5a
	Acephate	0.5	7	6.21b	0.37b	0.67b	9	25.8b
		1.0	10	6.26b	0.30b	0.62b	10	21.0b
	Dimethoate	0.5	16	6.42b	0.26bc	0.41b	15	23.8b
		1.0	6	5.66b	0.13c	0.34b	6	20.15
Delayed	Water		10	4. 15a	0.12a	1.02a	ا	ı
	Acephate	. 0.5	6	4.62a	0.08a	0.89a	1	i
	Dimethoate	0.5	6	4.81a	0.04a	0.90a	t	ı

If ANOVA significant, P<0.05, means within a column for each experiment followed by the same letter are not significantly different, Duncan's multiple range test, P<0.01.

Ø

b No data collected.

Table XXVIII. Numbers of filled seeds, <u>C. oregonensis</u> galls and total insect damage in cones sprayed with carbaryl and methoxychlor at 2 orchard sites in 1979.

Insecticde	Concn (%)	Site	No.	-	ged seeds l/axial slice Total insect
Insecticue	(%)	31 te	trees	Galls <sup>a</sup>	damage <sup>a</sup>
Carbaryl (DU)	0		8	6.63	7.59
	2.0	Koksilah	8	6.38	7.27
Carbaryl (EC)	0		8	3.68a	4.86a
	0.5		8	1.16b	1.94b
	1.0	Lake Cowichan	8	0.99ь	1.43b
Methoxychlor (EC)	0		10	0.48	0.70
	0.5		9	0.18	0.46
	1.0	Lake Cowichan	9	0.33	0.54

If ANOVA significant,  $P \le 0.05$ , means within a column for each treatment followed by the same letter are not significantly different, Duncan's multiple range test,  $P \le 0.05$ .

At Lake Cowichan, carbaryl EC caused significant reductions in the numbers of midge galls and total seeds damaged by insects (Table XXVIII). The percentage reductions for 1.0% were 69 and 71% for gall midge and total damage, respectively. These levels of control are not sufficient for carbaryl EC to be used in seed orchards. Methoxychlor did not significantly affect the numbers of midge galls or total seeds damaged by insects (Table XXVIII).

Timing of application was a significant factor in the effectiveness of acephate but not dimethoate against <u>C. oregonensis</u> (Table XXIX). Applications of acephate after conelets became pendant were significantly less effective than earlier applications; earlier applications appeared to decrease in effectiveness the later the application was made. This trend may indicate that acephate is more effective as a contact insecticide or that it has more systemic activity in younger conelets. For dimethoate, the best apparent developmental stages for treatment were after the conelets had closed and were turning, but before they reached the pendant position. However, time of treatment did not significantly affect the amount of reduction, probably because of the small sample size and because the systemic effect and period of effectiveness prior to degradation of the insecticide ensured continual and persistent action. Dimethoate reduced <u>C. oregonensis</u> galls significantly more than did acephate overall.

Against  $\underline{M}$ . spermotrophus, both insecticides were effective and timing of application had no effect (Table XXIX).

Two sprays of dimethoate were no better than one in reducing numbers of midge galls or total seeds damaged by insects (Table XXX). Both treatments reduced galls and total damaged seeds significantly. This result

Table XXIX. Mean numbers of <u>C. oregonensis</u> galls and <u>M. spermotrophus</u> larvae exposed per axial slice in cones treated with 0.5% acephate and 0.5% dimethoate at 5 stages of cone development at Dewdney in 1979.

Insecticide	Stage of conelet development at time of treatment	C. oregonensis	M. spermotrophus
Control		2.63 a	1.54 a
Acephate	0pen	0.03 c	0.05 b
	Closed and turning	0.18 c	0.28 b
	Horizontal	0.42 c	0.17 b
	Pendant	1.24 b	0.25 b
	Pendant + 5 days	1.30 ь	0.26 b
Control	_	2.63 a	1.54 a
Dimethoate	Open	0.20 ь	0.25 b
	Closed and turning	0 ь	0.03 b
•	Horizontal	0 ь	0.24 b
	Pendant	0.19 b	0.25 b
	Pendant + 5 days	0.11 b	0.01 b

Means within a column for each experiment followed by the same letter are not significantly different, Duncan's multiple range test, P< 0.01.

Table XXX. Numbers of filled seed, <u>C. oregonensis</u> galls and total insect damage in cones treated with single and double applications of 0.5% dimethoate at Snowdon in 1980.

No •	No •	Mean no. seeds	s exposed/axial slice
applications	trees	a Filled Gal	a Total a lls insect damage
Control	16	1.13 a 4.8	39 a 7.29 a
Single	30	5.02 b 0.1	13 ь .28 ь
Double	30	4.63 b 0.2	20 ь .31 ь

Means followed by the same letter are not significantly different, Duncan's multiple range test,  $P \le 0.01$ .

supports the finding in the timing experiment that dimethoate is effective when applied at any stage of development until the pendant position is reached.

Hydraulic sprays of dimethoate were more effective than airblast sprays for controlling <u>C. oregonensis</u> and total insect damage (Table XXXI). The only application by airblast sprayer that caused a significant reduction in the number of galls was at 2.24 kg a.i./ha, and this rate was not as effective as hydraulic sprays.

Summarizing the experimental trials, dimethoate and acephate were the most effective insecticides tested for controlling damage by  $\underline{C}$ . Oregonensis and other insects; the other insecticides were not effective enough for use in seed orchards. Dimethoate was the most effective agent against  $\underline{C}$ . Oregonensis. The optimal stage of conelet development for application of dimethoate is after the conelets have closed and are turning, but before they reach the pendant position, as suggested by Johnson and Hedlin (1967). Single sprays are as effective as 2 sprays, and hydraulic sprays are more effective and efficient than airblast sprays.

## 6.2.1.3.2 Operational trials

Operational sprays of both acephate and dimethoate resulted in significant reductions in <u>C</u>. <u>oregonensis</u> galls and total insect damage, and in significant increases in the number of filled seeds (Table XXXII). The increases in numbers of filled seeds per axial slice corresponded to increases in numbers of extractible filled seed. The mean number of extracted filled seeds per cone was 12.6 and 23.2 for control and sprayed trees, respectively, at Quinsam in 1979, 15.2 and 20.8, respectively, at Snowdon in 1979, and 11.9 and 23.8, respectively, at Quinsam in 1980. The

Table XXXI. Mean numbers of filled seeds, <u>C. oregonensis</u> galls and total insect damage exposed per axial slice in cones treated with dimethoate applied with airblast and hydraulic sprayers at Quinsam in 1980.

Treatment	Sprayer	No. trees	Mean no. Filled seed <sup>a</sup>	exposed Galls <sup>a</sup>	/axial slice Total insect damagea <sup>a</sup>
Control	_	20	0.91 a	4.94 a	6.93 a
0.56 kg/ha	Airblast	10	1.27 ab	4.71 a	5.61 a
1.12 kg/ha	Airblast	10	1.09 a	5.44 a	6.19 a
2.24 kg/ha	Airblast	10	1.60 ab	3.42 b	4.05 b
0.5% to drip point	Hydraulic	20	1.78 ъ	1.72 c	2.38 c

Means within a column followed by the same letter are not significantly different, Duncan's multiple range test,  $P \le 0.05$ .

Douglas-fir seed orchards in B.C. All means for sprayed cones are significantly different from total insect damage exposed per axial slice in operationally sprayed and untreated cones in Table XXXII. Mean numbers and percentage change of filled seeds, C. oregonensis galls and their respective controls, t-test, P  $\leq$  0.05.

				F111(	Filled seed	ပါ ပါ	Damaged oregonensis	seed	[ota]
Insecticide	Year	Orchard	Treatment	×	(%) Increase	۱×	(%) Reduction	۱×	(%) Reduction
Acephate	1978	Tahsis	Control	5.36		3.99	ı	5.01	I
			Sprayed	7.49	70	0.43	89	0.67	87
	1979	Tahsis	Control	1.39	ı	4.11	1	6.54	I
			Sprayed	4.01	189	2.36	21	2.85	26
	1979	PFP	Control	1.03	ı	9.03	ı	10.92	ı
			Sprayed	2.92	184	7.13	77	8.06	99
Dimethoate	1979	Ouinsam	Control	3.09	ı	2.69	1	3.62	
		,	Sprayed	5.84	89	0.78	71	0.92	7.5
	1979	Snowdon	Control	3.81	ı	1.36		1.84	1
	)		Sprayed	5.20	37	0.20	85	0.23	88
	1980	Ouinsam	Control	0.78	ı	4.79	ı	6.37	ı
			Sprayed	3.01	286	1.23	74	2.04	89
	1980	Tahsis	Control	•	ı	3.45	1	4.25	ı
			Sprayed	•	54	0.82	9/	0.88	79
	1981	Ouinsam	Control	2.81	ı	0.69	1	3.02	ı
		•	Sprayed	4.16	48	0.09	87	0.84	72
	1981	Snowdon	Control	3.10	1	0.23	ı	1.78	ı
			Shraved	£ 8 7	07	C	100	0.22	88

differences are all significant (t-test,  $P \leq 0.01$ ). Dimethoate generally caused greater reductions than acephate, which is consistent with the results of the experimental trials; however, there was considerable variation in reductions of  $\underline{C}$ . oregonensis galls and total insect damage achieved with both insecticides.

Reasons for the variation in results with acephate are not clear. Although rain after insecticide application, can cause insecticide dilution and run-off, the relationship between effectiveness and weather was not The weather was warm and dry for 5 days after application at Tahsis in 1978 where the reduction of total insect damage was 87%, whereas it rained the day of application plus the next 9 days at PFP in 1979 where the reduction in total insect damage was 56%. However, it was dry and warm for several days after the application at Tahsis in 1979 where the reduction was only 26%. The lower reductions at Tahsis in 1979 were in part due to poor timing of application; nearly 1/3 of the trees had cones already in the pendant position. Acephate is not as effective when applied to pendant conelets (section 6.2.1.3.1). Poor timing is probably one of several factors that caused the poor levels of control achieved, since 2/3 of the trees were at the recommended stage of cone development for treatment. For example, little is known about the interaction between the physiology of conifers and systemic insecticides.

The effectiveness of dimethoate was less variable than that of acephate and the relationship between weather and effectiveness was more consistent. The most effective applications, at Snowdon in 1979 and 1981 (Table XXXII), were followed by several days of dry weather. The least least effective application was at Quinsam in 1980 where 40% of the sample trees were treated on 2 May when precipitation occurred immediately after

insecticide application, while the others were treated one week later when the weather was warm and dry. The reduction in total insect damage achieved by the later application was significantly greater than the early application, 85 vs 22% (t-test,  $P \le 0.01$ ).

Weather will undoubtedly pose a problem during future operational applications because precipitation occurs regularly about the time the conelets reach the stage of development suitable for treatment.

Theoretical benefit: cost ratios for applications to individual trees were calculated to determine the number of cones on a tree and increased seed yield necessary to make an application cost effective based on the following assumptions. The base cost of application used in the analysis was \$1.64 per tree, the calculated cost for the operational applications at Quinsam and Snowdon in 1981 (Table XXXIII). The sprayer used in these applications was a hydraulic sprayer mounted on a self-propelled manlift (Active Machine Works Ltd., Kelowna, B.C.) (Fig. 38). The increase in seed yield (extractible, filled seeds) per cone due to treatment was assumed to be 12, the value obtained in the small-scale trial at Koksilah in 1978. Multiples of the base cost and the base seed yield were used to show the importance of application cost and increased seed yield. Seed value was assumed to be \$50, \$100, \$250 or \$500 per kilogram and the number of filled seeds per kilogram was assumed to be 100,000, approximately the mean number of seeds per kilogram listed by Dobbs et al. (1976). The cost of orchardproduced Douglas-fir seed in B.C. was estimated at \$150 per kilogram in 1980 (J. Konishi<sup>1</sup>, pers. comm.).

The theoretical benefit: cost ratios listed in Table XXXIV indicate that insecticide applications for cone and seed insect control are economically justifiable in Douglas-fir seed orchards provided a treated

Table XXXIII. Breakdown of the insecticide application costs at 2 seed orchards, Quinsam and Snowdon, in 1981 using a manliftmounted hydraulic sprayer (T. Crowder<sup>8</sup>, per. comm.).

Item	Cost (\$)
Labour: 17 1/2 h @ \$9.30/h	162.75
Dimethoate 4E, 22.5L @ \$13.62/L	306.45
Sprayer rental, 12 h @ \$4.00/h	48.00
TOTAL	517.20
Cost per tree, 316 trees treated	1.64

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Figure 38. Hydraulic sprayer mounted on self-propelled manlift used at Quinsam and Snowdon for cone and seed insect control.



Table XXXIV. Theoretical benefit: cost ratios for insecticide applications to individual trees in Douglas-fir seed orchards in B.C., assuming a seed value of \$50/kg and 100,000 seeds/kg.

Cost of application per tree (\$)	Increased yield per cone	No. cones per tree	Increased yield per tree	Value of increased yield per tree (\$)	Benefit:cost ratio
1.64	6	2,000	12,000	6.00	3.66
		1,000	6,000	3.00	1.83
		500	3,000	1.50	0.92
		100	600	0.30	0.18
1.64	12	2,000	24,000	12.00	7.32
		1,000	12,000	6.00	3.66
		500	6,000	3.00	1.83
		100	1,200	0.60	0.37
1.64	24	2,000	48,000	24.00	14.63
		1,000	24,000	12.00	7.32
		500	12,000	6.00	3.66
		100	2,400	1.20	0.74
3.28	6	2,000	12,000	6.00	1.83
	-	1,000	6,000	3.00	0.92
		500	3,000	1.50	0.47
		100	600	0.15	0.09
3.28	12	2,000	24,000	12.00	3.66
		1,000	12,000	6.00	1.83
		500	6,000	3.00	0.92
		100	1,200	0.60	0.18
3.28	24	2,000	48,000	24.00	7.32
	_ ,	1,000	24,000	12.00	3.66
		500	12,000	6.00	1.83
		100	2,400	1.20	0.37

threshold number. The benefit: cost ratio must be greater than one for an insecticide application to be cost effective. In a similar analysis, DeBarr (1971) showed that insecticide applications were similarly justifiable in pine orchards in the southern United States.

In young seed orchards entering the production phase, it is not uncommon for a minority of trees to produce most of the crop. Treating only trees with moderate of heavy crops maximizes the benefit: cost ratio by reducing the amount of insecticide used and the amount of labour required to apply the spray while protecting the bulk of the crop. This approach is currently followed in B.C. Douglas-fir seed orchards and has been suggested for use in pine orchards in the southern United States (Yates 1977).

The actual numbers of cones a tree must bear at harvest to recover the cost of the insecticide application are indicated in Table XXXV. These numbers are dependent on the increase in seed yield due to the application and on seed value. Treatment of trees with less than the threshold number of cones for the specific circumstances is not justified. Yates' (1977) caution that the crop size must be considered when deciding whether or not to spray in pine orchards also applies in Douglas-fir orchards. Thus, it is essential to have a technique for estimating the numbers of cones that trees bear. Some allowance for conelet abortion should be made when projecting these required harvest counts back to counts at the time of application.

The amount of increased seed yield per cone necessary to recover the application cost is indicated in Table XXXVI. The higher the seed value or the larger the number of cones on the tree, the smaller is the necessary increase in seed yield per cone. Insecticide applications for control of light infestations are economically justifiable if the cone crop is large

Table XXXV. Number of cones a Douglas-fir tree must bear at harvest for recovery of insecticide application costs.

Increased seed yield	Application costs per	No. of	cones re	nuired at	t appoific	
	, <del>,</del> ,	No. of cones required at specific seed values (\$/kg)				
per cone	tree (\$)	\$50	\$100	\$250	\$500	
					<del></del>	
6	0.82	274	137	555	28	
	1.64	547	274	110	55	
	3.28	1094	547	219	110	
	6.56	2188	1094	438	219	
12	0.82	137	68	28	14	
	1.64	274	137	55	28	
	3.28	547	274	110	55	
	6.56	1094	547	219	110	
24	0.82	68	26	14	7	
	1.64	137	55	28	14	
	3.28	274	110	55	28	
	6.56	547	219	110	55	

Table XXXVI. Amounts of increased seed yield (numbers of extractible filled seed) per cone necessary to recover the cost of an insecticide application to a Douglas-fir tree, assuming 100,000 seeds/kg.

Cost of application	No. cones on tree	Increase in no. seeds/cone required at specific seed value (\$/kg)			
(\$)		\$50	\$100	\$250	\$500
1.64	2,000	1.64	0.82	0.33	0.16
	1,000	3.28	1.64	0.66	0.33
	500	6.56	3.28	1.31	0.66
	100	32.80	16.40	6.56	3.28
3.28	2,000	3.28	1.64	0.66	0.33
	1,000	6.56	3.28	1.31	0.66
	500	13.12	6.56	2.62	1.31
	100	65.60	32.80	13.12	6.56

enough, whereas treating trees with only 100 cones is justifiable only when seed values are high since it is unrealistic to expect increased yields of 32 or 65 seeds per cone considering current seed yields of  $\leq$  25 filled seed per cone (M. Crown<sup>2</sup> pers. comm.). The interaction between infestation level and insecticide efficacy must result in an increase in yield greater than the threshold value for the application to be justifiable. Efficacies of 85% or more can be attained with dimethoate, depending on weather. When deciding whether or not to spray, it is necessary to know the level of infestation present in the orchard, the damage caused by such an infestation level, and the amount of damage that can be tolerated without taking control actions. Thus, it is essential to have sampling techniques for estimating infestation level and for predicting damage that will be done if no control action is taken.

Phytotoxicity was associated with applications of dimethoate. The degree of needle burn, associated with the operational sprays was correlated with clone or family (Table XXXVII). For example, 7.4% of all trees sprayed at Tahsis in 1980 suffered moderate to severe needle burn, but this figure rose to 100% for clone 214. One clone (218) was susceptible at 2 different orchard sites and at the same orchard in 2 consecutive years. This relationship indicates that some Douglas-fir clones and members of some families are relatively more sensitive than others to poisoning by dimethoate. Hybrids involving one sensitive parent, such as clone 218, produced some offspring that were also sensitive, indicating further that sensitivity is at least partially genetic, although environmental factors and health of grafts and seedlings may also have effects, since not all ramets or seedlings from one family were equally sensitive to burn (Table

Table XXXVII. Numbers of Douglas-fir trees and clones or families moderately or severely burnt by Dimethoate 4E in 3 B.C. seed orchards, 1979-80.

		No. clones or families (trees)		Susceptible Clone or Fami Code No. trees No. tr		or Family No. trees
Orchard	Year	Sprayed	Burnt <sup>a</sup>	h	sprayed	burnt <sup>a</sup>
Quinsam	1979	91(211)	2(3)	218c 276c	2	2 1
	1980	87(189)	2(4)	218c 276c	1 3	1 3
Snowdon	1979	27(104)	0(0)	-	-	
	1980	34(163)	4(4)	546f 548f 556f 566f	3 2 2 3	1 1 1
Tahsis	1980	120(1061)	16(79)	180c 206c 210c 214c 216c 218c 502c 542c 605c 639c 218/215x 218/216x 218/220x 218/windx 180/windx 216/220x		3 2 3 17 3 7 8 3 5 2 2 7 9 4 3

a moderately or severely

b c = clone, f = family, x = cross

XXXIII). The degree of needle burn was not correlated with position in the orchard.

It is not known if the trees were sensitive to the active ingredient or to another constituent of the insecticide formulation, such as the emulsifier. Previous studies (Johnson and Rediske 1965; Johnson and Meso 1966; Hedlin 1966; Johnson and Zing 1967) that indicated little or no phytotoxicity at the concentrations applied, used technical grade dimethoate or the Cygon formulations and not the formulation used in this study (Dimethoate 4E). Differences in levels of phytotoxicity could also be due to differences in the trees treated. In the previous studies a small number of "wild" trees were treated, not identified clones or families as in this study. The proportion of clones or families that were moderately or severely burnt in the orchards was low, <13%, and may be even lower in non-orchard stands if susceptibility is increased by such practices as grafting.

Virtually all burnt needles dropped from the trees within 3 weeks after the application was made. Two severely burnt trees died, both at Tahsis. The other severely burnt trees survived because the vegetative buds flushed 1-2 weeks after dimethoate application. Application of dimethoate after vegetative flush, for control of Cooley spruce-gall aphid, Adelges cooleyi (Gillette) (Homoptera: Phylloxeridae), could result in considerable mortality within the sensitive clones or families. The consequences of treating sensitive trees just before vegetative flush in consecutive years is not known.

The burning of these orchard trees points out the need for an expanded arsenal of registered insecticides for use against cone and seed insects in Douglas-fir orchards. If alternative registered insecticides do

not become available, clones or families sensitive to operationally used concentrations of dimethoate may not be suitable for planting in seed orchards. The need to avoid insecticide-sensitive clones or families is an undesirable complicating factor in a tree improvement program.

## 6.2.2 Fatty acid derivatives

# 6.2.2.1 Introduction

Fatty acid derivatives (including salts or soaps) have been known for their insecticidal properties for many years (Seigler and Popenoe 1925; Tattersfield and Gimingham 1927), but outside of the home garden, they have seldom been used as practical control agents since 1940. They are gaining attention as alternatives to conventional insecticides because of their low toxicity to nontarget organisms. Recent studies have shown that the fatty acid salts, potassium oleate and potassium caprate, were effective against several forest aphids and lepidopterans (Puritch 1974, 1975, 1978).

The purpose of this study was to determine the efficacy of several fatty acid derivatives for use against <u>C</u>. <u>oregonensis</u> and to determine if these compounds were phytotoxic.

#### 6.2.2.2 Materials and Methods

Trials were carried out at Koksilah and Lake Cowichan in 1979, and at Dewdney in 1980. At Koksilah, 4 branches bearing at least 20 conelets on each of 10 trees received a single application to the drip point of 0.5% potassium oleate, 1.0% potassium oleate, or water. Each branch was treated when the conelets were open to receive pollen (24 and 25 April), the critical time for application of contact insecticides for control of  $\underline{C}$ . oregonensis. The applications were made with a 3.79-L compression sprayer.

At Lake Cowichan, each of the 3 treatments were applied to 10 randomly selected trees (27 April) with a 10-L, mechanical pump, backpack sprayer.

At Dewdney, 5 fatty acid derivatives were each applied at 3 concentrations, as follows: potassium oleate, potassium caprate and potassium undecylenate at 1.0, 0.5 and 0.1%, methyl coconate [a mixture of the methyl esters of lauric (50.1%), myristic (16.5%), palmitic (7.8%), caprylic (7.3%), oleic (5.7%), stearic (3.6%) and linoleic (1.5%) acids] and lauryl alcohol at 0.25, 0.10 and 0.05%. Each spray was applied to 5 randomly selected trees when the conelets were open (April 25 and 27) with the same sprayer used at Lake Cowichan. The concentrations used are known to be insecticidal against other insects (Badertscher 1931; Bousquet et al. 1935; Bradbury and Armstrong 1955; Fleming 1934; Puritch 1974, 1975, 1978; Tattersfield and Gimingham 1927). In addition, 5 trees were treated with 0.5% dimethoate, the standard insecticide for Douglas-fir cone and seed insects, and 5 untreated trees were marked as controls.

To evaluate the effects of the sprays on conelets in 1979, the treated branches at Koksilah and 4 selected branches per treated tree at Lake Cowichan were examined 2 weeks after treatment, and the numbers of healthy and aborted conelets were counted. In 1980, treated trees were examined 3 weeks after treatment and counts for whole trees were taken.

To evaluate the effects on <u>C. oregonensis</u>, 10 to 20 cones per treatment per tree were collected at the time of cone harvest (August 18-23) at each site. These cones were processed in the usual manner. Tree averages were then calculated for use in statistical analyses. Trees that produced fewer than 10 harvestable cones (per treatment) were not included in analyses; 4 trees treated with 1.0% and 1 tree treated with 0.5%

potassium oleate in 1980 were not included. Analysis of variance and Duncan's multiple range test was used in data analysis.

### 6.2.2.3 Results and Discussion

Potassium oleate caused large increases in the abortion rate of conelets at both sites in 1979 (Table XXXVIII). Differences in effect among concentrations were significant at both sites.

At Dewdney in 1980, all concentrations of potassium oleate and potassium caprate, and the 2 highest concentrations of potassium undecylenate, caused large significant increases in the abortion rate of conelets (Table IXL). The lowest concentrations of potassium undecylenate and all concentrations of methyl coconate, lauryl alcohol and dimethoate had no significant effect on the abortion rate. The differences in effect among potassium oleate concentrations were all significant. The 2 highest concentrations of potassium caprate did not differ in effect, but both were significantly more toxic than the lowest concentration. The 2 phytotoxic concentrations of potassium undecylenate were equal in effect. Foliage was not visibly damaged by any of the fatty acid derivatives tested.

There have been few reports of phytotoxicity of fatty acid derivatives to conifers in general, and no reports specifically concerned with phytotoxicity to conelets. Spraying 1-year-old seedlings of 8 species of conifers, including Douglas-fir, with up to 3.5% potassium oleate to the drip point did not cause foliage damage or stunt growth (Ross and Puritch 1979, unpublished data). Foliage damage to grand fir, Abies grandis (Dougl.) Lindl., was caused by 5% potassium oleate applied to the drip point

Table XXXVIII. Effects of potassium oleate on the abortion rate of Douglas-fir conelets and on the numbers of filled seeds and  $\underline{C}$ .  $\underline{Oregonensis}$  galls exposed per axial slice at Koksilah and Lake Cowichan in 1979.

		Aborted	Mean no. expo	no. exposed/axial slice		
	Concn	conelets	Filled			
Site	(%)	(mean %) <sup>a</sup>	seeds <sup>a</sup>	Galls <sup>a</sup>		
Koksilah	Control	1.8 a	3.31	8.51 a		
	0.5	64.6 b	3.32	3.25 b		
	1.0	94.0 c	3.39	4.31 b		
Lake Cowichan	Control	9.2 a	3.08	6.66 a		
	0.5	53.0 b	4.10	2.41 b		
	1.0	92 <b>.</b> 4 c	3.56	1.71 b		

Means within a column at each site followed by the same letter are not significantly different, Duncan's multiple range test,  $P \leq 0.05$ .

Table IXL. Effects of 5 fatty acid derivatives and dimethoate on the abortion rate of Douglas-fir conelets and on the numbers of filled seeds and Contarinia oregonensis galls exposed per half-cone, Dewdney, 1980.

	Concn	Aborted conelets	Mean exposed/ax Filled	
Treatment	(%)	(mean %)a	seeds <sup>a</sup>	Galls <sup>a</sup>
Control	_	1.8	5.3	1.8
Potassium oleate	0.10	25.1 a	4.7	1.1
	0.50	52.3 b	4.2	1.1
	1.00	95.5 c	4.5	1.8
Potassium caprate	0.10	26.5 a	5.5	0.9
	0.50	57.2 b	3.4	0.8
	1.00	68.8 b	4.6	0.7
Potassium undecylenate	0.10	15.8	4.8	1.5
	0.50	78.6 a	3.7	2.5
	1.00	74.8 a	2.4	1.6
Methyl coconate	0.05	2.0	5.6	1.1
	0.10	8.5	5.4	0.9
	0.25	8.1	4.1	1.1
Lauryl alcohol	0.05	5.4	6.0	0.9
	0.10	8.1	5.5	1.1
	0.25	9.8	6.1	1.3
Dimethoate	0.05	15.7	8.9 a	0 a

<sup>&</sup>lt;sup>a</sup> Means with a column followed by a letter are significantly different from control. Concentration means followed by a different letter are significantly different, Duncan's multiple range test,  $P \le 0.05$ .

during the growing season (G.S. Puritch<sup>10</sup>, pers. comm.) but not by concentrations of up to 15% during the dormant season (Puritch 1975). Douglas-fir conelets which are open to receive pollen are apparently more sensitive to potassium oleate than is foliage.

Fatty acid derivatives, especially methyl esters containing the saturated fatty acids between  $C_6$  and  $C_{10}$ , are known to reduce growth of plants in other taxonomic groups and have been used as chemical pruning agents (Tso 1964; Williams and Moser 1974; Verecke 1975; Byers and Barden 1976). The methyl coconate used in this experiment contained low concentrations of fatty acids in the  $C_6$ - $C_{10}$  range, which may explain why this mixture of methyl esters did not affect the conelets. Methyl coconate did not increase the abortion rate of conelets in white spruce, Picea glauca (Moench) Voss (J.R. Sutherland  $^{11}$ , pers. comm.).

The aborting of conelets caused by the fatty acid salts may be due to changes in the cuticular waxes, allowing dessication of the conelets. Fatty acid derivatives have been shown to affect cuticular waxes in sweet cherries (Harrington et al. 1978) and chrysanthemums (Nelson and Reid 1971).

Spraying conelets at various points in their development showed that they were affected less by potassium oleate as they matured. Conelets were sensitive from the time they opened until they had been pendant for 2

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days, but not when they had been pendant for 1 month, even with concentrations of up to 2% (the highest concentration tested).

Reducing the numbers of conelets could be useful when trees produce more conelets than they can support to maturity. To prevent large-scale losses in these instances, an agent for measured thinning of the crop would be useful. The fatty acid salts, especially potassium oleate, may have considerable potential for such use.

The numbers of <u>C. oregonensis</u> galls per cone were significantly reduced by potassium oleate at both sites in 1979 (Table XXXVIII). The reductions at Koksilah were 49 and 62% for 1.0 and 0.5% potassium oleate, respectively, and at Lake Cowichan, 74 and 64% for 1.0 and 0.5%, respectively. The differences between concentrations were not significant at either site. At Dewdney in 1980, none of the fatty acid derivatives affected the number of galls, whereas dimethoate-treated conelets were gall free (Table IXL), indicating that none of the derivatives were as effective as dimethoate for reducing <u>C. oregonensis</u> damage. Reasons for the differences between years in effectiveness of potassium oleate are not known.

The numbers of filled seeds were not affected significantly at any site even though the number of galls was reduced (Table XXXVIII). Increased numbers of filled seeds normally correspond to reductions in C. oregonensis damage when effective insecticides, such as dimethoate, are used.

The fatty acid derivatives tested for use against <u>C. oregonensis</u> are unsuitable because they were toxic to conelets or not effective in reducing pest damage. In general, increasing the abortion rates of conelets has negative economic impacts on seed production unless the increase in abortions caused by an insecticide is offset by reducing the proportion of seed crops destroyed by the midge. For example, dimethoate sometimes causes

abortion rates of 10-15%, but is used because it usually reduces insect damage by  $\geq$  85%. Unfortunately, none of the derivatives used in these tests provided this combination of low phytotoxicity with high efficacy against  $\underline{C}$ . oregonensis. Potassium oleate, the only derivative that did cause significant reductions in gall midge damage, caused many times more abortions. In addition, the reductions in insect damage did not increase the numbers of filled seeds.

### 7.0 CONCLUDING DISCUSSION

To date, <u>C. oregonensis</u> has been the key pest in coastal Douglas-fir seed orchards in B.C. Components of a practical pest management system were developed during this research, especially in the areas of population monitoring, damage prediction and control, for use against this pest in seed orchards.

Sampling techniques for estimating populations of Douglas-fir conelets (section 5.1) and predicting <u>C. oregonensis</u> damage (section 5.3) allow for deciding whether or not an insecticide application is justified. Both of these variables must be considered because there is no point in applying an insecticide if the cone crop is too small to recover the application costs or if the expected damage is less than the threshold value such that application costs can be recovered.

An important component of pest management systems is knowledge of the economic threshold, i.e. the density at which control measures should be applied to prevent a pest population from reaching the density that will cause economic damage (Stern et al 1959). This threshold allows for optimal economic use of insecticides (Stern 1973). It is difficult to determine the economic injury level for <u>C. oregonensis</u> because it is difficult to determine seed value. The only seed value currently available is the production cost which is a function of the size of the crop and management techniques used. Crop size varies dramatically among orchards and years. Seeds produced from a small crop are more expensive than seeds from large crops managed with the same procedures. Little seed has been produced in orchards to date, and this seed is quickly used by the producing agency. Market values will not be known until genetic gains in orchard-produced

seeds can be quantified and amounts produced in orchards are large enough for market sales to occur.

The techniques developed for estimating population sizes of Douglas-fir conelets and <u>C. oregonensis</u> eggs can be time consuming during a period when time is at a premium. The period between time of damage prediction sampling and optimal time for insecticide application is often less than I week. Thus, time is a critical commodity. The sequential technique for damage prediction reduces considerably the time needed for estimating damage. If precise estimates are not required, the time needed to estimate crop size could be reduced by sampling only trees that appear to bear approximately the threshold number of conelets for crop management to be justified. For example, if the threshold number is 200 conelets, estimating crop size with the technique on a tree bearing 1000 conelets should not be necessary. Visual classification by experienced personnel should indicate that the tree bears more than the threshold number. Similarly, a tree bearing only 25 conelets should not require quantification.

Observations of reproductive behaviour indicated the presence of a sex pheromone in <u>C. oregonensis</u> (Table III). This substance has potential use in population monitoring and for indicating the dates of initial and maximum adult midge activity. Currently, studies are being carried out to identify the attractant(s). Placement of sex attractant traps is important because the midges mate on or near the duff where they overwinter. It would be necessary to place traps in nearby stands of Douglas-fir which may present a problem with vandalism in some circumstances because midges from adjacent areas invade orchards. Host attractants, if they exist as for other cone and seed insects (Asher 1970; Kinzer et al. 1972), may hold more

potential for population monitoring than sex attractants because they could be used in the orchards and because they monitor populations of females rather than males, theoretically a more direct measure of potential damage since females attack (oviposit in) the conelets. Trapping of adults with either a sex attractant or host volatiles should reduce the time necessary for cone gall midge population monitoring compared to methods based on egg counts and have the additional advantage of being non-destructive.

A bioassay technique using heptane extracts of female ovipositors was developed (section 3.3.2.2) but needs further refinements. Male responsiveness may be increased past the maximum achieved in these studies (42%) by using virgin males which have not been exposed to calling females prior to use in bioassays. Habituation resulting in reduced male response due previous exposure to pheromone is known in Lepidptera (Shorey and Gaston 1964; Traynier 1970; Farkas et al. 1975) and Coleoptera (Vick et al. 1973) but has not been reported in Diptera. Problems associated with ensuring virginity and lack of previous exposure include reduced incidence of adult emergence due to handling in individual rearings and an inability to determine the sex of midges prior to the adult stage.

The only practical control technique currently available for protecting a crop is the application of dimethoate, the only insecticide registered against Douglas-fir cone and seed insects in B.C. (Richmond et al. 1975). None of the other insecticides tested during this research were as effective as dimethoate and most did not reduce <u>C. oregonensis</u> damage significantly (section 6.2). Several insecticides were shown by other researchers to be effective (Miller 1980), but most are more toxic to mammals than dimethoate. Currently, the B.C. Ministry of Forests prefers to avoid using pesticides in their orchards that are highly toxic to mammals.

However, the susceptibility of some Douglas-fir clones to dimethoate points out the need for other registered insecticides.

Contact insecticides can be used only as preventative sprays because they are effective only when applied to conelets open to receive pollen. Preventative sprays may have uses in an orchard pest management program for C. oregonensis when infestations are likely to be high, i.e. when a light to significant crop occurs in nearby stands the year following a moderate or heavy crop in these stands. Systemic insecticides allow time for a demonstration of the need for an insecticide application, because they may be applied after C. oregonensis oviposition is completed and egg population estimation is possible. Thus, they are attractive tools in orchard pest management. Delayed seed-cone bud burst can reduce the number of crops that must be protected with insecticides but the effectiveness of delayed bud burst is determined by weather and varies so dramatically that it cannot be relied upon (section 6.1). Insect control should not be considered a factor when deciding whether or not to install an overhead irrigation system because of this lack of consistent control. Any negative effects on insect infestations should be considered a bonus.

The impact of cone harvest as control technique should not be underestimated (Annila 1976). All cones should be harvested, including unwanted cones produced on rootstocks. Tahsis consistently suffered heavier insect losses than PFP, located about 1 km away, until they changed in 1978 to a policy of harvesting all cones, as is done at PFP.

Parasitoids and predators appear to be of limited usefulness in orchards because of their low incidence and their inability to prevent damage. Other biological agents, such as entomophagous fungi, may have potential as control agents. Timonin et al. (1980) showed that Beauvaria

bassiana (Bals.) Vuill. and Metarrhizium anisopliae (Metch.) Sor. cause mortality in spruce cone and seed insects in the laboratory, but no successful field trials have been reported.

The sex attractant and, if one exists, the oviposition marker pheromone (section 3.3.2.3) also have potential as control agents. Both types of pheromones have been used in mass trapping and disruption trials to reduce damage by other insects (Jacobson 1972; Birch 1974; Katsoyannos and Boller 1976; Roeloffs 1979).

Ultimately, the most effective control technique may be orchard isolation which would reduce the number of gall midges invading an orchard. If possible, orchards should be isolated from stands of the same species. The amount of isolation from other stands required for effective pest control is not known since the distances <u>C. oregonensis</u> (or other Douglas-fir cone and seed insects) can disperse are not known. It would be difficult to isolate Douglas-fir orchards by significant distances in areas suitable for cone production in B.C. It may be possible to isolate orchards through destruction of cone crops in nearby stands when they occur and the eventual elimination of <u>C. oregonensis</u> populations in the vicinity of the orchard. Fatty acid salts may be useful agents in this regard (Tables XXXVIII, IXL).

The pest management system for <u>C. oregonensis</u> in Douglas-fir seed orchards developed during this research consists of estimating conelet crop sizes and <u>C. oregonensis</u> infestations on trees individually and, when necessary, spraying the trees with dimethoate.

Total numbers of conelets can be estimated by counting the number on 6 whorl branches in each of the upper and mid crown thirds, multiplying calculated means, after transforming by  $\log_{10} (x + 1)$ , by the number of

producing branches. At current application costs (\$1.64/tree), a tree should produce 200-250 (transformed value of 2.301-2.398) cones at harvest for an insecticide application to be economically justifiable. An allowance for conelet abortion, specific to each orchard, should be made when deciding whether or not to spray a tree.

Predictions of seed loss to <u>C. oregonensis</u> can be made by determining the numbers of egg-infested scales per conelet. Using the sequential plan developed during this research, the number of conelets required from a tree is determined by crossing of "stop sampling lines" illustrated in Figs. 31 and 32. The critical densities for 10 and 20% seed loss were estimated to be 2.0 and 4.0 infested scales per conelet, respectively. Means for trees bearing cones in all 3 levels of the number of eggs or infested scales can be estimated accurately by sampling the mid crown only. A maximum of 8 conelets would be necessary to determine whether or not an insecticide application is necessary for these trees. Otherwise, all producing crown levels should be sampled.

Dimethoate should be applied when the conelets are closed and turning but before they reach the pendant position with a hydraulic sprayer at a rate of 0.5-1.0% until the cone-bearing regions of the trees are soaked. Orchard trees should be tested for susceptibility to dimethoate poisoning (Table XXXVII) before being operationally sprayed for the first time, so that potential phytotoxicity problems will be known in advance.

The pest management procedures developed during this research were aimed only at <u>C. oregonensis</u>, although the efficacies of the test insecticides and delayed flowering were determined for all Douglas-fir cone and seed insects present. A pest management system should be expanded to include other pests. The importance of a management system

aimed at the whole complex of pests is underlined by the situation at PFP in 1981 where 24% of the seeds were infested by the Douglas-fir seed chalcid (R. Heath<sup>12</sup>, per. comm.) in spite of a dimethoate application aimed at reduction of gall midge damage. Ultimately, any pest management system should be integrated with all orchard management techniques.

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