HOST SELECTION, IMPACT AND CHEMICAL ECOLOGY OF THE WESTERN CONIFER SEED BUG, *LEPTOGLOSSUS OCCIDENTALIS* HEIDEMANN (HEMIPTERA: COREIDAE).

by

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ABSTRACT

The western conifer seed bug, *Leptoglossus occidentalis* Heidemann, is a poorly understood pest of British Columbia seed orchards. Two Douglas-fir and two lodgepole pine orchards located on Vancouver Island and Vernon, respectively, were surveyed for *L. occidentalis* during the years 1992-1995 and 1993-1995, respectively. Analysis by conventional dispersion statistics disclosed that *L. occidentalis* exhibits a pronounced clonal preference in its host selection. Applying Poisson regression analysis to the data reaffirmed this conclusion. Visual reflectance from foliage and conelets was not different between trees of preferred and non-preferred clones. Host preference was greater for trees of intermediate height and cone density, indicating that host selection by *L. occidentalis* is complex and cannot be described by a simple linear relationship. Adults and nymphs of *L. occidentalis* share their seed resource with the Douglas-fir seed chalcid, *Megastigmus spermotrophus* Watchl. Laboratory experiments showed that *L. occidentalis* adults discriminate between sound and chalcid-infested seed. Nymphs discriminate at low levels of infestation but a few will feed on the larvae in a no-choice situation. Both adults and nymphs give off an alarm pheromone when disturbed. In adults, the pheromone is a five-component blend of: hexyl acetate, heptyl acetate, hexanal, octyl acetate, and hexanol. Nymphs possess a single component pheromone: (*E*)-2-

hexenal. Both adults and nymphs will respond to alarm pheromone given off by either life stage. Field trapping experiments disclosed that males produce an aggregation pheromone to which both sexes respond. The life cycle of *L. occidentalis* can be better understood. Adults seek hosts in the spring, making several choices. In sequential order these are: selection of an appropriate clone, choosing an acceptable cone density, evaluation of a preferred height, and determining the level of chalcid-free seed. Once chosen, mating and oviposition occur, which may be facilitated by pheromones and host kairomones, respectively. Nymphs hatch and feed throughout the summer, also avoiding chalcid-infested seed, and molt through five instars. Both adults and nymphs utilize an alarm pheromone to avoid predation and warn con-specifics of danger. In the fall the newly molted adults aggregate, by means of the aggregation pheromone, and overwinter in houses and other man-made structures.

DEDICATION

For Kaitlyn Lillith

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General Introduction

The Order Hemiptera is one of the most diversified and little understood orders in the Class Insecta. Hemipterans inhabit both land and water and feed on every family of plant species on earth. It has been argued that prohemipterans were originally carnivorous and hygrophilous (Cobben 1978, 1979); however, other arguments are in favor of prohemipterans being phytophagous and terrestrial (Sweet 1979, Zrzavú 1992). The Order itself is composed of two sub-Orders: Homoptera, a diverse grouping of many types of insects, the most well-known of which are aphids; and Heteroptera, the true bugs. Within the Heteroptera occur many beneficial insects, such as predatory mirids (Niemczyk 1978, Arnoldi *et al.* 1992, Braman and Beshear 1994), and many pests, e.g. assassin bugs which vector Chagas disease in humans (Borror *et al.* 1989, WHO 1991), lygus bugs (Michailides *et al.* 1987, Schowalter and Stein 1987), and squash bugs (Nechols 1988) that damage agricultural and forest crops. This dissertation focuses on one heteropteran in the family Coreidae, the western conifer seed bug, *Leptoglossus occidentalis* Heidemann.

The genus *Leptoglossus* comprises the leaf-footed bugs, insects characterized by a proximal leaf-shaped expansion of their metathoracic tibia. They inhabit three continents and feed on a wide range of plant

species. In the angiosperms, they feed on the fruits, bolls, stems, berries and joints of plants from 43 genera found in 22 orders. Leaf-footed bugs also feed on the developing cones, seeds, buds, shoots and staminate flowers of gymnosperms in five genera within two families (Schaefer and Mitchell 1983). *Leptoglossus* spp. feed by inserting stylets into the tissue, secreting enzymes to digest the starches and carbohydrates and then siphoning the liquefied cells into their digestive tract (Miles 1986). In many situations, mechanical damage is believed to be done to the tissues; however, this does not appear to always be the case (Miles 1986). *Leptoglossus* spp. have been documented as being pests in the United States, Australia, South America and Argentina (Fernando 1957), but in no instance is their biology well understood.

Leptoglossus occidentalis is native to western North America and ranges from southern California north to central British Columbia and as far east as Idaho (Hedlin *et al.* 1981). It was recently discovered in Ontario (Katovich and Kulman 1987, Marshall 1991) and is considered a household nuisance there. It feeds on conifers in many genera, including *Abies, Picea, Pinus* and *Pseudotsuga*. It was not recognized as a pest until Koerber (1963) published the first account of this species feeding on the cones of coniferous trees. Since then, its role as a pest of conifer seed has been repeatedly acclaimed (Krugman and Koerber 1969, Ebel and Yates 1974, Ruth 1980, Hedlin *et al.* 1981, Schowalter *et al.* 1985,

Shea *et al.* 1986, Summers and Ruth 1987, Pasek and Dix 1988, Schowalter and Sexton 1990, Connelly and Schowalter 1991, Turgeon and de Groot 1994, Schowalter 1994, 1996). However, no study has conclusively demonstrated or described the relationship between damaged seed and insect numbers. Furthermore, very little is understood about the interactions of *L. occidentalis* with their hosts or with each other. Knowledge of such interactions would doubtless aid in characterizing *L. occidentalis* as a pest, and could possibly lead to new monitoring and control practices.

My original objective was to elucidate certain aspects of the chemical ecology of *L. occidentalis*, in an attempt to discover an ecologically sound management system to replace the current use of insecticides. However, many aspects of the proposed studies posed insurmountable difficulties (at least during the short time-frame of a Ph.D. research project). In addition, initial problems in finding populations of *L. occidentalis* drew me of necessity into examining various aspects of its natural distribution and host selection. As a result of this examination, various hypotheses concerning the use of semiochemicals as a means to select hosts, find mates, warn conspecifics and prepare for overwintering were formulated. Therefore, this thesis evolved as two separate parts, one emphasizing plant-insect interactions

and host selection, and the other selected aspects of pheromone-based communication.

Upon introduction to this species, I briefly examined the life cycle in Canada and found no discrepancy with that reported by Hedlin *et al.* (1981) or Hanson (1984). I observed much about its distribution within the seed orchards and the actual numbers of insects present. In Part I, Chapter 1, I describe the sampling plan which was developed, the distribution and clonal host preference of *L. occidentalis* within seed orchards and its impact. I initiated a study to verify the identification of the characteristic damage done by this species and to evaluate the potential for it to cause economic damage to an orchard. As the distribution of *L. occidentalis* within orchards was not random, I hypothesized that host selection by *L. occidentalis* may depend on various physical properties of the trees (Chapter 2). *Leptoglossus occidentalis* is not the only seed-feeding species to inhabit seed orchards. Therefore, its host selection may also be affected by the presence of another pest species, the Douglas-fir seed chalcid. In Chapter 3, I determine if *L. occidentalis* can discriminate between chalcid-infested seed and filled seed and then compare host selection preferences of these two species in the field. In Chapter 4, I use Poisson regression to evaluate further the relative importance of tree characteristics upon the host selection preferences of *L. occidentalis*. To summarize my results, I

propose and describe a hypothetical decision tree detailing the potential host selection decisions that must be made by *L. occidentalis* (Chapter 5).

While examining the life cycle and distribution of *L. occidentalis* in Part I, the potential for this species to utilize semiochemicals for warning conspecifics, seeking overwintering sites and locating mates became apparent. In Part II of this dissertation, I examine various aspects of the chemical ecology of this species. Many Heteroptera are mistakenly called 'stink bugs' due to an offensive and profuse scent they emit when disturbed (Borror *et al.* 1989). I test the hypothesis that *L. occidentalis* utilizes its defensive secretion as an alarm pheromone in Chapter 6. Prior to overwintering, some Heteroptera and Coleoptera have been observed to aggregate in large numbers (Harper and Lilly 1982, Schowalter 1986, Zack 1990, Negron and Riley 1991, Segelken 1994). In British Columbia, residents complain each year about the numbers of *L. occidentalis* entering their homes in the fall. In Chapter 7, I describe one of these aggregations, detail its occurrence and test the hypothesis that it is mediated by pheromones. To conclude the thesis, an integrating discussion demonstrates how the reported discoveries contribute to an overall understanding of the biology of *L. occidentalis* and suggest further areas for biological inquiry.

Part I : Distribution, Host Selection and Impact

Chapter 1
Clonal Preference and Impact by *Leptoglossus occidentalis*

Introduction

The life cycle of *L. occidentalis* has been well documented (Hedlin *et al.* 1981, Hanson 1984). Adults overwinter and emerge in early spring to begin laying eggs on the foliage of coniferous trees. Eggs hatch approximately two weeks later and the young instars feed on the foliage and developing cones. There are five instars, and first generation adults can be found in late July. All life stages feed on the cones and are capable of contributing to the damage observed at the end of the season. *Leptoglossus corculus* (Say), found in the southeastern USA, has been observed to demonstrate a preference for specific host genotypes (J.C. Nord, pers. comm., 147 Rock Springs Rd. NE, Eatonton, Georgia, USA, 31024). Adults of both species select host trees in spring and oviposit on them. Nymphs hatch and develop on these trees (ramets) throughout the season.

In the United States, *L. occidentalis* is considered to be a serious pest in seed orchards (Koerber 1963; Hedlin *et al.* 1981). Feeding by the

western conifer seed bug, *L. occidentalis* occurs when a bug inserts its stylets through the cone scales and into the seed. Studies on western white pine, *Pinus monticola* Dougl. ex D. Don, coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, and ponderosa pine, *Pinus ponderosa* Doug. ex. Laws., have found losses of 70-80% (Connelly and Schowalter 1991 and Schowalter 1994, 1996), 50% (Schowalter 1996) and 41% (Pasek and Dix 1988), respectively. In Canada, *L. occidentalis* is reported as causing seed losses of between 36% and 41% on Douglas-fir (Hedlin *et al.* 1981 and Ruth 1980).

Coastal Douglas-fir and lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, are two species involved in tree improvement programs in British Columbia. Douglas-fir is extensively planted following harvesting on the coast. Lodgepole pine seed is utilized for replanting in the B.C. interior, as well as for export to various countries. As the major objective of a seed orchard is the production of seeds that will eventually result in phenotypically desirable trees (Di-Giovanni and Kevan 1991), significant losses of seed in either species reduce the number of high quality trees being produced for replacement planting. The potential for this pest to cause considerable damage in Canadian seed orchards is of concern, but conclusive impact studies have not been conducted in Canada.

My objectives were to determine the distribution of *L. occidentalis* populations within clonal Douglas-fir and lodgepole pine seed orchards, and to evaluate the importance of *L. occidentalis* as a pest species. I use the term distribution to refer to the location of *L. occidentalis* individuals at one point in time.

Materials and Methods

Surveys in 1992 were carried out in Saanichton, B.C. in three coastal Douglas-fir seed orchards, Mt. Newton 1 and 2, first and second generation orchards, respectively, located at Mt. Newton Seed Orchards, Fletcher Challenge Canada (now Timber West Ltd.), and Nootka, located at Tahsis Seed Orchards, Canadian Pacific Forest Products. In 1993, 1994 and 1995, the survey was repeated at Mt. Newton 1 and 2, and extended to include two lodgepole pine seed orchards, Kal 1 and 2, reference numbers 230 and 307, respectively, located at Kalamalka Seed Orchards, B.C. Forest Service, Vernon, B.C.

Trees (ramets) were selected from a list using a computer-generated random sample selection program (Visual Basic) for each seed orchard and identified by their coordinates within the orchard and clone number. In two orchards, Mt. Newton 1 and 2, three cone-bearing branches, visually selected at a distance, were thoroughly searched for

L. occidentalis adults and nymphs during mid-July. For comparison, the entire tree was then surveyed, using ladders or lifts when necessary.

In 1993, trees were selected at random for bagging to exclude *L. occidentalis*, providing an uninfested control. Cones on two branches, each bearing ~ 6 cones, were bagged collectively in late May using either machine sewn light-weight cotton bags, 48 cm long and 26 cm wide or nylon pollination bags, 56 cm long and 27 cm wide. Bags were placed not just over the cones, but a distance up the main cone-bearing branch, enclosing small branches that supported the bag and prevented it from touching the cones. As a result, *L. occidentalis* could not feed through the bags.

A 25-cone sample was collected randomly from selected trees during commercial harvest in 1992 and 1993. Cones were air-dried in paper bags under greenhouse conditions. Seed were extracted by shaking, de-winged by gentle abrasion and winnowing, and then x-rayed (Faxitron Model 804) at 19 kvolts for 1 min. Radiographs were developed in an instant processor (Kodak Industrex Model P-1). Seeds were categorized as either filled, empty, partially-filled or infested by the Douglas-fir seed chalcid, *Megastigmus spermotrophus* Wachtl (for Douglas-fir only).

Insect densities were transformed by $\sqrt{x+0.5}$ to stabilize the variances prior to General Linear Model (GLM) or regression analysis (SAS Institute 1988). In 1992, the number of insects per branch were regressed against the total number of insects per tree to determine the reliability of branch sampling. Analysis of variance was used to evaluate the influence of clone on *L. occidentalis* density. As the number of replicates within clones was extremely variable, the assumptions of the model were violated and F values somewhat suspect. Therefore, I also evaluated the influence of clone on *L. occidentalis* using Morisita's Index of Dispersion (Standardized Morisita's Coefficient), an analysis that is more robust and descriptive, calculated the negative binomial k (Krebs 1989) for both adults and nymphs considered separately, and compared the proportion of trees infested within clones, with mean number of insects per clone using linear regression. Impact was assessed by regressing insect numbers against percentage of partially-filled seed (SAS Institute 1988). Bagged cone samples were analyzed using the paired differences test (Zar 1984). In all cases $\alpha = 0.05$. An estimate of the number of insects per tree required to effect damage was calculated from figures obtained from B.C. Forest Service personnel and a laboratory feeding rate developed by Hanson (1984).

Results

No relationship between numbers of *L. occidentalis* on branches and the entire tree was disclosed when all branch samples were considered (Fig. 1). It was common for three-branch samples to miss the insects present on the tree completely. When three-branch samples with zero or one insect encountered were excluded from the data set, the relationship was highly significant (Fig. 1).

Use of dispersion indices to describe the distribution of *L. occidentalis* within Douglas-fir and lodgepole pine seed orchards disclosed that the populations were consistently clumped (Standardized Morisita's Coefficient > 0.5), reflecting an apparent preference for certain host clones (Figs. 2-4). Moreover, in 18 of 20 instances, there was a significant positive relationship between the mean number of insects per ramet and the proportion of sampled trees within that clone that were infested (Table 1), indicating that if one tree is inhabited by *L. occidentalis*, most of the other trees within that clone will also be inhabited. All k values were < 1 indicating that, for descriptive purposes, the distribution of *L. occidentalis* over the clones can be fit to a negative binomial (Figs. 2-4). Although the Douglas-fir clones bearing *L. occidentalis* were not the same in each year (in large part because many clones do not bear cones every year), seven clones were infested in both

Figure 1: Comparison between numbers of *Leptoglossus occidentalis* found on three sampled branches of 86 coastal Douglas-fir trees sampled in 1992 and numbers found on total tree. Although the relationship was not significant for all branch samples (all triangles, r^2=0.17, *P*<.0001), a significant linear relationship occurred when two or more insects were found on sampled branches (filled triangles, y= .82x+.168, r^2=0.76, *P*<.0001).

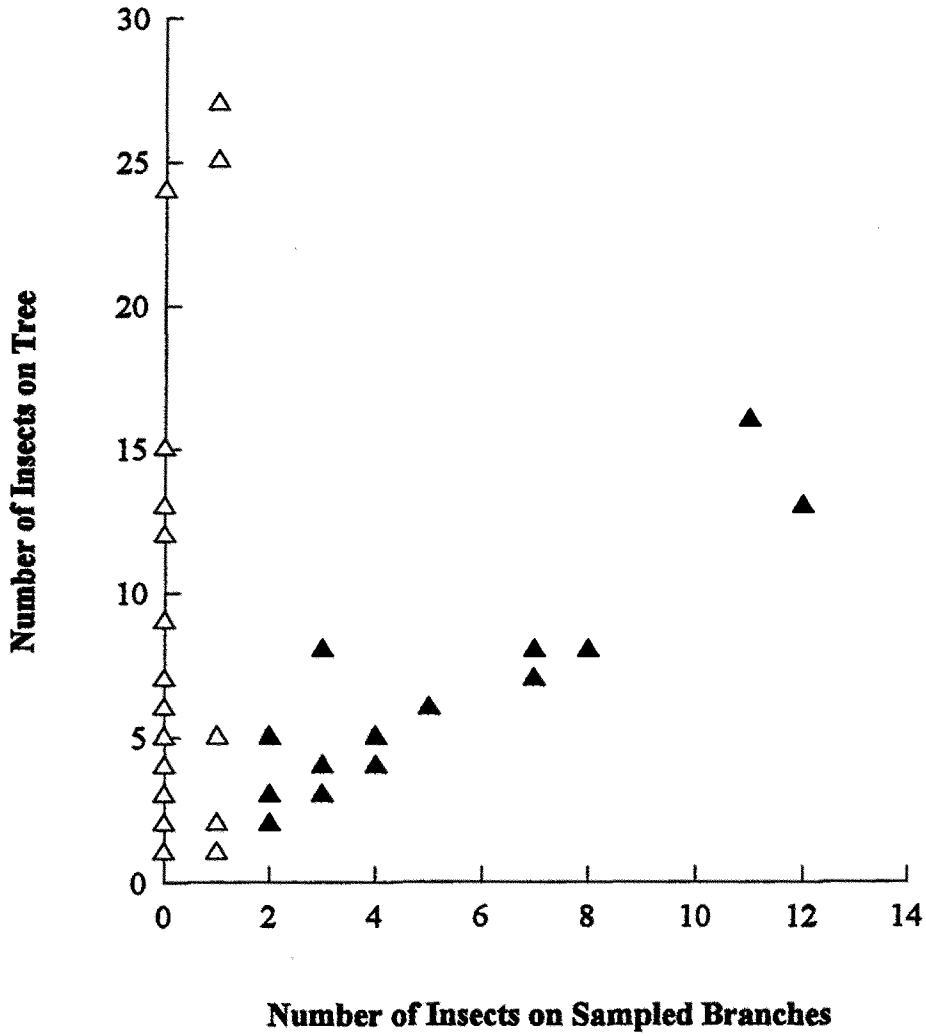

30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
Number of Insects on Tree
Number of Insects on Sampled Branches

Figure 2: Mean numbers of *L. occidentalis* per tree (ramet) for Douglas-fir clones in first generation orchards sampled during 1992-1994. For clarity, many clones harboring no *L. occidentalis* are not shown, including (no. clones in parentheses): Mt. Newton 1- 1992 (36), 1993 (33) and 1994 (30); Nootka - 1992 (25). In each subfigure the value of Standardized Morisita's Coefficient (SMC) are shown as an indication of a clumped distribution (>0.5) of clones harboring *L. occidentalis*. Degrees of freedom for each year are: Mt. Newton 1 (1992) - 64; (1993) - 55; (1994) - 52; Nootka (1992) - 16. Clones represented consecutively in 1992 and 1993 are designated by **. Those represented consecutively in 1992, 1993 and 1994 are designated by ***.

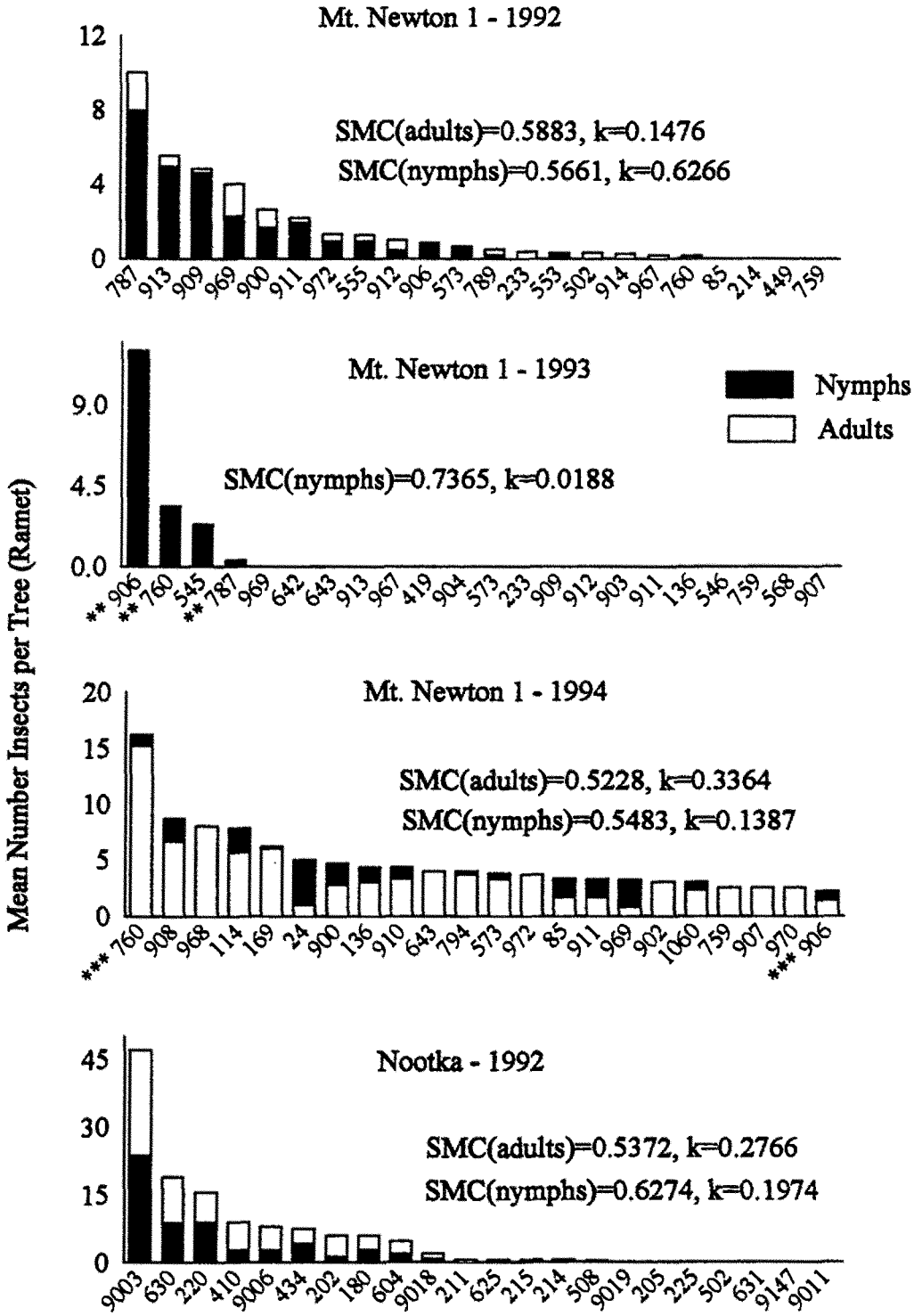

Mt. Newton 1 - 1992
SMC(adults)=0.5883, k=0.1476
SMC(nymphs)=0.5661, k=0.6266
787 913 909 969 900 911 972 555 912 906 573 789 233 553 502 914 967 760 85 214 449 759
Mt. Newton 1 - 1993
Nymphs
Adults
SMC(nymphs)=0.7365, k=0.0188
** 906 ** 760 545 ** 787 969 642 643 913 967 419 904 573 233 909 912 903 911 136 546 759 568 907
Mt. Newton 1 - 1994
SMC(adults)=0.5228, k=0.3364
SMC(nymphs)=0.5483, k=0.1387
*** 760 908 968 114 169 24 900 136 910 643 794 573 972 85 911 969 902 1060 759 907 970 *** 906
Nootka - 1992
SMC(adults)=0.5372, k=0.2766
SMC(nymphs)=0.6274, k=0.1974
9003 630 220 410 9006 434 202 180 604 9018 211 625 215 214 508 9019 205 225 502 631 9147 9011
Mean Number Insects per Tree (Ramet)
Clone

Figure 3: Mean numbers of *L. occidentalis* per tree (ramet) for Douglas-fir clones in a second generation orchard sampled during 1992-1994. For clarity, many clones harboring no *L. occidentalis* are not shown, including (no. clone in parentheses): Mt. Newton 2 - 1992 (70), 1993 (42) and 1994 (25). In each subfigure the value of Standardized Morisita's Coefficient (SMC) are shown as an indication of a clumped distribution (>0.5) of clones harboring *L. occidentalis*. Degrees of freedom for each year are: Mt. Newton 2 (1992) - 74; (1993) - 75; (1994) - 52. Clones represented consecutively in 1992 and 1993 are designated by **. Those represented consecutively in 1992, 1993 and 1994 are designated by ***.

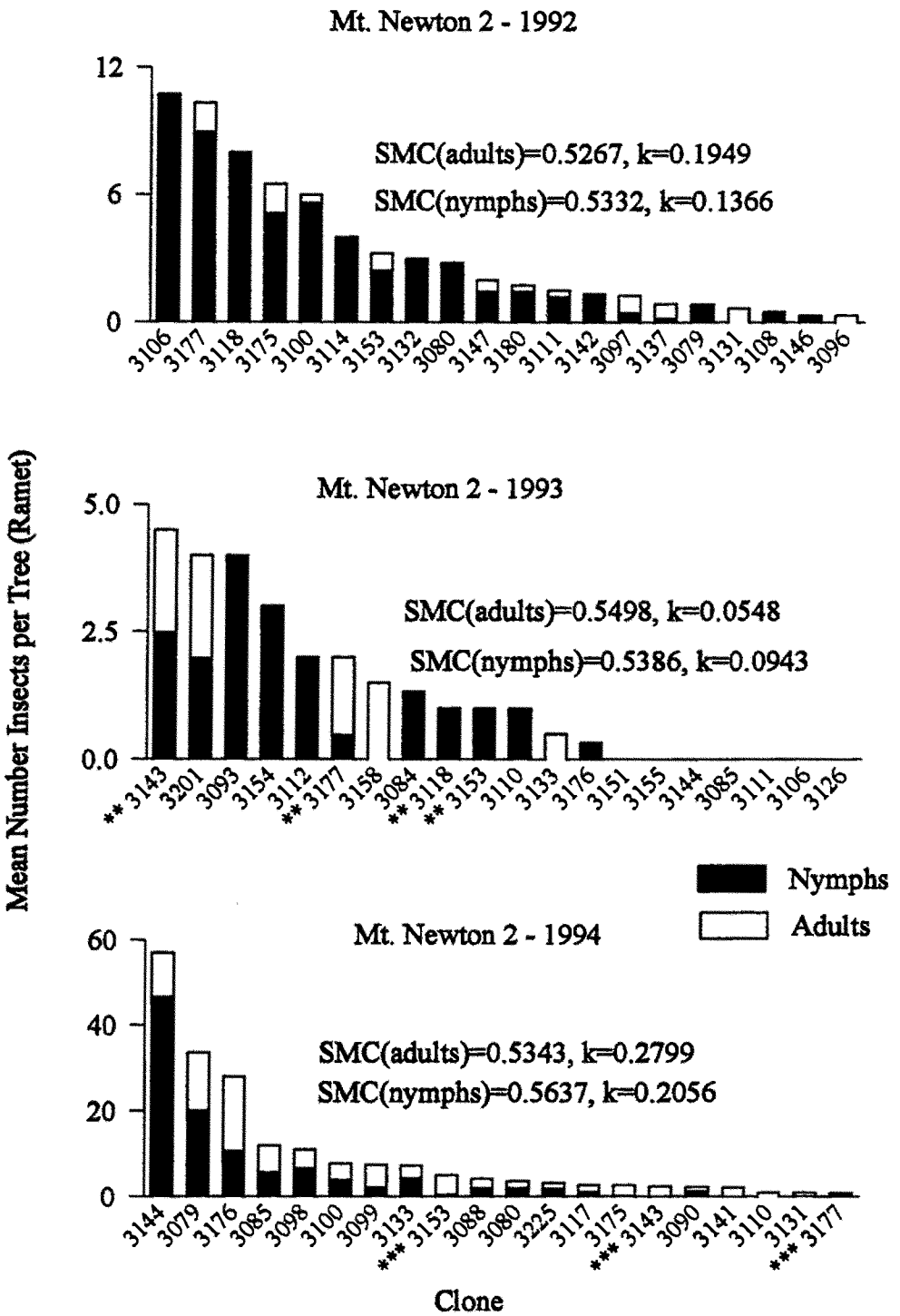

Mt. Newton 2 - 1992
12
6
0
SMC(adults)=0.5267, k=0.1949
SMC(nymphs)=0.5332, k=0.1366
3106 3177 3118 3175 3100 3114 3153 3132 3080 3147 3180 3111 3142 3097 3137 3079 3131 3108 3146 3096
Mean Number Insects per Tree (Ramet)
Mt. Newton 2 - 1993
5.0
2.5
0.0
SMC(adults)=0.5498, k=0.0548
SMC(nymphs)=0.5386, k=0.0943
** 3143 3201 3093 3154 3112 ** 3177 3158 3084 ** 3118 ** 3153 3110 3133 3176 3151 3155 3144 3085 3111 3106 3126
Nymphs
Adults
Mt. Newton 2 - 1994
60
40
20
0
SMC(adults)=0.5343, k=0.2799
SMC(nymphs)=0.5637, k=0.2056
3144 3079 3176 3085 3098 3100 3099 3133 *** 3153 3088 3080 3225 3117 3175 *** 3143 3090 3141 3110 3131 *** 3177
Clone

Figure 4: Mean numbers of *L. occidentalis* per tree (ramet) for lodgepole pine orchards sampled during 1993-1994. For clarity, many clones harboring no *L. occidentalis* are not shown, including (no. clones in parentheses): Kal 1 - 1993 (86) and 1994 (78); Kal 2 - 1993 (38) and 1994 (22). In each subfigure the value of Standardized Morisita's Coefficient (SMC) are shown as an indication of a clumped distribution (>0.5) of clones harboring *L. occidentalis*. Degrees of freedom for each orchard are: Kal 1 (1993) - 41; (1994) - 100; Kal 2 (1993) - 115; (1994) - 101. Clones represented consecutively in 1993 and 1994 are designated by **.

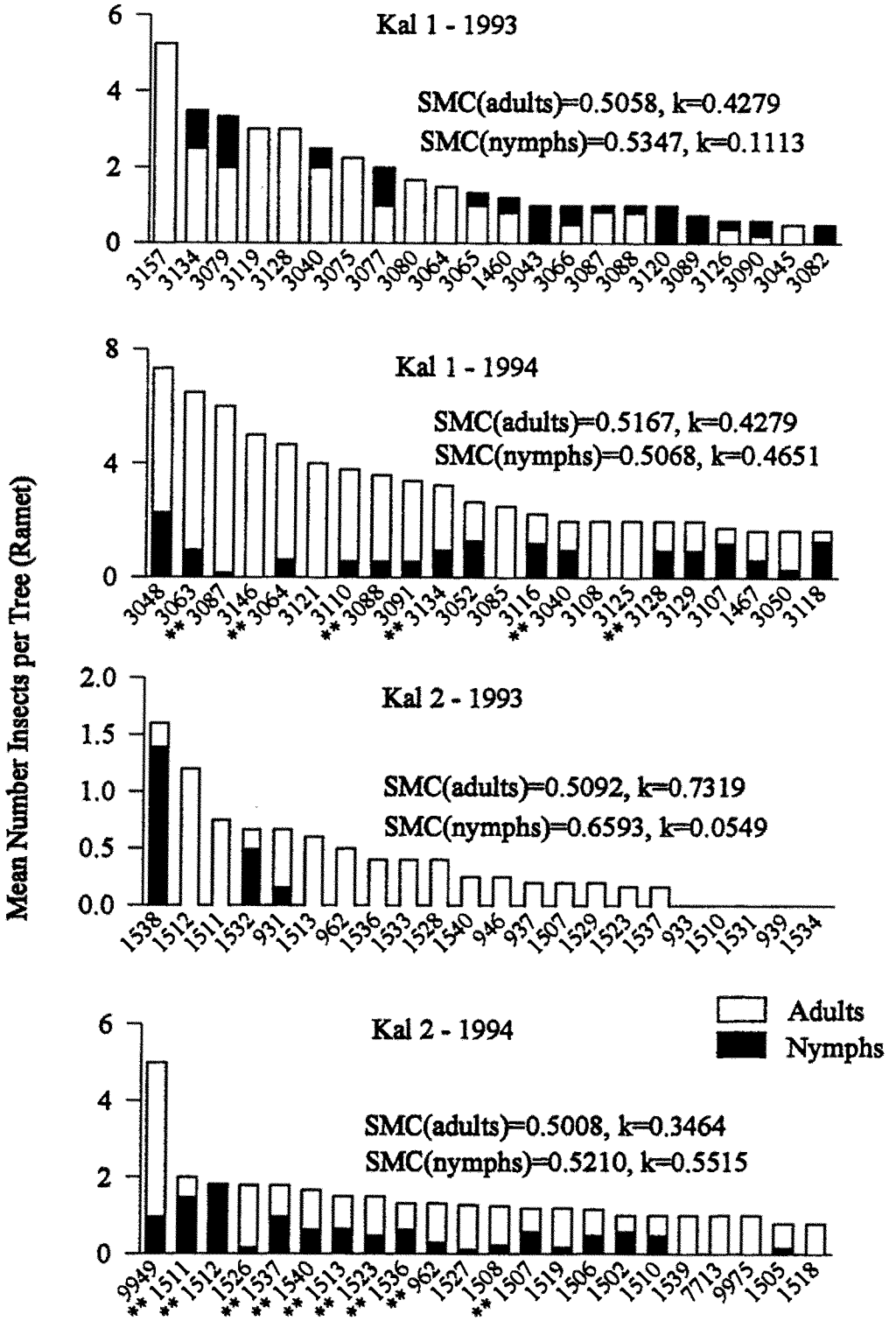

Kal 1 - 1993
SMC(adults)=0.5058, k=0.4279
SMC(nymphs)=0.5347, k=0.1113
3157 3134 3079 3119 3128 3040 3075 3077 3080 3064 3065 1460 3043 3066 3087 3088 3120 3089 3126 3090 3045 3082
Kal 1 - 1994
SMC(adults)=0.5167, k=0.4279
SMC(nymphs)=0.5068, k=0.4651
3048 3063 ** 3087 3146 ** 3064 3121 3110 ** 3088 3091 ** 3134 3052 3085 3116 ** 3040 3108 3125 ** 3128 3129 3107 1467 3050 3118
Kal 2 - 1993
SMC(adults)=0.5092, k=0.7319
SMC(nymphs)=0.6593, k=0.0549
1538 1512 1511 1532 931 1513 962 1536 1533 1528 1540 946 937 1507 1529 1523 1537 933 1510 1531 939 1534
Kal 2 - 1994
SMC(adults)=0.5008, k=0.3464
SMC(nymphs)=0.5210, k=0.5515
9949 ** 1511 ** 1512 1526 ** 1537 ** 1540 ** 1513 ** 1523 ** 1536 ** 962 1527 1508 ** 1507 1519 1506 1502 1510 1539 7713 9975 1505 1518
Adults
Nymphs
Mean Number Insects per Tree (Ramet)
Clone

Table 1: Regression equations and r^2 values describing the relationship between mean numbers of *L. occidentalis* per tree (ramet) and corresponding proportion of trees infested. Clones represented by only one tree were eliminated from the analysis. Mt. Newton 1 - 1993 is not included as only four trees harbored insects that year.

Orchard	Year	Life stage	Regression equation	r^2	*P*
Mt. Newton 1	1992	nymphs	y =.10x+.149	0.82	< 0.0001
		adults	y =.41x+.119	0.77	< 0.0001
	1994	nymphs	y =.81x+.790	0.56	< 0.0001
		adults	y =1.2x+.980	0.35	< 0.0001
Mt. Newton 2	1992	nymphs	y =.07x+.267	0.80	< 0.0001
		adults	y =.57x+.041	0.77	< 0.0001
	1993	nymphs	y =.08x+.387	0.08	< 0.3137
		adults	y =.36x+.234	0.73	< 0.0001
	1994	nymphs	y =.57x+1.09	0.11	< 0.0572
		adults	y =.79x+1.04	0.21	< 0.0069
Nootka	1992	nymphs	y =.03x+.344	0.54	< 0.0001
		adults	y =.04x+.293	0.69	< 0.0001
Kal 1	1993	nymphs	y =.01x+.208	0.33	< 0.0001
		adults	y =.01x+.234	0.70	< 0.0001
	1994	nymphs	y =.76x+.735	0.63	< 0.0001
		adults	y =.73x+.751	0.66	< 0.0001
Kal 2	1993	nymphs	y =.29x+.344	0.91	< 0.0001
		adults	y =.35x+.124	0.52	< 0.0012
	1994	nymphs	y =.89x+.695	0.86	< 0.0001
		adults	y =.68x+.728	0.84	< 0.0001

1992 and 1993 and four clones were infested in all three years (Figs. 2-3). For lodgepole pine, 20 clones were infested in both 1993 and 1994 (Figs. 4).

There was no relationship between seed bug numbers and percentage of partially-filled seed (Fig. 5) indicating that *L. occidentalis* probably caused only a small fraction of this damage and suggesting that abiotic factors (Owens *et al.* 1990) or feeding by another insect may account for some of this type of damage.

In 1993 there was no difference between bagged and non-bagged cones in their content of partially-filled seed [Mt. Newton 1, mean = 37.61% (bagged) and 24.16% (non-bagged), n=36, P=0.37; Mt. Newton 2, mean = 19.93% (bagged) and 18.44% (non-bagged), n=27, P=0.34; Kal 2, mean = 12.1% (bagged) and 13% (non-bagged), n=24, P=0.22].

Laboratory feeding studies have shown that *L. occidentalis* of most life stages will on average feed on one seed per day (Hanson 1984). This feeding rate was used to predict the numbers of seed bugs required for a 120-day feeding season to cause 5, 10, 15 or 20% damage (Table 2). On the basis of these conservative figures, *L. occidentalis* populations in coastal Douglas-fir seed orchards in

Figure 5: Relationship between percentage partially-filled seed and numbers of *Leptoglossus occidentalis* on a tree. Data obtained from four orchards over a two year sampling period.

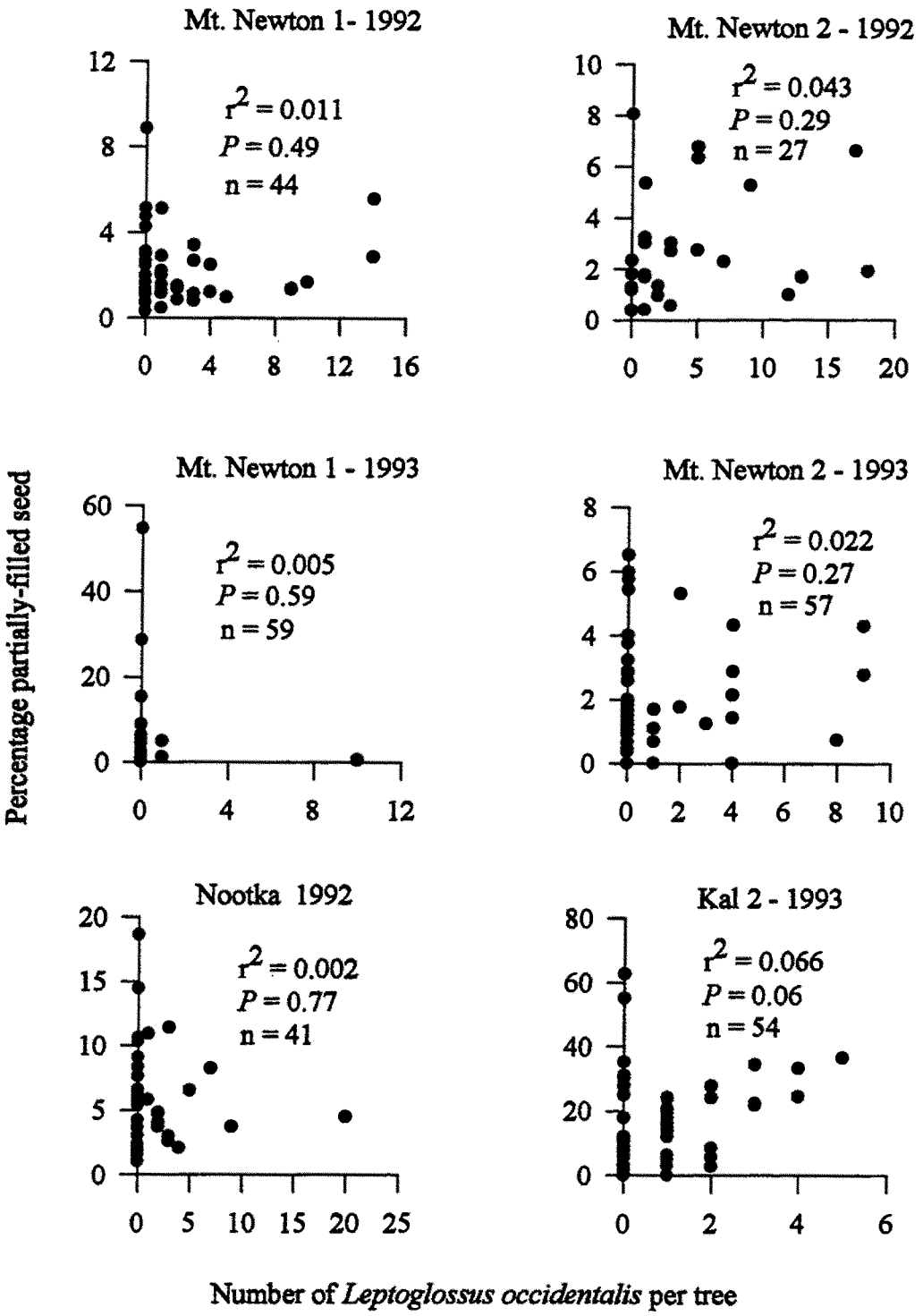

Mt. Newton 1- 1992
$r^2 = 0.011$
$P = 0.49$
n = 44
Mt. Newton 2 - 1992
$r^2 = 0.043$
$P = 0.29$
n = 27
Mt. Newton 1 - 1993
$r^2 = 0.005$
$P = 0.59$
n = 59
Mt. Newton 2 - 1993
$r^2 = 0.022$
$P = 0.27$
n = 57
Nootka 1992
$r^2 = 0.002$
$P = 0.77$
n = 41
Kal 2 - 1993
$r^2 = 0.066$
$P = 0.06$
n = 54
Percentage partially-filled seed
Number of *Leptoglossus occidentalis* per tree

Table 2: Calculated numbers of *L. occidentalis* required to cause 5-20% damage to Douglas-fir and lodgepole pine seed based on Hanson's (1984) feeding rate of one seed per bug per day, and extended over an estimated 120 days of favorable weather for feeding from 1 May to 31 August in British Columbia. Estimates of cones per tree and filled seed per cone based on data supplied by D. Reid and B. Barber, B.C. Forest Service, Saanichton, and Victoria, respectively, who obtained records from the Tree Seed Centre, B.C. Forest Service, Surrey, B.C.

Species	Source of seed	Cones per tree	Filled seed per cone	Filled seed produced	Percent damage	Seed damaged	Insects required per tree
Douglas-fir	Seed orchard	3,000	40	120,000	5	6,000	50
					10	12,000	100
					15	18,000	150
					20	24,000	200
	Wild stand	2,800	14	39,200	5	1,960	17
					10	3,920	33
					15	5,880	49
					20	7,840	66
Lodgepole pine	Seed orchard	2,000	15	30,000	5	1,500	13
					10	3,000	25
					15	4,500	38
					20	6,000	50

Table 2 (Continued)

Species	Source of seed	Cones per tree	Filled seed per cone	Filled seed produced	Percent damage	Seed damaged	Insects required per tree
Lodgepole pine	Wild stand	8,300	20	166,600	5	8,300	70
					10	16,600	139
					15	24,900	208
					20	33,200	277

B.C. (Figs. 2 and 3) are not high enough to be of concern to the seed orchard manager.

Discussion

My results indicate that sampling for *L. occidentalis* requires whole tree searches. Because *L. occidentalis* lays 2-20 eggs per clutch and the nymphs aggregate to feed on cones in close proximity to the empty chorions (Koerber 1963; J.C. Nord, pers. comm.), the insects tend to be clumped within a tree. As a result of this patchiness, selecting a limited number of branches for sampling of *L. occidentalis* will probably not be effective at low population levels. The data in Fig. 1 and reported patchiness caused by aggregated eggs and feeding nymphs suggest that a sequential sampling scheme could be developed for *L. occidentalis*. A sequential sampling scheme would be effective in this case as low numbers of insects would indicate that further sampling is required. High insect numbers would probably indicate that the population had been found and thus sampling could cease.

Leptoglossus occidentalis was found to be clumped within a seed orchard, corresponding to location of preferred clones. In every year surveyed, some clones of both Douglas-fir and lodgepole pine had a high mean number of *L. occidentalis* per tree (ramet) while many had few or

none (Figs. 2-4, Table 1). The demonstrated clonal preference by *L. occidentalis* is similar to observations suggesting a similar clonal preference expressed by *L. corculus* in southern Georgia pine orchards (J.C. Nord, pers. comm.). Preference of clones for oviposition has also been shown for the Douglas-fir seed chalcid, *Megastigmus spermotrophus* Watchl., possibly on the basis of cone length, a highly heritable trait (Schowalter and Haverty 1989; Rappaport and Roques 1991). Many other species also demonstrate a clonal preference. Schowalter and Haverty (1989) found the Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae), to exhibit a clonal preference in western Oregon. It was further demonstrated that resistance by Douglas-firs was a dominant, heritable trait. Askew *et al.* (1985) found clonal variation in susceptibility to four species of coneworm, *Dioryctria* spp. (Lepidoptera: Pyralidae) in loblolly pine, *Pinus taeda* L., in the southeast U.S.A. Rappaport and Wood (1994) found specific host genotypes to have higher attack rates by the Douglas-fir twig beetle, *Pityophthorus orarious* Bright (Coleoptera: Scolytidae), in northern California. Preference for specific provenances and cultivars has also been demonstrated. Schowalter and Stein (1987) found *Lygus hesperus* Knight (Heteroptera: Miridae) impact to be related to seedling provenance in Douglas-fir nurseries. Provenance effects were also found by Mendel (1984) for *Matsucoccus josephi* (Homoptera: Margarodidae). In agriculture, squash bugs, *Anasa trista* (DeGeer) (Heteroptera: Coreidae),

were found to prefer certain cultivars of squash and cucurbits (Bonjour *et al.* 1990).

As documented for aphids, and other homoptera, plant genotype can play a large role in determining the numbers, quality, morph type and sex of the offspring produced (Eastop 1972 , Moran 1981, Service 1984, Weis and Campbell 1992). I hypothesize that the gradient of host preference expressed by *L. occidentalis* (Figs. 2-4) may reflect a preferential deposition of eggs on those genotypes which offer the best chance for survival and success of the next generation of nymphs. The ball gallmaker, *Eurosta solidaginis* (Diptera: Tephritidae) (Anderson *et al.* 1989), a butterfly, *Euphydryas editha* (Lepidoptera: Nymphalidae) (Ng 1988) and a bud cone moth, *Zeiraphera canadensis* Mutuura & Freeman (Lepidoptera: Tortricidae) (Quiring and Butterworth 1994) are also documented as preferring some plant genotypes for oviposition over others.

Laboratory feeding studies show that *L. occidentalis* will cause seed to appear partially-filled, slightly shriveled or almost completely empty with a small amount of debris left in the seed (Hanson 1984). Seed appearing as any of these in x-ray analyses was considered 'partially-filled' and caused by *L. occidentalis*. Even by this liberal criterion, damage by *L. occidentalis* was <5% in

Douglas-fir in 1992 and 1993 and ~ 14% in lodgepole pine in 1993.

While bagging was only done in 1993, percentages of partially-filled seed were as high in 1993 as in 1992 when *L. occidentalis* populations were considerably higher within the seed orchards. These results support the hypothesis that populations of *L. occidentalis* in 1992 and 1993 were not high enough to cause significant losses of seed in both Douglas-fir and lodgepole pine. Populations of *L. occidentalis* were slightly higher in 1994; however no impact studies were conducted during this year.

For lodgepole pine at Kalamalka, where summer temperatures are much warmer than in coastal orchards where Douglas-fir seed is produced, numbers of *L. occidentalis* (Fig. 4) were slightly lower than numbers required to cause 5% losses (Table 2). The higher loss and greater numbers of insects present in lodgepole pine than Douglas-fir orchards suggests that *L. occidentalis* could be a greater problem of lodgepole pine than Douglas-fir, but in neither case would control measures be warranted. At more southerly latitudes than in B.C. *L. occidentalis* can occur in very high numbers and has been documented to cause significant losses on Douglas-fir (Schowalter

et al. 1985, Schowalter and Sexton 1990, Schowalter 1994, 1996) and on other species (Koerber 1963, Krugman and Koerber 1969, Shea *et al.* 1986, Pasek and Dix 1988, Connelly and Schowalter 1991, Schowalter 1994, 1996). While in Canada, no insecticidal controls are necessary, *L. corculus* in the southeastern U.S.A., requires frequent insecticidal control (DeBarr *et al.* 1982). Therefore, I hypothesize that in years with favorable weather, or in the event of even slight global warming, *L. occidentalis* may achieve the population numbers in B.C. required to cause significant damage to seed orchard seed.

Chapter 2

Physical Characteristics as a Potential Mechanism of Host Selection

Introduction

Physical attributes are used as indicators of host quality by many forest insects such as bark beetles (Shepherd 1965, Jenkins 1983, Raffa and Berryman 1983, Rappaport and Wood 1994, Turgeon *et al.* 1994), sawflies (Craig *et al.* 1990), seed chalcids (Roques 1987, Rappaport and Roques 1991, Rappaport *et al.* 1993) and lepidoptera (Kinghorn 1954). Visual cues such as tree silhouette, size and shape, cone size, shape, and color and contrast between foliage and cone color all assist cone feeding insects in recognizing and selecting suitable hosts (Turgeon *et al.* 1994). Selection of suitable hosts has been postulated as being a linear relationship whereby insect abundance increases with plant vigor (*Plant Vigor Hypothesis*, Price 1991) or with an increase in plant stress (*Plant Stress Hypothesis*, White 1969). In both cases, the physiological state and morphological form of the tree is responsible for a particular host being considered attractive by a pest species.

It is unknown if *L. occidentalis* selects hosts based upon tree characteristics. Ovipositing by female *L. occidentalis* on trees with adequate food supplies, e.g. cones, would be adaptive to ensure survival of the offspring. As *L. occidentalis* is a strong flier, I hypothesize that it would be fairly easy for females to survey all or a large part of an orchard and each year to select those trees bearing high numbers of cones. As tall trees tend to be healthy and also to produce cones, selecting trees based on their silhouette may also aid in the selection of adequate food reserves. All trees within a clone develop their ovulate cones at the same time. The onset of this development can differ between clones by up to three weeks. As *L. occidentalis* feeds on the developing ovulate cones, timing oviposition to coincide with the development of their food resource may be important. I speculate that *L. occidentalis* may favor those clones developing late in the season to avoid sudden changes in spring temperatures which would be detrimental to the survival of nymphs. And finally, preference for certain trees may be due to their visual reflectance. Some trees may reflect more light and thus be more apparent than others to host seeking *L. occidentalis*. I hypothesize that all these factors: cone crop, tree height, conelet development and visual reflectance may be cues used to facilitate host selection by *L. occidentalis* in seed orchards.

Trees were selected from a list using a computer-generated random sample selection program (Visual Basic) for each seed orchard surveyed in 1992 (Chapter 1) and identified by their coordinates within the orchard and clone number. Tree height was either estimated to the nearest half metre or obtained from the orchard manager. Cone density was either ranked from 0-5 (0=no cones, 5=very heavy cone crop) or actual number of cones per tree obtained from the orchard manager. The ranked categories take into consideration both tree height and number of cones present. A rating of 5 would mean that the tree has >80% of its branches bearing cones while a tree rated 1 would have <10% of its branches bearing cones.

In 1993 and 1994, cone bearing trees were stratified by clone, based on results obtained during 1992, and a computer-generated random sample of approximately half of these trees was selected for total-tree survey of *L. occidentalis* adults and nymphs (Krebs 1989). In 1995, all trees within orchards Mt. Newton 1 and 2 and Kal 1 and 2 were surveyed. Trees bearing no cones were excluded from the sampling pool based on observations in 1992 that such trees do not harbor *L. occidentalis*. Due to poor weather that delayed both crop and insect population development, surveying in 1993 and 1994 was not conducted

until mid-August, which coincided with commercial harvest. Surveying in 1995 occurred during July.

Timing of conelet development is categorized as being early, late or mid-way during the developmental season. Data for each clone were obtained from the orchard manager. Conelet development is genetically determined; thus clones which 'flower' early in one year will always flower early.

To examine the effect of cone density, insect numbers were ranked and then correlated with cone density using a one-tailed Spearman Rank Correlation Analysis in SAS (Zar 1984, SAS Institute 1988). Number of cones per tree, obtained from the orchard manager, were regressed against insect numbers for data collected at Kalamalka in 1995. In all cases, only weakly significant relationships were disclosed. To examine the effect of tree height, insect numbers, transformed by $\sqrt{(x+0.5)}$ to stabilize the variances prior to regression analysis (Zar 1984), from Mt. Newton and Kalamalka were regressed against tree height. Graphical examination of data for both cone density and tree height indicated a possible quadratic relationship; however, *post hoc* regressions were significant but not predictive. It was then hypothesized that the distribution of *L. occidentalis* across categories of cone density or tree height may be a result of the number of trees within the categories. To

compare the frequency of trees with insects with the frequency of trees within the same cone category or tree height, a goodness of fit test was performed. For orchards where cone density was ranked into categories, total numbers of trees per category and total numbers of trees harboring adults or nymphs per category were tabulated. Proportions for each category were calculated and compared with expected proportions using chi-square analysis (Zar 1984). In orchards where tree height or actual cone numbers were available, categories were created by dividing the range of measurements into 9-11 categories. In all cases $\alpha = 0.05$.

To evaluate whether *L. occidentalis* prefers trees which flower late in the season, a chi-square test was performed. Frequency of trees harboring adults or nymphs within each flowering category were compared to expected values at $\alpha = 0.05$.

In May of 1996, conelet and foliage samples were obtained from preferred clones 3143, 3153, and 3177, and non-preferred clones 3188, 3105 and 3081 located at Mt. Newton Seed Orchards. A Kary 17 reflectance spectrophotometer (Lapis and Borden 1995) was used to determine whether the amount of light reflected by these plant parts differed between clone. Three to five trees per clone were sampled and analyzed over the entire range of visible light, 350 - 700 nm. Mean reflectance curves were generated for each clone and analyzed using

General Linear Model Analysis of Variance and Scheffe's Mean Separation Test (Zar 1984) for each point of data collected.

Results

A significant ($P<0.05$) but weak correlation between numbers of *L. occidentalis* per ramet and cone density was obtained for only three of the seven orchards surveyed in 1992 - 1994 [Mt. Newton 1 (1992) n=31, r = -0.632; Mt. Newton 2 (1992) n=55, r = 0.347; and Mt. Newton 2 (1993) n= 119, r=0.271). All remaining orchards were not significant ($P>0.05$) [Mt. Newton 1 (1994) n=17, r=0.444; Mt. Newton 2 (1994) n=19, r=0.029; Kal 1 (1993) n = 30, r=0.298; Kal 2 (1993) n=17, r=0.159]. Mt. Newton 1 (1993) was not analyzed because there were only 4 trees harboring insects. No *L. occidentalis* were found on trees bearing no cones and trees with high cone densities did not necessarily harbor greater numbers of insects than those with few cones. In both Kal 1 and Kal 2 in 1995, the relationship between numbers of *L. occidentalis* and numbers of cones per tree was significant (P ranged from 0.0001 to 0.0003); however, the predictability of this relationship was very low (r^2 values ranged from 0.009-0.025).

Post hoc regression analyses to evaluate a potential quadratic relationship between numbers of *L. occidentalis* and numbers of cones per tree or tree height were highly significant (all *P* values < 0.0001) but had little predictive power (r^2 values ranged from 0.016-0.026). The significant *P* values are possibly due to the large data sets used, n=1323 and 1275, which provide an accurate estimate of a linear model but one which is not precise (low r^2 values).

Chi-square analysis disclosed significant relationships between numbers of *L. occidentalis* and numbers of cones per tree in seven of 10 instances for nymphs and in five of 10 instances for adults (Figs. 6, 7, results from Mt. Newton 2 - 1994 and 1995 not significant and not shown). In all cases, the trees chosen by *L. occidentalis* for feeding (adults) or oviposition (nymphs) tend to be those with moderate cone densities.

There was no linear relationship between numbers of *L. occidentalis* and tree height for Mt. Newton or Kalamalka (*P* ranged from 0.09 to 0.86). Similar to cone density at Kalamalka (Fig. 7), the relationship appeared to be quadratic wherein the majority of *L. occidentalis* could be found on trees of medium height (Fig. 8, 9). *Post hoc* regressions were either significant (*P* ranged from 0.0001 - 0.03) with low r^2 values (ranging from 0.02-0.09) or insignificant (*P* ranged from

Figure 6: Relationship between the proportion of Douglas-fir trees in four cone density categories harboring *L. occidentalis* and proportion of trees within each category at Mt. Newton 1 and 2. Significant chi-square values indicate that distribution of adults and nymphs across cone density categories is independent of the number of trees within each category.

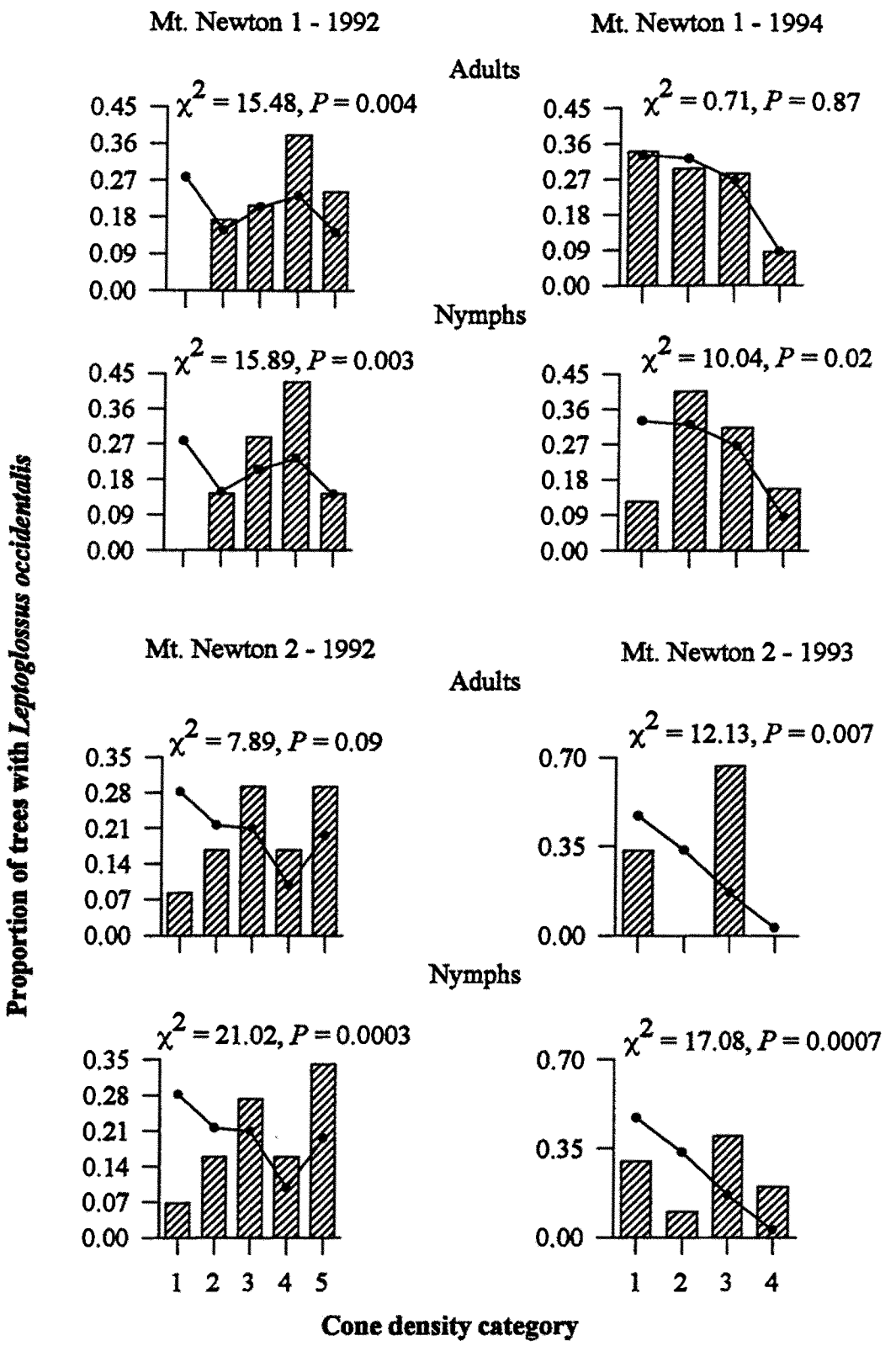

Mt. Newton 1 - 1992
Mt. Newton 1 - 1994
Adults
$\chi^2 = 15.48, P = 0.004$
$\chi^2 = 0.71, P = 0.87$
Nymphs
$\chi^2 = 15.89, P = 0.003$
$\chi^2 = 10.04, P = 0.02$
Mt. Newton 2 - 1992
Mt. Newton 2 - 1993
Adults
$\chi^2 = 7.89, P = 0.09$
$\chi^2 = 12.13, P = 0.007$
Nymphs
$\chi^2 = 21.02, P = 0.0003$
$\chi^2 = 17.08, P = 0.0007$
Proportion of trees with *Leptoglossus occidentalis*
Cone density category
Distribution of trees within each cone density category

Figure 7: Relationship between the proportion of lodgepole pine trees in a given cone density category harboring *L. occidentalis* and the proportion of trees within each category at Kalamalka 1 and 2. Significant chi-square values indicate that distribution of adults and nymphs across cone density categories is independent of the number of trees within each category.

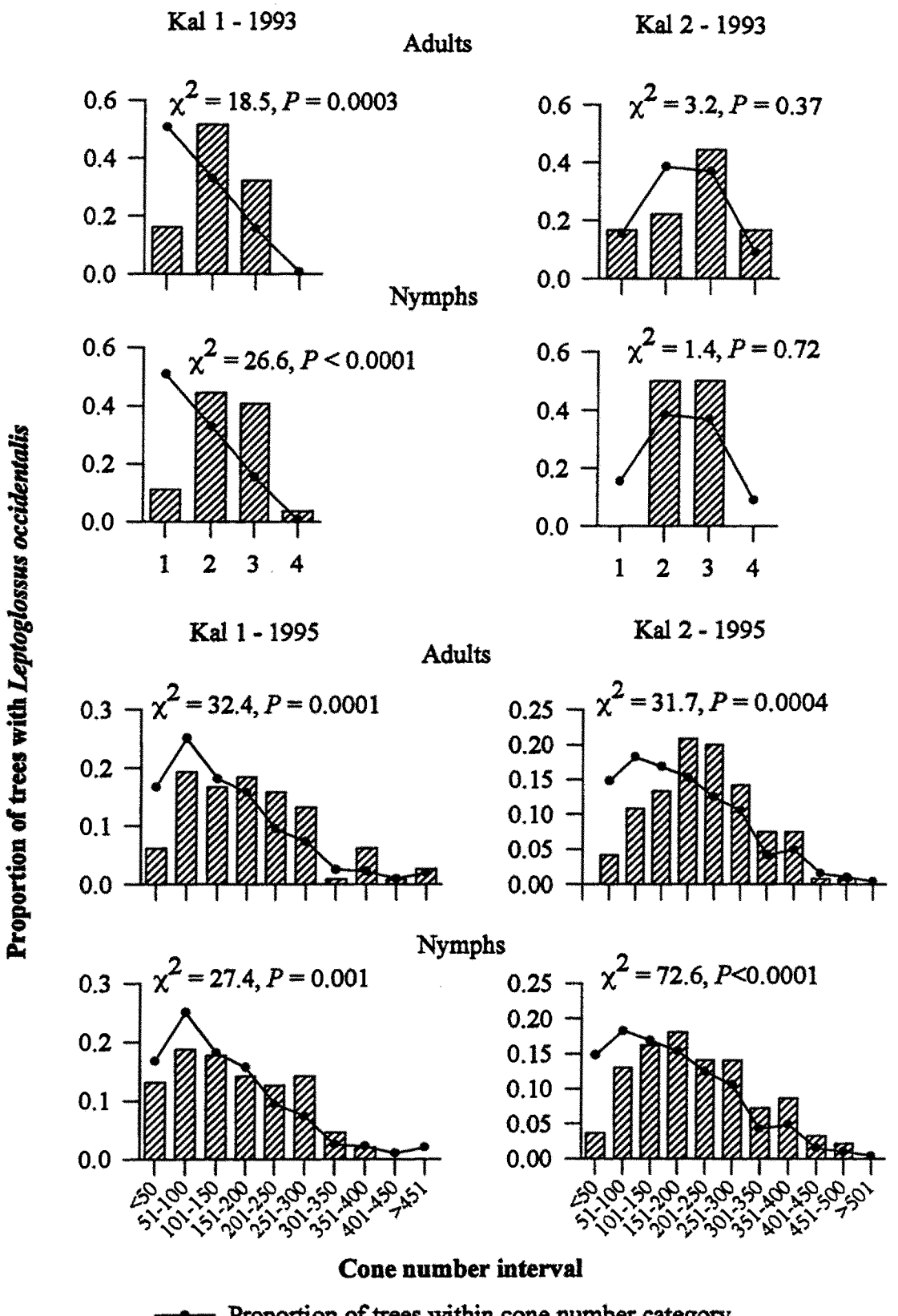

Kal 1 - 1993
Adults
$\chi^2 = 18.5, P = 0.0003$
Kal 2 - 1993
$\chi^2 = 3.2, P = 0.37$
Nymphs
$\chi^2 = 26.6, P < 0.0001$
$\chi^2 = 1.4, P = 0.72$
Kal 1 - 1995
Adults
$\chi^2 = 32.4, P = 0.0001$
Kal 2 - 1995
$\chi^2 = 31.7, P = 0.0004$
Nymphs
$\chi^2 = 27.4, P = 0.001$
$\chi^2 = 72.6, P < 0.0001$
Proportion of trees with *Leptoglossus occidentalis*
Cone number interval
Proportion of trees within cone number category

Figure 8: Relationship in 1992 and 1993 between the proportion of Douglas-firs (Mt. Newton 1 and 2) and lodgepole pines (Kal 1 and 2) in a given height category harboring *L. occidentalis* and the proportion of trees within each tree height category. Significant chi-square values indicate that the deviations of adults and nymphs is independent of the number of trees with each category.

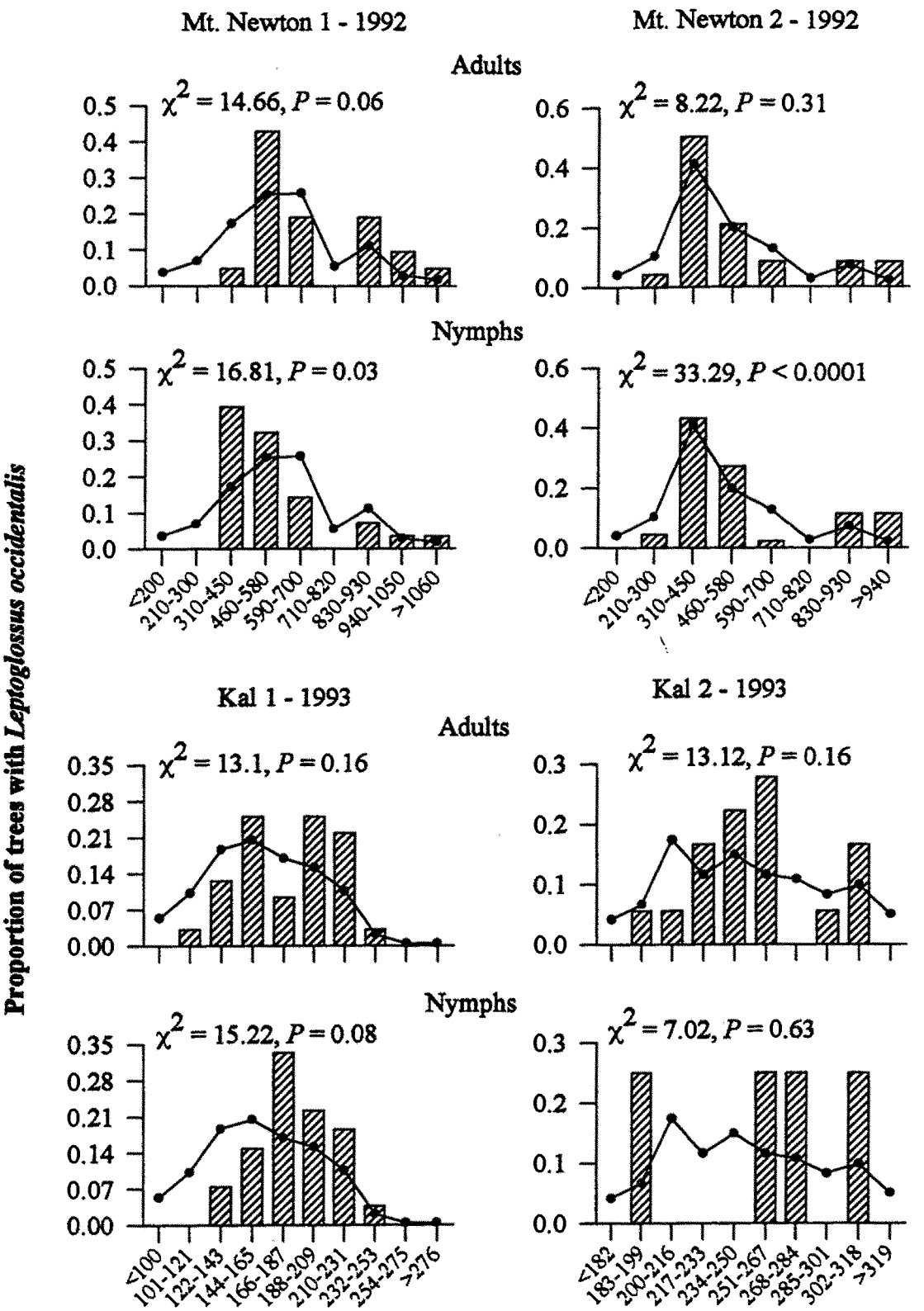
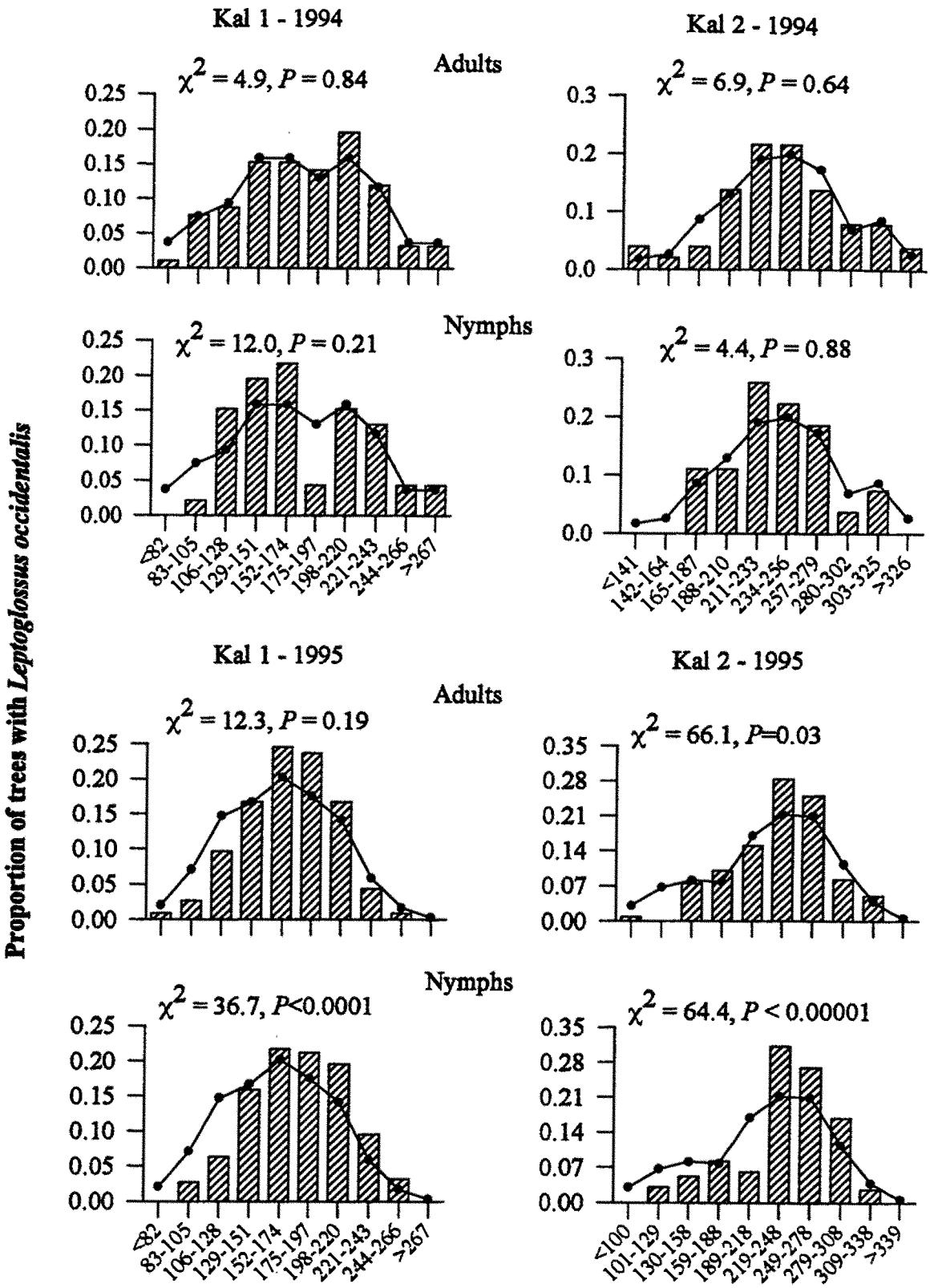

Mt. Newton 1 - 1992
Mt. Newton 2 - 1992
Adults
$\chi^2 = 14.66, P = 0.06$
$\chi^2 = 8.22, P = 0.31$
Nymphs
$\chi^2 = 16.81, P = 0.03$
$\chi^2 = 33.29, P < 0.0001$
<200
210-300
310-450
460-580
590-700
710-820
830-930
940-1050
>1060
>940
Kal 1 - 1993
Kal 2 - 1993
Adults
$\chi^2 = 13.1, P = 0.16$
$\chi^2 = 13.12, P = 0.16$
Nymphs
$\chi^2 = 15.22, P = 0.08$
$\chi^2 = 7.02, P = 0.63$
<100
101-121
122-143
144-165
166-187
188-209
210-231
232-253
254-275
>276
<182
183-199
200-216
217-233
234-250
251-267
268-284
285-301
302-318
>319
Proportion of trees with *Leptoglossus occidentalis*
Tree height interval / cm
Proportion of trees within tree height category

Figure 9: Relationship in 1994 and 1995 between the proportion of lodgepole pine trees in a given height category harboring *L. occidentalis* and the proportion of trees within each tree height category. Significant chi-square values indicate that the deviations of adults and nymphs is independent of the number of trees with each category.


Kal 1 - 1994
Kal 2 - 1994
Adults
$\chi^2 = 4.9, P = 0.84$
$\chi^2 = 6.9, P = 0.64$
Nymphs
$\chi^2 = 12.0, P = 0.21$
$\chi^2 = 4.4, P = 0.88$
Kal 1 - 1995
Kal 2 - 1995
Adults
$\chi^2 = 12.3, P = 0.19$
$\chi^2 = 66.1, P = 0.03$
Nymphs
$\chi^2 = 36.7, P < 0.0001$
$\chi^2 = 64.4, P < 0.00001$
Proportion of trees with *Leptoglossus occidentalis*
Tree height interval / cm
Proportion of trees within tree height category

0.29 - 0.79) with low r^2 values (ranging from 0.007 - 0.021). In all cases, the precision of the model was low. Chi-square analysis disclosed significant deviations between the distributions of *L. occidentalis* and tree height categories in four of eight instances for nymphs, but only one of eight instances for adults (Figs. 8, 9).

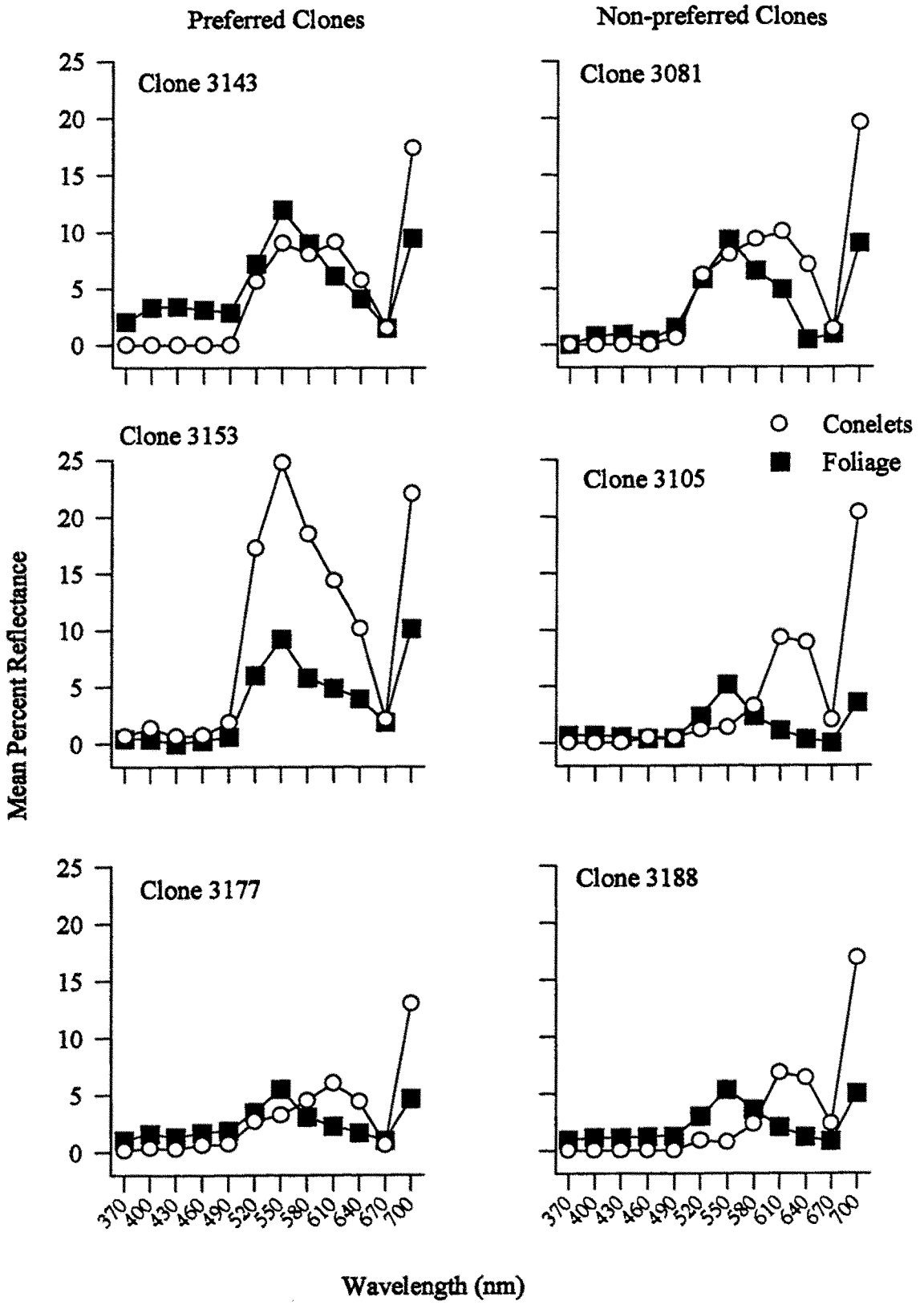
No relationships between early, mid or late flowering time and incidence of *L. occidentalis* for either Mt. Newton or Kalamalka could be determined (χ^2 values ranged from 0.09-5.50, $\chi^2_{crit\ 0.05,2} = 5.99$). Equal proportions of trees were infested with *L. occidentalis* over all flowering times.

In all but one instance, conelets reflected more light than foliage (Fig. 10). Conelets also tended to reflect over a greater range of wavelengths than did foliage (520-670 nm and 550-640 nm, for conelets and foliage, respectively). However, there were no distinct differences in reflectance between preferred and non-preferred clones.

Discussion

My results do not support either hypothesis put forth by Price (1991) or White (1969) where a linear relationship is predicted between insect abundance and host quality. Neither eggs, nymphs nor adults

Figure 10: Mean light reflectance curves for conelets and foliage from preferred and non-preferred clones located at Mt. Newton Seed Orchard. In all cases, the mean reflectance per clone was used to generate the curves.


Preferred Clones
Non-preferred Clones
Clone 3143
Clone 3081
Clone 3153
Clone 3105
Clone 3177
Clone 3188
Conelets
Foliage
Mean Percent Reflectance
Wavelength (nm)
0
5
10
15
20
25
370
400
430
460
490
520
550
580
610
640
670
700

were ever found on trees without cones, suggesting that presence of potential nymphal food is an important factor in host selection by *L. occidentalis*. However, there was no strong linear relationship between insect numbers and size of cone crop (Figs. 6, 7). For cone-bearing trees, the relationship between numbers of insects and numbers of cones was not linear, as expected, but appeared to be quadratic in nature, wherein adults apparently selected trees with moderate cone crops for feeding and oviposition. In only three orchard years (Mt. Newton 2 - 1994 and 1995, Kal 2 - 1993), of the 10 orchard-years analyzed, were there no significant deviations of numbers of both adults and nymphs from the distribution of the trees across cone category. In two instances, Mt. Newton 1 - 1994 and Mt. Newton 2 - 1992, there were significant chi-square for nymphs but not adults. In these cases, inclusion of males (males and females were not distinguished during the surveys) may have obscured the relationship.

It may be of adaptive advantage for *L. occidentalis* to select clones which have consistent annual cone crops, rather than periodic 'bumper' crops. If variation in cone crop size is great, insect damage can be expected to be sporadic (Mattson 1971). *Leptoglossus occidentalis* apparently selects its preferred trees during early spring when the conelets are being pollinated (Owens 1974), prior to conelet abortion

(pers. obs.). I hypothesize that trees bearing high numbers of conelets may experience a higher percentage of abortion than trees bearing few conelets, thus reducing the actual cone density to levels lower than those on trees that have consistently moderate crops. Griffith (1968) found a consistent negative relationship between flower production and cone production among Douglas-fir trees grown at the University of British Columbia Malcolm Knapp Research Forest, Maple Ridge, B.C., and suggested that abortion may be highest on trees bearing the most conelets. Byram *et al.* (1986) found cone production to be under moderate genetic control, but that clones changed rank from year to year with respect to overall production within an orchard. Thus, selection between cone-bearing clones may be based on cues that signal high host suitability, such as moderate cone density, with the result that *L. occidentalis* may select some clones in successive years but may shift the order of preference, as well as add and delete clones, from year to year (Figs. 2-4).

Trees that produce large cone crops tend to be the most stressed (Wheeler and Keeley 1987). An increase in Douglas-fir seed and cone production can reduce overall growth (Eis *et al.* 1965, El-Kassaby and Barclay 1992), a result of a trade-off in energy allocation between reproduction and somatic growth. High quantities of secondary metabolites, known to be repellent to many insect species (Rosenthal and

Janzen 1979, Berenbaum and Seigler 1992, Hobson 1995), are produced by stressed trees, and may also be repellent to *L. occidentalis*. Trees bearing large numbers of cones tend to have small cones, few seeds per cone and poor germination rates (Wheeler and Jech 1990). Large, heavy seeds are most likely to germinate and produce strong, healthy seedlings (Wheeler and Jech 1990, St. Clair and Adams 1991). For *L. occidentalis*, which invests much time in feeding, small seeds may not provide a good return on investment. Trees bearing moderate numbers of large cones with a high number of seed per cone would thus be more suitable hosts for *L. occidentalis* than prolific cone producers. Furthermore, a high variation in number of cones per tree and filled seeds per cone was found between clones in Eurasian Scots pine, *Pinus sylvestris* L. (Boes *et al.* 1991). This suggests that even if trees are producing many cones, uniformity of the cone crop and numbers of filled seeds per cone may be affected more by genetics than by environmental factors.

In orchards where the effect of clone could not be removed, more insects were found on trees of median height than on the tallest trees (Figs. 8, 9). This observation appears to contradict the 'bigger is better' school of host selection (Price 1991, Palumbo *et al.* 1991a, 1991b), and indicates that certain genotypes, regardless of height, represent unsuitable habitats. Tall trees tend to be older, of higher quality and

better nutrient status, and to have a larger surface area, silhouette and cone crop than shorter trees.

While other cone and seed insects time their attack in accordance with host phenology (Hedlin *et al.* 1981), *L. occidentalis* were as likely to feed on clones flowering early or late in the spring. Unlike other insects that only have a limited window of opportunity to oviposit on their host (e.g. seed chalcids, *Megastigmus* spp.), or only live a short time following eclosion or overwintering (e.g. cone gall midges, *Contarinia* spp.), *L. occidentalis* lives well into the summer following overwintering and thus has a very large window of opportunity. As flowering phenology has no discernible effect on numbers of filled seeds per cone, seed weight or percent moisture content (Edwards and El-Kassaby 1988), there may be no adaptive advantage in selecting trees by flowering time.

Because conelets tended to be more reflective than foliage at wavelengths >500 nm (Fig. 10), reflectance may have a significant role in guiding *L. occidentalis* toward trees bearing cones, and within trees to specific feeding sites. However, preferred clones were neither more nor less reflective than non-preferred clones (Fig. 10). Other insects, e.g. *Heteropsylla cubana* Crawford (Lapis and Borden 1995), *Rhagoletis pomonella* (Walsh) (Owens and Prokopy 1986), whiteflies (Kring 1972) and aphids (Kring 1972, Kennedy 1976), are attracted to surfaces which

reflect wavelength frequencies that mimic the reflectance of suitable host plants. In these cases, reflectance makes their hosts more apparent than plants which reflect at other wavelengths. While some cone maggots, *Strobilomyia* spp. are most attracted to fluorescent yellow traps reflecting light in wavelengths from 350 to 650 nm (Jenkins and Roques 1993), there is no evidence available on the spectral response of *L. occidentalis*. For many insects, the behavioral action spectrum sensitivity peak is around 450 nm (Truman 1976). In the alfalfa weevil, *Hypera postica* (Gyllenhal), spectral sensitivity is highest in the red region above 650 nm (Meyer 1977). Insects have pigments with absorption peaks near 440 and 510 nm plus an unltraviolet peak from 340-390 nm (Wolken 1971). On the basis of the data in Fig. 10, and the lack of knowledge on *L. occidentalis* spectral sensitivity, it is unlikely that spectral reflectance is involved in the clonal preference exhibited by *L. occidentalis*. However, visual reflectance may be used as a long-range cue to distinguish cone-bearing trees from those not bearing food resources, a mechanism found in a larch cone fly, *Lasiomma melania* Ackl, (Roques 1987).

Chapter 3

Infestation of Host by

Megastigmus spermotrophus Wacthl.

Introduction

Leptoglossus occidentalis does not exist in any seed orchard in isolation. In Douglas-fir, there are five other major species which feed on the cones and seed: the Douglas-fir cone moth, *Barbara colfaxiana* (Kearfott) (Lepidoptera: Olethretidae), the fir coneworm, *Dioryctria abietivorella* (Grote) (Lepidoptera: Tortricidae), the Douglas-fir cone gall midge *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) and a seed chalcid, *Megastigmus spermotrophus* Wachtl (Hymenoptera: Torymidae). All of these choose Douglas-fir trees within the seed orchard for oviposition and feeding. This species complex is common in Douglas-fir within the western United States and Canada (Schowalter *et al.* 1985, Schowalter and Haverty 1989).

Lepidoptera leave behind obvious evidence of infestation in the form of deformed cones and frass (Hedlin *et al.* 1981). In contrast, *M. spermotrophus*, and *L. occidentalis* damage the seed directly and do little or no visible damage to the cone. It is currently not possible to evaluate

damage by these two species without x-raying the seed (Baron 1967 and Belcher 1973), conducting an enzyme test (Buszewicz and Holmes 1957, Campbell and Shea 1990) or using a floating technique (Brown 1967, Lebrun 1967 and Simak 1973) following harvest. Estimates of damage resulting from *L. occidentalis* feeding range from 41-80% (Pasek and Dix 1988, Connelly and Schowalter 1991 and Schowalter 1994, 1996) while estimates of 10 to 60% are reported for *M. spermotrophus* (Hedlin *et al.* 1980, Schowalter *et al.* 1985, Dombrosky and Schowalter 1988, Rappaport and Roques 1991).

Very little is known concerning the interaction of these pest species. It would be of high adaptive advantage for species to recognize already infested food resources and focus their own search elsewhere when resources are readily available. In years of low resource availability, the species arriving at the orchard earliest would have an advantage over late-arriving species, possibly by competitive exclusion. Most of the species associated with Douglas-fir cones arrive at the orchards relatively early (Schowalter 1994). To avoid competition, *L. occidentalis* might be expected to avoid those cones inhabited by the lepidopteran species, based solely on visual or olfactory cues.

Megastigmus spermotrophus exhibits a clonal preference in Douglas-fir in France (Rappaport and Roques 1991), but its preference has not been well documented in North America (Hedlin and Ruth 1978,

Schowalter and Haverty 1989). No study has examined the host selection preferences of *L. occidentalis* and *M. spermotrophus* within the same host. It is unknown if *L. occidentalis* is an obligate herbivore, or like numerous other Hemiptera (Niemczyk 1978, Ruberson *et al.* 1986), is a facultative carnivore. If *L. occidentalis* were capable of feeding on *M. spermotrophus* larvae inside the seed, late arrival would not adversely affect *L. occidentalis*, and *M. spermotrophus* may benefit from arriving late. Field surveying and evaluation of seed for *L. occidentalis* damage enables data to be collected on *Megastigmus* spp. at the same time. I hypothesized that if *L. occidentalis* can distinguish chalcid-infested seed from sound seed, then their host preferences would be mutually exclusive and the first species to arrive in the orchard may then competitively exclude the other from valuable hosts.

As these two species are commonly found in the same seed orchards in North America, my objectives were: 1) to determine if *L. occidentalis* can discriminate between *Megastigmus*-infested and filled seed, 2) to assess whether an appreciable difference in seed weight could be attributed to *L. occidentalis* feeding; and 3) to compare the distributions of *L. occidentalis* and *M. spermotrophus* in the field.

Materials and Methods

Douglas-fir seed were obtained from the Tree Seed Centre, B.C. Forest Service, Surrey, B.C. Samples of seed were x-rayed (Faxitron, Model 804) at the Pacific Forestry Centre (PFC), Canadian Forest Service, Victoria, B.C. for 1.5 min at 19 kv. Seed were categorized as either filled or infested by *M. spermotrophus* and placed in separate bags.

Three laboratory feeding experiments (Exp.) were conducted to test the hypotheses that adult (Exp. 1,2) and nymphal (Exp. 3) *L. occidentalis* can discriminate between chalcid-infested and uninfested seed. In Exp. 1, seed were assigned in lots of 20 so that there were six treatments consisting of 0, 20, 40, 60, 80, and 100% chalcid-infested seed. Seed were individually marked using a black felt-tipped pen, re-x-rayed and then weighed individually using an analytical balance. Exp. 2 was conducted because the numbers of feeding adult bugs were low in Exp. 1. Seed in Exp. 2 and 3 were assigned in lots of 10. Insects for Exp. 1 and 3 were obtained from a laboratory colony maintained at a 15:9 h (L:D), 60% R.H., and with a temperature rising to 30°C in the day and falling to 15°C at night. Insects for Exp. 2 were collected from the field in June and held in the laboratory until used.

Treatments were replicated eight and 10 times for both adult male and female *L. occidentalis* in Exp. 1 and 2, respectively, and 10 times in

Exp. 3. Two types of controls (n=8, 10 and 10, respectively) consisted of uninfested or 100% chalcid-infested seed that were not exposed to seed bugs. Exp. 1 was run from January 12 - 25, 1994 with each replicate of 20 seeds being placed in a 150 mm diam. plastic Petri-dish with a moistened dental cotton wick and a single adult *L. occidentalis*. Petri dishes were arranged in a completely randomized design on a laboratory bench at 20°C, 60% R.H. and 16:8 h (L:D). Seed bugs were allowed to feed for two weeks; any individual that died within this period was replaced. Feeding behavior was observed several times per day on alternate days. Exp. 2 was carried out from June 13 - August 29, 1995 with a pair of 10-day long replicates for each sex being run at a time. Each individual replicate was placed in a wood and mesh covered cage measuring 10x10x10 cm for 10 days under laboratory conditions as described above. Each insect was had a water wick to provide moisture; seeds were placed in a small Petri disk (60 mm diam.) on the floor of the cage. Exp. 3 was carried out from August 8 - 20, 1996 with two sets of five replicates being run for seven days. Because nymphs are gregarious, groups of three nymphs were placed in 150 mm diam. plastic Petri-dishes with moistened dental cotton wicks. The dishes were randomly arranged on a bench near the laboratory colony to ensure continuity of conditions. Instars 3-5 were used for this experiment as they are hardier than 1st or 2nd instars. All dead insects were replaced to ensure equal exposure to the seeds during the study period. Immediately after being

exposed to seed bugs, seed were x-rayed once more and categorized as full or partially full.

To determine the viability of damaged and sound seed used in this study, germination trials were conducted with filled and partially-filled seed as per International Seed Testing Association standards, using seeds from Exp. 1. Seed were placed in germination trays on moist toweling by replicate and stratified in the dark for three months at 5°C. They were transferred to a germination chamber and held for two weeks under a 30:20°C, and 18:6 h (L:D) photoregime. Seed were then categorized as germinated or moldy.

To test the hypothesis that *L. occidentalis* and *M. spermotrophus* might avoid competition by exhibiting opposite host preferences, the distribution of the two species was examined during July, 1992, for *L. occidentalis*, and post harvest, September, for *M. spermotrophus*, in three Douglas-fir seed orchards on Vancouver Island, B.C. Fifty-one trees representing 10 clones in a first generation Douglas-fir orchard at Mt. Newton Seed Orchards (Mt. Newton 1), 45 trees representing 12 clones in a second generation Douglas-fir orchard also at Mt. Newton (Mt. Newton 2) and 31 trees representing seven clones in a first generation orchard at Nootka, Canadian Pacific Forest Products Ltd. were chosen. A 25-cone sample was obtained from these trees during commercial harvest on August 25. Cones were placed in paper bags, air-dried in the greenhouse

and shaken to extract the seed. Seed were de-winged by gentle abrasion and winnowing, and x-rayed at 19 kvolts for 1.5 min on a Faxitron Model 804. Seeds filled with *M. spermotrophus* larvae were counted and percentage infestation per tree calculated. These percentages were compared with numbers of *L. occidentalis* obtained in whole tree counts for the same trees (Figs. 2-4).

Weight loss or gain of seed in 0 and 100% chalcid-infested treatments from Exp. 1 were compared with those of the unexposed controls using General Linear Models (GLM) and the Dunnett's one-way t-test (Day and Quinn 1989). Weights of partially-filled and filled seed were compared. Weight loss or gain between seed types (*Megastigmus*-infested, partially-filled, filled seed) were compared using General Linear Models and Scheffe's mean separation test. Percent germination was transformed by arcsin$\sqrt{x}$ to stabilize the variances and compared between treatments using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. Percentage *M. spermotrophus* infestations were transformed using arcsin$\sqrt{x}$ and *L. occidentalis* counts transformed with $\sqrt{x+0.5}$ to stabilize the variances prior to analysis. Clonal preferences were then analyzed using GLM and compared between clones using Duncan's multiple range test. Numbers of *L. occidentalis* (Figs. 2-4) were regressed against percentage infestation by *M. spermotrophus* using GLM. In all cases $\alpha=0.05$.

Results and Discussion

Leptoglossus occidentalis adults discriminated between chalcid-infested seed and filled seed. In both experiments, partially-filled seed, characteristic of *L. occidentalis* feeding (Hedlin *et al.* 1981), occurred for both sexes in the 0, 20 and 40% treatments of seed infested by *M. spermotrophus* (Table 3). Only one male was observed probing an infested seed. In Exp. 2, two males also fed at the 60% treatment level. Post treatment x-rays for both experiments revealed no evidence that any *M. spermotrophus* larva had been fed on by *L. occidentalis*. Mortality of *L. occidentalis* was relatively constant at 16-24% amongst all treatments. These data are consistent with a refusal to feed at all when infestation by *M. spermotrophus* is high. In Exp. 3, nymphs also discriminated between chalcid-infested and full seed when chalcid infestation levels were <100%. However, in two 100% chalcid-infested replicates, feeding on *M. spermotrophus*-infested seed occurred (Table 4). This may be due to nymphs possessing lower fat reserves than adults and, when faced with starvation, a propensity to accept lower quality food. Mortality was fairly constant ranging from 23-35% amongst all treatments.

The results in Tables 3 and 4 support the hypothesis that *L. occidentalis* adults and nymphs can discriminate between chalcid-infested and uninfested seed, and indicate that adults are not facultative predators on seed chalcids. Whether nymphs would feed consistently on

Table 3: Feeding by adult *L. occidentalis* on Douglas-fir seed with variable levels of infestation by *M. spermotrophus*.

Exp.	Treatment	Infestation level of *M. spermotrophus*	Number of insects that fed		Mean percentage of uninfested seeds fed on	
			Females	Males	Females	Males
1	8 insects of	0	1	1	60	25
	each sex	20	4	3	18	14
	tested singly	40	1	3	33	6
	for feeding	60	0	0	0	0
	on 20 seeds	80	0	0	0	0
	over 14 days	100	0	0	-----	-----
2	10 insects of	0	1	3	30	56
	each sex	20	2	3	81	45
	tested singly	40	0	1	0	66
	for feeding	60	0	2	0	75
	on 10 seeds	80	0	0	0	0
	for 10 days.	100	0	0	-----	-----

Table 4: Feeding by nymphal *L. occidentalis* on Douglas-fir seed with variable levels of infestation by *M. spermotrophus*.

Treatment	Infestation level of *M. spermotrophus*	Number of replicates where feeding occurred	Mean percentage of uninfested seeds fed on	Mean percentage of chalcid-infested seed fed on
10 groups of	0	6	9	-----
3 nymphs	20	2	8.7	0
(Instars 3-5)	40	3	10	0
feeding on 10	60	2	7.5	0
seeds for	80	2	10	0
7 days.	100	2	-----	3

the larvae of *M .spermotrophus* is not known. These results do not support the hypothesis that nymphal *L. occidentalis*, like other hemiptera (Coppel and Jones 1962 and Mukerji and Le Roux 1965), require animal protein to successfully develop to adulthood.

Partially-filled seed fed on by *L. occidentalis* weighed significantly less than seed infested by *M. spermotrophus* or filled seed, which gained weight slightly over two weeks, probably because of imbibed water (Table 5). Germination of filled seed was not significantly different between treatments ($F=4.16$, $P=0.08$), averaging 61% in the insect-exposed treatments and 50% in control treatments. Surprisingly, 18% of 43 partially-filled seed also germinated. Thus, although *L. occidentalis* feeding consumed approximately 45% (mean weight loss of 4.55±2.05 mg) of the endosperm and embryo tissue, there were sufficient reserves left in many seeds to permit germination. The remaining damaged seed which did not germinate had 52% of their contents consumed (mean weight loss of 5.87±2.39 mg from seed originally weighing on average 11.22±2.46 mg). Rowan and DeBarr (1974) found the same phenomenon in slash pine seed, *Pinus elliotii* Engelm., damaged by the southern conifer seed bug, *Leptoglossus corculus* Say. Kermode and Bewley (1985) found castor beans, *Ricinus communis* L., to germinate even when subjected to a drying treatment which reduced the amount

Table 5: Weight loss or gain over a two-week period by filled Douglas-fir seed or seed damaged by *M. spermotrophus* and *L. occidentalis* in 0 and 100% chalcid-infested treatments.

Seed Category	Controls, seed not exposed to *L. occidentalis*			Seed exposed to *L. occidentalis*		
	Number of Seeds	Weight (mg) (mean ± SE)		Number of Seeds	Weight (mg) (mean ± SE)	
		Initial weight	Loss(-) or gain(+)		Initial weight	Loss(-) or gain(+)[a]
Seed infested by *M. spermotrophus*	160	5.99 ± 0.08	- 0.14 ± 0.02	315	6.25±0.08	- 0.068±0.03 b
Filled seed	160	10.39 ± 0.18	+ 0.134 ± 0.02	300	10.32±0.13	+ 0.24±0.02 a*
Partially-filled seed fed on by *L. occidentalis*	---	---	---	17	11.24±0.56	- 6.52±0.43 c*

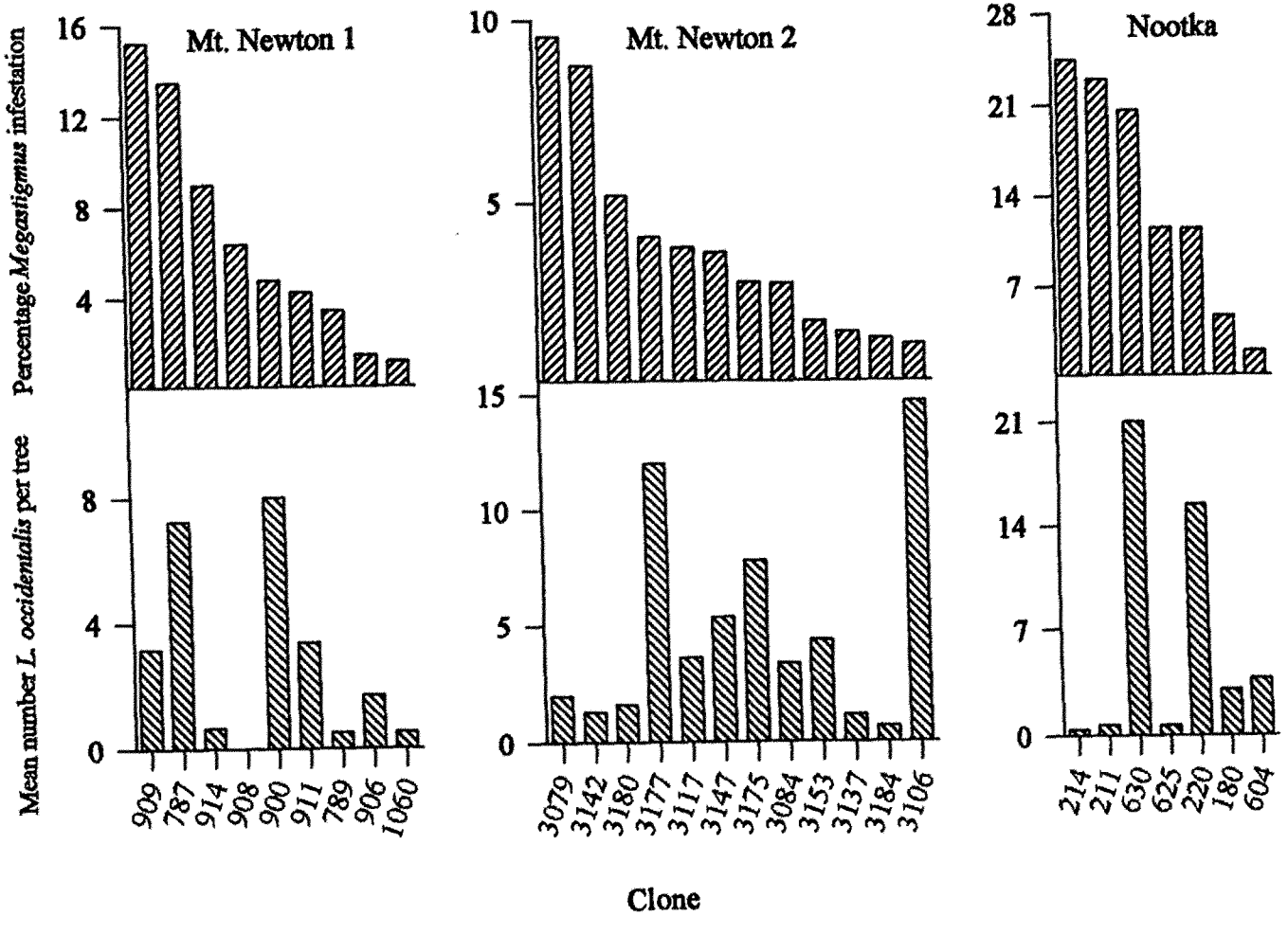
[a] Means within column followed by different letters are significantly different, Scheffe's test, $P < 0.0001$. Means for weight loss or gain for chalcid-infested and filled seed followed by * are significantly different from weight change in control seed in the same category, Dunnett's one way t-test, $P < 0.0001$.

of endosperm present. Similar phenomena have been observed for acorns fed upon by filbert weevils, *Curculio occidentis* (Casey) and moths, *Melissopus latiferreanus* (R.G. Bennett, B.C. Forest Service, Victoria, pers. comm.). I do not know how vigorous seedlings from partially-filled seed would be, but it is possible that such seedlings represent a significant proportion of the 20% of Douglas-fir seedlings that are routinely culled in nursery production (D. Summers, B.C. Forest Service, Green Timbers Nursery, Surrey, B.C., pers. comm.).

Megastigmus spermotrophus exhibited a pronounced clonal preference in all three orchards surveyed in 1992 (Fig. 11), with clone accounting for 38-52% of the variation in chalcid infestation. These data on *M. spermotrophus* in its native range support those obtained in France by Rappaport and Roques (1991). They suggest that seed orchardists could select for less-preferred clones if preferences do not shift from year to year as they do for *L. occidentalis*, and genetic diversity in the seed crop is not sacrificed.

No relationship was found between percentage chalcid infestation and numbers of *L. occidentalis* (Fig. 11). While *L. occidentalis* and *M. spermotrophus* did not prefer the same clones, their preferences overlapped in the field. In the orchards surveyed, populations of both species may have been too low (≤ 24% infestation for *M. spermotrophus*

Figure 11: Clonal preferences of *M. spermotrophus* and *L. occidentalis* in three Douglas-fir orchards on Vancouver Island, surveyed in 1992. Data for *L. occidentalis* adapted from Chapter 1. Relationship between percentage infestation by *M. spermotrophus* and counts of *L. occidentalis* as follows: Mt. Newton 1, $F=0.05$, $P<0.833$, $r^2=0.009$; Mt. Newton 2, $F=0.24$, $P<0.629$, $r^2=0.005$; Nootka, $F=0.02$, $P<0.889$, $r^2=0.0006$.


Mt. Newton 1
Mt. Newton 2
Nootka
Percentage *Megastigmus* infestation
Mean number *L. occidentalis* per tree
16
12
8
4
0
10
5
15
28
21
14
7
909
787
914
908
900
911
789
906
1060
3079
3142
3180
3177
3117
3147
3175
3084
3153
3137
3184
3106
214
211
630
625
220
180
604
Clone

and ≤ 20 bugs/tree for *L. occidentalis*) to have allowed a segregating effect based on clonal preference to be disclosed.

Megastigmus spermotrophus utilized the distal regions of Douglas-fir cones in France (Rappaport and Roques 1991), while the central zone was infested primarily by lepidoptera. A related species, *M. aculeastus nigroflavus*, was shown to preferentially infest large seed (achenes) of wild and cultivated species of the genus *Rosa* over smaller seed (Nalepa and Grissell 1993). That *L. occidentalis* can benefit from feeding on larger seed, and as I have observed *L. occidentalis* feeding on all areas of a cone, I hypothesize that *L. occidentalis* is probably in direct competition with *M. spermotrophus* for seed. However, when low infestations by *M. spermotrophus* occur, I hypothesize that *L. occidentalis* could infest the same clones(trees) and feed preferentially on the proximal region of the cones to avoid chalcid-infested seed. When chalcid populations are high *L. occidentalis* might be displaced from those clones most preferred by *M. spermotrophus*, reinforcing the rejection of less-preferred clones by the seed bug.

Because both seed bugs and seed chalcids require uninfested seed (Tables 3, 4) and because their clonal preferences overlap but are not identical (Fig. 11), I conclude that impacts from these two species are segregated and additive.

Chapter 4
Host Selection : A New Model

Introduction

Utilizing an analysis of variance (ANOVA) or general linear model (GLM) on insect count data has always been problematic. In many cases, as demonstrated in Chapter 1, transformations are required prior to analyzing the data using ANOVA or GLM and use of indices, such as k values from the negative binomial distribution, are suspect due to interpretative limitations (Taylor *et al.* 1979). Very few analysis methods can adequately handle data sets with variables which are both continuous and categorical, a disproportionate number of zero counts and a low and unequal number of replicates. In situations such as these, more complex analytical methods are required.

A suitable analysis method is one which is consistent with a theoretical model of the behavior of an organism being studied. Three steps which should be followed to determine an appropriate method of analysis are: 1. a precise theory of the behavior of the organism which generates the data must be stated, 2. an appropriate distribution, as implied by the behavior, selected and 3. a statistical analysis based on

the distribution located and used to analyze the data. By applying these steps to my data I will show Poisson regression to be the appropriate analysis.

For the first step, the host selection behavior of *L. occidentalis* in a seed orchard must be examined. In the spring, *L. occidentalis* adults leave overwintering sites and fly to an orchard where acceptable hosts are located. Theoretically, if all trees within a seed orchard were genotypically and phenotypically the same or if host selection did not depend on tree characteristics, each tree can be considered acceptable and no host preference can be observed. *Leptoglossus occidentalis* is a strong flier (Schowalter 1984) and can possibly survey a large section of an orchard prior to selecting a host. Therefore, any insect entering an orchard has a large number of trees to choose from. I hypothesize that selection of an appropriate tree for feeding and oviposition is based on tree characteristics, such as height and cone density. Once an acceptable host is selected, mating and oviposition occur. The number of eggs oviposited may be an indicator of how preferred the tree is. I assume that each *L. occidentalis* individual behaves in the same way, which allows me to consider the observed number of bugs on each tree to be draws from an identical distribution.

To meet the requirements of step two, a picture of the data resulting from the behavior must be described. With respect to *L. occidentalis*, the probability that an insect chooses a particular tree, from an orchard of identical trees, is 1/n, where n is the number of trees within the orchard. Given that n is large, the probability of an individual tree being chosen is small. The number of insects choosing a particular tree within an orchard of identical trees therefore follows a Poisson distribution. If *L. occidentalis* exhibits a host preference, one would expect to see larger probabilities associated with preferred trees. For the orchards studied, a few insects had many trees to choose from. As a result, there were many trees which harbored no insects at all and only a few trees which had insects on them. A picture of this data set would be many zero counts and few high numbered counts. As in many similar studies, when the number of positive responses is small in comparison to the total number of individuals being studied, the Poisson model should provide a reasonable approximation to the exact distribution of the data (Frome *et al.* 1973, Breslow and Day 1987, Lawless 1987).

For the third step, a review of the literature is required. Other situations where individuals experience repeated events, and Poisson regression has been used to analyze the data, can be found in cancer research (Boffetta *et al.* 1992, Koivusalo *et al.* 1994, Morrison *et al.* 1994, Plu-Bureau *et al.* 1994), epidemiology (Frome and Checkoway 1985),

mortality studies (Mackenbach *et al.* 1992, Tolbert *et al.* 1992, Schouten *et al.* 1993, Segal and Neuhaus 1993), environmental impact studies (Alexander *et al.* 1992, Ray *et al.* 1992, Schwartz 1994), medicine (Gail *et al.* 1980, Bartoszynski *et al.* 1981, Farewell and Sprott 1988, Blackwelder 1993, Lavange *et al.* 1994), equipment reliability studies (Crow 1974, Ascher and Feingold 1984), economics (Hausman *et al.* 1984) and sociology (Heckman and Singer 1985). In all cases, the behavior of the organism or entity being studied has followed a Poisson distribution. As a result, use of Poisson regression to analyze the data, which is based on the Poisson distribution, is appropriate.

There are four important reasons to use the Poission distribution in cases such as these (Dean 1988): 1) it can be derived theoretically as the distribution of counts using simple assumptions; if events occur randomly and independently with the average rate of occurrence being constant, then the number of events that occur in a fixed time interval has a Poisson distribution. 2) it is the limiting form of certain distributions including the binomial and the superposition of many arbitrary counting processes. 3) it is a member of the exponential family so that a unique, unbiased, minimum variance estimator of the mean of the distribution exists, this estimator being a function of the sufficient statistic and 4) it frequently provides an adequate approximation to the distribution of random counts. A fifth reason for using the Poisson

distribution is that it enables more meaningful biological interpretation of a model and its parameters than merely formal goodness-of-fit testing (Douglas 1994). Insect counts, replete with zero counts, have been described using many distributions such as the negative binomial, Neyman type A or Polya-Aeppli (Kemp 1987). In Chapter 1, the negative binomial distribution, and its exponent parameter *k*, was used to describe the distribution of *L. occidentalis* within orchards. However, as *k* has been shown to be valuable as a descriptive statistic only (Taylor *et al.* 1979), evaluation of variables is not possible. In this case other distributions, such as the Poisson, provide a better basis for analysis.

My objectives were to develop and utilize a suitable analysis method to describe the host selection process of *L. occidentalis* and test the hypothesis that tree characteristics change the probability of a tree being selected. Three steps required to justify use of a distribution to analyze a data set have been outlined and evaluated with respect to my data. Use of the Poisson distribution provides a better fit to the data generating process than the normal distribution and thus is the appropriate distribution upon which analysis should be based.

Organization and Analysis of the Data

Data sets were compiled for two Douglas-fir orchards, Mt. Newton 1 and 2, first and second generation orchards, respectively, located at Mt. Newton Seed Orchards, Timber West Ltd., Saanichton, B.C., and two lodgepole pine orchards, Kal 1 and 2, reference numbers 230 and 307, respectively, located at Kalamalka Seed Orchards, B.C. Forest Service, Vernon, B.C. Mt. Newton 1 and 2 were surveyed consecutively from 1992-1994 and the following data was collected from each tree: tree height, cone density, gibberellin use and flowering time (early, mid or late). Kal 1 and 2 were surveyed consecutively from 1993-1995 and the following data collected: tree height, cone density or actual cone numbers, yield, half-cone seed counts and flowering time. Insect surveys, for adults and nymphs, occurred in either July or August. For all data sets analyzed, the number of trees per clone was at least 3, but varied to as high as 41 trees per clone in the lodgepole pine orchards.

Limdep (Limited Dependent Variables) version 7.0 (Greene 1995) obtained from Econometric Software Inc., Belport, New York, and installed on a PC was used to analyze the data. Each data set was analyzed three times using a different set of variables. The first analysis used clone and tree characteristics as the explanatory variables, the

second analysis was restricted to only clone and the third analysis was again restricted to only tree characteristics as explanatory variables. Insect counts for adults and nymphs were analyzed separately. Flowering time data, although obtained for each orchard, was not included in the analysis due to a collinearity (correlation with clone) problem. To determine the effect of flowering time the clone variable would have had to been removed. As I deemed the clone variable to be more important in the model and flowering time was not found to be significant (Chapter 2), flowering time was not included.

To compare the goodness of fit of these models, log-likelihood values, generated by Limdep, were compared in two ways. The first comparison was between log-likelihood values generated by OLS and Poisson regression. Limdep automatically runs an OLS (ordinary least squares, or linear regression, which is analogous to an analysis of covariance) analysis first to aid in determining starting values in the iterations of the Poisson regression. Comparison of the log-likelihood values generated by both regression analyses determines which model, linear or Poisson regression, has a better fit with the data. The criteria for this better fit is a log-likelihood value which is closer to zero. The second log-likelihood comparison was done examining the unrestricted model (clone and tree characteristics analysis) with restricted models (clone or tree characteristics analysis). The Likelihood Ratio Test

(Davidson and MacKinnon 1993) generates a G value which has a chi-square distribution and a *p* value which indicates whether imposing a restriction significantly changes the fit of the model. For example, restricting the analysis to only clone is in effect a look at how the tree characteristics impact on the model, because the tree characteristics, in this analysis, have zero effect. If the *p* value is significant, it indicates that tree characteristics are important in the model and without them, the model is significantly changed from the unrestricted (where all variables are included) model. A non-significant *p* value indicates that including tree characteristics in the model does not significantly increase the explanatory power of the model.

For the analysis, I used a constant term to represent trees harboring a high number of insects. This is referred to as the base case throughout this chapter. Selecting a base case for comparison as one harboring a high number of insects is not typically done. It is easier to interpret comparisons if the base case represents the worst case scenario, in this case, those trees harboring the lowest number of or zero insects. However, there are many trees harboring no insects so selecting a base case becomes difficult. Poisson regression results are relative, not absolute like they are in ANOVA, and relative to zero, any tree with more than zero bugs has a relative count of infinity. This yields large coefficient values which are difficult to interpret. To avoid this problem, I

chose as a base case a clone harboring a relatively large number of adult and nymph *L. occidentalis*. The same base case was used for adults and nymphs.

To determine the coefficient values, Limdep utilizes the Newton method to maximize the log of the likelihood function. For these analyses, at least fifty iterations of the model are calculated before final estimates are obtained for each parameter. The final result, in the form of coefficients, are generated for each parameter. The basic form of the Poisson model for discrete random variable, Y, and observed frequencies, y_i, i=1,.....,N, where $y_i \geq 0$, and regressors x_i,

$$\text{prob}\,(Y=y_i) = (e^{-\lambda}\lambda_i^{y})/(y_i!),\ y=0,1,....$$

$$\text{where} \qquad \ln\lambda_i = \beta' x_i$$

$$\text{and}\ \ \beta' x_i = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} + L + \beta_k x_{ki}$$

In this model, λ_i is both the mean and variance of y_i. In less mathematical terms, β represents the coefficient generated for each variable added to the model. Using these coefficients, a λ value can be calculated for each tree, based on individual tree characteristics, which represents the estimated mean number of insects expected. The analysis also generates a Z score which indicates whether the variable is significant in the model. Relative risks for each variable were generated using the equation: Rel. risk = $\exp(\beta_1)/\exp(\beta_c)$ (Maldonado and Greenland 1993) where β_1 is the coefficient for the variable and β_c is the coefficient of

the base case. However, setting $\exp(\beta_c)$ to 1 results in the equation being merely divided by one and calculation of the relative risk becomes: Rel. risk = $\exp(\beta_1)$. All relative risk values generated are compared to this base case. And finally, as a graphical aid only, the estimated mean number of *L. occidentalis* per tree was compared with the actual mean for each orchard, year and life stage to determine if the model would be able to reproduce means similar to the actual values.

Interpretation of the Results

Categorical and continuous data within the model were handled differently with respect to interpreting the relative risks. Categorical data, *i.e.* cone density, had each category compared with the highest cone density value of 5 for Mt. Newton 1 and 2 and 4 for Kal 1 and 2. In only one case (Kal 1 - 1993) was the lowest cone density rating (1) used due to the highest cone rating having a very low number of replicates. All relative risks generated refer to the amount of increase, if greater than 1, or decrease, if less than 1, compared with the highest cone density value. As an example, if a cone density of 2 generates a relative risk of 9.55, this means that trees with a cone density of 2 are 9.55 times more likely to have insects on them than trees with a cone density of 5. Conversely, if cone density of 2 generates a relative risk of 0.65, this means that trees with a cone density of 2 are less likely, by 0.65 times, to have insects on them compared to trees with a cone density of 5.

Continuous data, *i.e.* tree height, does not generate relative risk in relation to only one lower valued situation. Relative risks generated for continuous variables refer to the amount of increase in insect numbers which can be expected with an increase in one unit of measure, *i.e.* a metre. So, if the relative risk of tree height equals 1.07 for a particular orchard, for every metre of tree height, it would be expected that the number of insects would increase by 1.07 times, *i.e.* ~ 7% increase.

Results and Discussion

Results obtained from the Poisson regression analysis were very good. In comparison with an OLS regression, Poisson regression gave values of the log-likelihood function which were closer to zero for both adults and nymphs (Tables 6 and 7, respectively), indicating that the data is more consistent with a Poisson regression model than with an OLS regression model. These results further justify using a Poisson regression model to analyze the data. In only one instance for adults (Mt. Newton 2 - 1994), and one instance for nymphs (Mt. Newton 2 - 1994) were the values of the log-likelihood function closer to zero for the OLS regression. In both cases the model was a restricted one including only tree characteristics. As the other models in all other orchards and years did not show this result, these results are considered anomalies.

Table 6: Log-likelihood function values calculated from two methods of analysis: OLS (ordinary least squares) regression and Poisson regression. Each value was generated from a model evaluating the relationship between adult *L. occidentalis* and various tree characteristics, separately (clone only or tree characteristics) or in combination (all variables). Values from Poisson regression closer to 0 than those from OLS regression indicate a better fit of the data to a Poisson distribution.

			Value of the log-likelihood function	
Orchard	Year	Model	OLS regression	Poisson regression
Mt. Newton 1	1992	All variables *	-175.38	-62.31
		Clone only	-176.51	-65.75
		Tree characteristics **	-205.98	-102.43
	1994	All variables	-514.59	-370.24
		Clone only	-520.67	-483.73
		Tree characteristics	-529.68	-508.61
Mt. Newton 2	1992	All variables	-99.72	-40.29
		Clone only	-107.69	-51.50
		Tree characteristics	-116.54	-67.02
	1993	All variables	-43.48	-7.62
		Clone only	-61.95	-16.75
		Tree characteristics	-82.03	-27.01
	1994	All variables	-367.27	-289.12
		Clone only	-368.29	-315.94
		Tree characteristics	-375.84	-414.82
Kal 1	1993	All variables	-143.40	-69.73

Table 6 (Continued)

Orchard	Year	Model	Value of the log-likelihood function: OLS regression	Value of the log-likelihood function: Poisson regression
Kal 1		Clone only	-150.13	-78.59
		Tree characteristics	-169.76	-102.88
	1994	All variables	-427.08	-274.33
		Clone only	-427.44	-275.41
		Tree characteristics	-467.98	-395.20
	1995	All variables	-671.92	-387.51
		Clone only	-680.99	-399.16
		Tree characteristics	-740.89	-490.21
Kal 2	1993	All variables	-95.71	-48.03
		Clone only	-96.54	-49.19
		Tree characteristics	-113.12	-73.58
	1994	All variables	-117.67	-101.99
		Clone only	-121.45	-104.35
		Tree characteristics	-129.18	-114.50
	1995	All variables	-97.03	-77.54
		Clone only	-100.99	-81.32
		Tree characteristics	-119.28	-102.17

* All variables - for Mt. Newton 1 and 2: clone, tree height, cone density and gibberellin application. For Kal 1 and 2: clone, tree height, cone density, yield, and half-cone seed counts.
** Tree characteristics - all characteristics listed above except clone.

Table 7: Log-likelihood function values calculated from two methods of analysis: OLS (ordinary least squares) regression and Poisson regression. Each value was generated from a model evaluating the relationship between nymphal *L. occidentalis* and various tree characteristics, separately (clone only or tree characteristics) or in combination (all variables). Values from Poisson regression closer to 0 than those from OLS regression indicate a better fit of the data to a Poisson distribution.

			Value of the log-likelihood function	
Orchard	Year	Model	OLS regression	Poisson regression
Mt. Newton 1	1992	All variables *	-354.88	-118.37
		Clone only	-367.92	-154.53
		Tree characteristics **	-367.59	-178.45
	1994	All variables	-362.83	-161.63
		Clone only	-367.32	-200.19
		Tree characteristics	-372.17	-236.95
Mt. Newton 2	1992	All variables	-295.69	-211.36
		Clone only	-299.07	-237.69
		Tree characteristics	-317.03	-357.41
	1993	All variables	-150.08	-34.08
		Clone only	-160.39	-49.99
		Tree characteristics	-174.03	-86.54
	1994	All variables	-404.19	-300.00
		Clone only	-405.41	-333.81
		Tree characteristics	-414.75	-495.11

Table 7 (Continued)

Orchard	Year	Model	Value of the log-likelihood function: OLS regression	Value of the log-likelihood function: Poisson regression
Kal 1	1993	All variables	-400.14	-121.61
		Clone only	-402.51	-133.81
		Tree characteristics	-442.44	-234.39
	1994	All variables	-271.19	-147.41
		Clone only	-273.32	-152.28
		Tree characteristics	-289.74	-193.34
	1995	All variables	-2629.83	-1390.51
		Clone only	-2643.48	-1489.10
		Tree characteristics	-2700.70	-1794.47
Kal2	1993	All variables	-60.95	-5.05
		Clone only	-65.12	-15.34
		Tree characteristics	-84.72	-27.75
	1994	All variables	-150.51	-70.19
		Clone only	-152.64	-75.40
		Tree characteristics	-169.61	-107.60
	1995	All variables	-666.15	-495.37
		Clone only	-670.90	-531.09
		Tree characteristics	-686.31	-649.66

* All variables - for Mt. Newton 1 and 2: clone, tree height, cone density and gibberellin application. For Kal 1 and 2: clone, tree height, cone density, yield, and half-cone seed counts.
** Tree characteristics - all characteristics listed above except clone.

Likelihood Ratio Test values comparing the unrestricted model to two restricted models shows that adding clone and tree characteristics significantly changes the model (Tables 8 and 9, adults and nymphs, respectively). For adults, the effect of adding clone to the model can be seen in the *p* values of restricted model 2 (analysis using only tree characteristics). In eight of 11 instances, clone was found to be highly significant for explaining the distribution of *L. occidentalis* within a seed orchard. For nymphs, the effect was even more dramatic and in 10 of 11 instances clone was a highly significant factor. Even more interesting is the effect of tree characteristics (restricted model 1). The effect of adding tree characteristics to the model was significant in eight of 11 instances for adults and in all instances for nymphs. These results suggest that for most orchards, both clone and tree characteristics are important in the host selection process of *L. occidentalis*. In years where addition of tree characteristics was not significant (non-significant *p* value in restricted model 1), addition of clone was significant, and conversely, if addition of clone was not significant, addition of tree characteristics was. There was only one instance where both restricted models were not significant (Kal 2 - 1994, adults) indicating that the data did not fit the model very well.

Table 8: Likelihood Ratio Test statistics for adult *L. occidentalis* in four orchards over four years of surveys. G values compare an unrestricted model with two restricted models: 1 (clone only) and 2 (tree characteristics). *P* values for restricted model 1 indicate the significance of including tree characteristics in the unrestricted model. *P* values for restricted model 2 indicate the significance of including clone in the unrestricted model.

		Unrestricted model[a]		Restricted model 1[b]				Restricted model 2[c]			
Orchard	Year	VLLF[d]	df	VLLF	df	G Value[e]	*P* value	VLLF	df	G Value	*P* value
Mt. Newton 1	1992	-62.31	43	-65.75	37	14.96	0.021	-102.43	6	80.24	<0.0001
	1994	-370.24	41	-483.73	36	162.52	<0.0001	-508.61	5	276.74	<0.0001
Mt. Newton 2	1992	-40.29	31	-51.50	25	29.81	<0.0001	-67.02	6	53.46	0.001
	1993	-7.62	49	-16.75	45	18.26	0.001	-27.01	5	38.78	0.695
	1994	-289.12	36	-315.94	31	86.06	<0.0001	-414.82	5	251.40	<0.0001
Kal 1	1993	-69.73	64	-78.59	60	17.72	0.001	-102.88	4	66.30	0.269
	1994	-274.33	59	-275.41	57	2.17	0.339	-395.20	2	241.74	<0.0001
	1995	-387.51	96	-399.16	94	23.29	<0.0001	-490.21	2	205.40	<0.0001

Table 8 (Continued)

Orchard	Year	Unrestricted model[a] VLLF[d]	df	Restricted model 1[b] VLLF	df	G Value[e]	*P* value	Restricted model 2[c] VLLF	df	G Value	*P* value
Kal 2	1993	-48.03	38	-49.19	32	2.31	0.889	-73.58	6	51.09	0.017
	1994	-101.99	32	-104.35	29	4.72	0.194	-114.50	3	25.02	0.677
	1995	-2665.40	38	-2679.47	37	28.13	<0.0001	-2914.15	1	497.50	<0.0001

[a]Unrestricted model - evaluates the relationship between insect numbers and several variables. For Mt. Newton 1 and 2 these variables include: clone, tree height, cone density and gibberellin use. For Kal 1 and 2 includes: clone, tree height, cone density, yield, and half-cone seed counts.
[b]Restricted model 1 - evaluates the relationship between insect counts and clone.
[c]Restricted model 2 - evaluates the relationship between insect counts and tree characteristics, listed above, excluding clone.
[d]VLLF - Value of the log-likelihood function
[e]G value - generated from the Likelihood Ratio Test (Davidson and MacKinnon 1993)

Table 9: Likelihood Ratio Test statistics for adult *L. occidentalis* in four orchards over four years of surveys. G values compare an unrestricted model with two restricted models: 1 (clone only) and 2 (tree characteristics). *P* values for restricted model 1 indicate the significance of including tree characteristics in the unrestricted model. *P* values for restricted model 2 indicate the significance of including clone in the unrestricted model.

		Unrestricted model[a]		Restricted model 1[b]				Restricted model 2[c]			
Orchard	Year	VLLF[d]	df	VLLF	df	G Value[e]	*P* value	VLLF	df	G Value	*P* value
Mt. Newton 1	1992	-118.37	43	-154.53	37	87.04	<0.0001	-178.45	6	120.16	<0.0001
	1994	-161.63	41	-200.19	36	144.86	<0.0001	-236.95	5	150.64	<0.0001
Mt. Newton 2	1992	-211.36	31	-237.69	25	200.79	<0.0001	-357.41	6	292.10	<0.0001
	1993	-34.08	49	-49.99	45	31.82	<0.0001	-86.54	5	104.93	<0.0001
	1994	-300.00	36	-333.81	31	67.91	<0.0001	-495.11	5	390.22	<0.0001
Kal 1	1993	-121.61	64	-133.81	60	24.40	<0.0001	-234.39	4	225.56	<0.0001
	1994	-147.41	59	-152.28	57	9.74	0.008	-193.34	2	91.85	0.002
	1995	-1390.51	96	-1489.10	94	197.19	<0.0001	-1794.47	2	807.93	<0.0001

Table 9 (Continued)

		Unrestricted model[a]		Restricted model 1[b]				Restricted model 2[c]			
Orchard	Year	VLLF[d]	df	VLLF	df	G Value[e]	*P* value	VLLF	df	G Value	*P* value
Kal 2	1993	-5.05	38	-15.34	32	20.59	0.002	-27.75	6	45.41	0.059
	1994	-70.19	32	-75.40	29	10.43	0.015	-107.60	3	74.82	<0.0001
	1995	-451.69	38	-453.65	37	3.93	0.047	-517.81	1	132.24	<0.0001

[a]Unrestricted model - evaluates the relationship between insect numbers and several variables. For Mt. Newton 1 and 2 these variables include: clone, tree height, cone density and gibberellin use. For Kal 1 and 2 includes: clone, tree height, cone density, yield, and half-cone seed counts.
[b]Restricted model 1 - evaluates the relationship between insect counts and clone.
[c]Restricted model 2 - evaluates the relationship between insect counts and tree characteristics, listed above, excluding clone.
[d]VLLF - Value of the log-likelihood function
[e]G value - generated from the Likelihood Ratio Test (Davidson and MacKinnon 1993).

Clone, tree height, cone density, half-cone seed counts, gibberellin injection, and yield were all found to be important in the models (Tables 10-20). In an analysis of variance model (see Chapter 1), none of these variables could be analyzed together within the same model and separate analyses did not disclose any consistently significant relationships. For any given year, the variables which were significant were not the same, nor was there any pattern of significance between the adults and nymphs. Starting with clone, most orchards had at least one clone being significant in the model. Significant in the model in this case means that the ratio between the coefficient and the standard error was found to be significantly different from 0. Clones not harboring insects had very large standard error values (>100) which, when divided into the coefficient values, returns very small ratio values, not significantly different from zero. Any clone harboring insects which is not significant in the model similarly has a very large standard error value and thus the ratio value is small. This ratio in essence takes into account the different variances within the clones and adjusts the significance test accordingly. Analysis of adults in Mt. Newton 2 - 1992 (Table 12), Kal 1 - 1993 (Table 15), Kal 2 - 1993 (Table 18) and Kal 2 - 1994 (Table 19) showed none of the clones to be significant to the model. Clones not harboring insects had relative risk values of <0.0001. These are not reported in the tables as the number of clones not harboring insects is very high, ~ 60% of all

Table 10: Relative risks, compared with the base case, for variables included in the Poisson regression model for Mt. Newton 1 - 1992, adults and nymphs. Clones with a relative risk <0.01 not shown in table (26 and 27 clones not shown, adults and nymphs, respectively). Variables significant in the model, $P<0.05$, denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	900	1.00 (base case)	900	1.00 (base case)
	911 *	0.42	972	0.37
	914	0.25	911 *	0.36
	972	0.18	969 *	0.36
	909 *	0.13	909 *	0.32
	789 *	0.12	906 *	0.30
	912 *	0.10	912 *	0.19
	967 *	0.09	233 *	0.14
	573 *	0.09	789 *	0.08
	760 *	0.08	573 *	0.04
	969 *	0.07	971	0.02
	971	0.03		
Height	metre	1.00	metre *	0.99
Cone rating	1	0.00	1	0.00
	2	1.16	2 *	6.46
	3	1.14	3 *	2.83
	4	1.36	4 *	5.91
	5	1.00 (base case)	5	1.00 (base case)
Gibberellins	Not used	1.00 (base case)	Not used	1.00 (base case)
	Used	0.00	Used	0.00

Table 11: Relative risks, compared with the base case, for variables included in the Poisson regression model for Mt. Newton 1 - 1994, adults and nymphs. Clones with a relative risk <0.01 not shown in table (10 and 19 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	760 *	2.83	969 *	4.73
	972	1.44	573	1.69
	233	1.03	900	1.49
	908	1.00 (base case)	114	1.28
	643	0.89	85	1.25
	169	0.70	911	1.18
	114	0.68	908	1.00 (base case)
	902	0.67	760	0.99
	970	0.54	906	0.79
	907	0.53	572	0.63
	794 *	0.45	233	0.61
	573 *	0.45	967	0.52
	85 *	0.37	419	0.42
	909	0.36	124	0.41
	763 *	0.34	623	0.41
	906 *	0.33	642	0.41
	572 *	0.33	794	0.36
	132 *	0.32	169	0.13
	900 *	0.31		
	911 *	0.30		

Table 11 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	759 *	0.28		
	903 *	0.26		
	791 *	0.20		
	60 *	0.18		
	967 *	0.17		
	969 *	0.13		
	568 *	0.11		
Cone rating	1 *	17.88	1	0.20
	2 *	13.38	2	1.06
	3 *	27.68	3	0.62
	4 *	6.73	4	1.01
	5	1.00 (base case)	5	1.00 (base case)
Gibberellins	Not used	1.00 (base case)	Not used	1.00 (base case)
	Used *	0.19	Used	0.00

Table 12: Relative risks, compared with the base case, for variables included in the Poisson regression model for Mt.Newton 2 - 1992, adults and nymphs. Clone with a relative risk <0.01 not shown in table (16 and 10 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3177	1.00 (base case)	3177	1.00 (base case)
	3131	0.52	3106	0.72
	3175	0.31	3100 *	0.43
	3084	0.23	3080 *	0.36
	3097	0.20	3175 *	0.30
	3153	0.18	3117 *	0.27
	3114	0.10	3153 *	0.16
	3100 *	0.10	3130 *	0.14
	3111 *	0.07	3132 *	0.11
	3130 *	0.06	3184 *	0.08
			3111 *	0.08
			3079 *	0.06
			3084 *	0.06
			3114 *	0.06
			3097 *	0.04
			3146 *	0.04
Height	metre	0.99	metre	0.99
Cone rating	1	<0.001	1 *	0.15
	2	1.07	2	0.69
	3	2.95	3 *	1.69

Table 12 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Cone rating	4 *	3.39	4 *	0.54
	5	1.00 (base case)	5	1.00 (base case)
Gibberellins	Not used	1.00 (base case)	Not used	1.00 (base case)
	Used	0.709	Used *	0.49

Table 13: Relative risks, compared with the base case, for variables included in the Poisson regression model for Mt.Newton2 - 1993, adults and nymphs. Clone with a relative risk <0.1 not shown in table (18 and 18 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *. Variables with insufficient data denoted with ND.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3143	1.00 (base case)	3130 *	4.98
	3177	0.75	3143	1.00 (base case)
	3158	0.36	3084	0.74
	3154	0.24	3177	0.20
	3112	0.14	3093	0.00
	3079	0.14	3154	0.00
	3132	0.14	3112	0.00
	3140	0.14	3153	0.00
	3145	0.14	3176	0.00
	3156	0.14	3158	0.00
	3106	0.11	3102	0.00
	3109	0.10	3111	0.00
	3125	0.10	3141	0.00
	3129	0.10	3151	0.00
	3134	0.10	3142	0.00
	3146	0.10	3148	0.00
	3185	0.10	3183	0.00
	3188	0.10	3186	0.00
Cone rating	1	1.00	1	1.00
	2	1.73	2	0.43

Table 13 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Cone rating	3	ND	3 *	4.82
	4	0.48	4	32.06
	5	1.00 (base case)	5	1.00 (base case)
Gibberellins	Not used	1.00 (base case)	Not used	1.00 (base case)
	Used	2.07	Used	ND

Table 14: Relative risks, compared with the base case, for variables included in the Poisson regression model for Mt.Newton2 - 1994, adults and nymphs. Clone with a relative risk <0.01 not shown in table (10 and 10 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3079	1.00 (base case)	3079	1.00 (base case)
	3100	0.88	3100	0.76
	3150	0.76	3080 *	0.23
	3088	0.54	3088 *	0.21
	3154 *	0.36	3114 *	0.20
	3141 *	0.34	3133 *	0.18
	3175 *	0.29	3090 *	0.16
	3143 *	0.23	3150 *	0.15
	3133 *	0.18	3130 *	0.14
	3080 *	0.16	3225 *	0.12
	3151 *	0.15	3117 *	0.11
	3146 *	0.13	3146 *	0.07
	3148 *	0.13	3148 *	0.06
	3130 *	0.11	3112 *	0.05
	3117 *	0.11	3177 *	0.05
	3225 *	0.11	3093 *	0.04
	3131 *	0.10	3175 *	0.03
	3090 *	0.08	3154 *	0.03
	3093 *	0.06	3131 *	0.02
	3114 *	0.06	3141 *	0.01

Table 14 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3112 *	0.03	3143 *	0.01
	3177 *	0.02	3132	0.00
Cone rating	1	1.00	1	1.00
	2 *	3.60	2 *	5.27
	3 *	4.20	3 *	3.52
	4 *	4.73	4	1.37
	5	1.00 (base case)	5	1.00 (base case)
Gibberellins	Not used	1.00 (base case)	Not used	1.00 (base case)
	Used *	0.34	Used	1.25

Table 15: Relative risks, compared with base case, for variables included in the Poisson regression analysis for Kall - 1993, adults and nymphs. Clones with a relative risk of <0.01 not shown in table (39 and 43 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3161	2.98	3157	1.61
	3147	1.66	3075	1.23
	3134	1.53	3079	1.00 (base case)
	3053	1.27	3119	1.00
	3089	1.25	3134	0.95
	3107	1.23	3040	0.75
	3129	1.22	3080	0.68
	3065	1.16	3065	0.60
	3054	1.10	3034	0.53
	3079	1.00 (base case)	3105	0.41
	3119	1.00	3126	0.31
	3162	1.00	1460 *	0.28
	3126	0.71	3088 *	0.23
	3087	0.68	3121	0.13
	3140	0.64	3087 *	0.09
	3040	0.57	3051 *	0.09
	3050	0.55	3138	0.08
	3090	0.54	3078 *	0.06
	1460	0.54	3090 *	0.05
	1466	0.40		

Table 15 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3088	0.34		
	3110	0.34		
	3091	0.28		
Height	metre	1.01	metre	1.00
Cone rating	1	1.00 (base case)	1	1.00 (base case)
	2 *	5.69	2 *	6.00
	3 *	7.87	3 *	4.82
	4	<0.0001	4	12.77

Table 16: Relative risks, compared with base case, for variables included in the Poisson regression analysis for Kall - 1994, adults and nymphs. Clones with a relative risk of <0.45 not shown in table (30 and 33 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3087 *	5.17	3122 *	4.93
	3063 *	5.03	1536	2.85
	3064 *	3.61	3052	2.66
	3091 *	2.33	3119	2.49
	3088 *	2.27	3126	1.94
	3139	1.85	3040	1.71
	3045	1.35	3091	1.11
	3123	1.25	3048	1.00 (base case)
	3077	1.25	3149	0.98
	3052	1.08	1467	0.95
	3082	1.06	3050	0.94
	3048	1.00 (base case)	3088	0.91
	3050	0.96	3034	0.88
	1467	0.85	3139	0.83
	1460	0.81	3140	0.79
	3040	0.80	3051	0.77
	3162	0.79	3064	0.71
	3140	0.78	3129	0.69
	3148	0.76	1460	0.69
	3065	0.75	3063	0.68

Table 16 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3066	0.72	3138	0.68
	3119	0.72	3053	0.56
	3138	0.62	3079	0.54
	3058	0.53	3058	0.51
	3090	0.49	3092	0.45
	1466	0.49		
	3134	0.47		
	3044	0.46		
Height	metre	0.99	metre	1.01
Seed	number/half cone	0.93	number/half cone *	1.52

Table 17: Relative risks, compared with base case, for variables included in the Poisson regression analysis for Kall - 1995, adults and nymphs. Clones with a relative risk of <0.45 not shown in table (60 and 38 clones not shown, adults and nymphs, respectively). Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	3128	1.58	3123 *	24.62
	3043	1.46	3113 *	15.05
	3129	1.45	3062 *	9.30
	3054	1.27	3058 *	6.99
	3033	1.26	3048 *	6.58
	3066	1.00(base case)	3156 *	6.10
	3089	0.83	3122	4.17
	3155	0.76	3109	4.16
	3161	0.75	3155	3.83
	3153	0.66	3136	3.42
	3040	0.63	3057	3.15
	3105	0.49	3108	3.12
	3139	0.46	3126	3.08
	3136	0.45	1460	2.99
	3122	0.44	3153	2.83
	3134	0.43	3080	2.70
	3075	0.39	3140	2.48
	3106	0.38	3034	2.46
	3126	0.37	3127	2.32
	1536	0.36	3050	2.16

Table 17 (Continued)

	Adults		Nymphs	
Variable	Value	Relative risk	Value	Relative risk
Clone	3112	0.31	3075	2.05
	3140	0.30	3049	1.98
	1460 *	0.28	3091	1.85
	3064	0.22	3092	1.79
	3088	0.21	3089	1.71
	3090 *	0.20	3116	1.65
	3053	0.18	3053	1.58
	3032	0.16	3148	1.55
	3044	0.13	3063	1.54
	3157 *	0.11	3118	1.50
	1461 *	0.09	3110	1.49
	3078 *	0.08	3088	1.42
	3051 *	0.08	3112	1.24
	3092 *	0.07	3172	1.23
	3115 *	0.06	3105	1.18
	3087 *	0.03	3149	1.05
			3139	1.03
			3066	1.00 (base case)
			3098	0.99
			3040	0.89
			3161	0.87

Table 17 (Continued)

	Adults		Nymphs	
Variable	Value	Relative risk	Value	Relative risk
Clone			3147	0.85
			3051	0.79
			1466	0.78
			3032	0.70
			1461	0.60
			3107	0.58
			3064	0.57
			3042	0.55
			3078	0.43
			3138	0.42
			1467	0.37
			3157	0.33
			3090	0.27
			3054	0.21
			3115	0.20
			3045	0.19
			3044	0.18
Height	metre	1.00	metre *	1.02
Cone	number/tree *	1.01	number/tree *	1.00

Table 18: Relative risks, compared with base case, for variables included in the Poisson regression model for Kal 2 - 1993, adults. Clones with a relative risk <0.20 not shown in table (19 clones not shown). Nymphs not included as data (n=9) was insufficient to obtain valid results.

Variable	Value	Relative Risk
Clone	1512	2.26
	1513	1.20
	1528	1.04
	931	1.00 (base case)
	1536	0.87
	1507	0.83
	962	0.78
	1511	0.57
	1529	0.51
	1533	0.40
	1532	0.39
	1537	0.38
	1538	0.23
	1523	0.20
Height	metre	0.99
Yield	bushels	0.96
Seed	number/half cone	1.11
Cone rating	1	1.00 (base case)
	2	0.63
	3	1.46
	4	1.25

Table 19: Relative risks, compared with base case, for variables included in the Poisson regression model for Kal2 - 1994, adults and nymphs. Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	1526	1.54	1512 *	16.89
	1523	1.32	1511 *	5.99
	1531	1.28	1537 *	5.59
	1536	1.13	1536	5.53
	1518	1.03	1510	4.23
	1506	1.02	1513	3.83
	1519	1.01	1508	2.95
	1540	1.00 (base case)	1504	2.46
	1520	0.92	1526	2.21
	1537	0.91	1502	2.16
	1505	0.88	1505	1.98
	1507	0.83	1507	1.81
	1511	0.75	1538	1.37
	1510	0.75	1529	1.19
	931	0.72	1540	1.00 (base case)
	1538	0.66	1523	0.89
	1508	0.57	1506	0.31
	1532	0.55	1527	0.00
	1528	0.54	946	0.00
	933	0.51	1532	0.00
	1514	0.50	933	0.00
	1502	0.49	1533	0.00
	1529	0.47	1503	0.00

Table 19 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	1533	0.43	1520	0.00
	1504	0.38	1518	0.00
	1503	0.26	1531	0.00
	1512	0.00	1528	0.00
	1527	0.00	1514	0.00
	946	0.00	931	0.00
	1513	0.00	1519	0.00
Height	metre	1.00	metre	0.99
Yield	bushels	0.95	bushels *	1.58
Seed	number/half cone	1.24	number/half cone *	1.45

Table 20: Relative risks, compared with base case, for variables included in the Poisson regression model for Kal2 - 1995, adults and nymphs. Variables significant in the model at α=0.05 denoted with *.

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	1540	1.27	1511	1.94
	1529	1.16	1514	1.35
	1510	1.05	1518	1.13
	1507	1.03	1512	1.07
	946	1.00 (base case)	1510	1.02
	1532	0.87	946	1.00 (base case)
	962	0.74	1507	0.88
	1514 *	0.70	933	0.87
	1533 *	0.68	1540	0.85
	1537 *	0.67	1537	0.81
	1505 *	0.62	1519	0.72
	1531 *	0.56	1529	0.70
	1511 *	0.55	1523	0.67
	1502 *	0.53	1508	0.65
	1513 *	0.51	1532	0.64
	1536 *	0.45	1520	0.57
	1501 *	0.44	1528	0.41
	1524 *	0.41	1513	0.41
	1523 *	0.41	962	0.38
	1527 *	0.41	1502	0.33
	1512 *	0.40	1501	0.33
	939 *	0.36	1533	0.32

Table 20 (Continued)

	Adults		Nymphs	
Variable	Value	Relative Risk	Value	Relative Risk
Clone	1518 *	0.35	939	0.30
	1538 *	0.33	1538	0.30
	931 *	0.29	1524	0.27
	1506 *	0.28	1505 *	0.21
	1519 *	0.28	931 *	0.20
	1516 *	0.28	1536	0.17
	1534 *	0.27	1531	0.16
	1526 *	0.26	1539 *	0.12
	937 *	0.25	1534 *	0.09
	933 *	0.24	1526	0.00
	1503 *	0.19	1516	0.00
	1528 *	0.15	1503	0.00
	1504 *	0.05	1504	0.00
	1539 *	0.05	1506	0.00
Cone	number/tree *	1.00	number/ tree *	1.00

clones in an orchard. Unlike an analysis of variance which is unable to discern significant clones in a model this large, the Poisson regression can.

Tree height was found to be significant in some orchards and not significant in others. In all the adult models (Tables 10-20), tree height was not significant. Relative risks for these orchards was either 1.00 or 0.99 indicating that increasing the tree height would not result in an increase in *L. occidentalis* numbers. For nymphs, the results are contradictory. In only two orchards, Mt. Newton 1 - 1992 (Table 10) and Kal 1 - 1995 (Table 17), was tree height found to be significant. The relative risks (Mt. Newton 1 - 1992 =0.99; Kal 1 - 1995 = 1.02) indicate that in one case, tree height results in fewer *L. occidentalis* being expected on the tree and in the other case, more *L. occidentalis* are expected with an increasing tree height. As tree height was only significant in two of 11 analyses and the results are contradictory, the relationship between *L. occidentalis* and tree height is unclear. The data in Figures 8 and 9 (Chapter 2) indicate a relationship between *L. occidentalis* and tree height where greater numbers of *L. occidentalis* can be found on trees which are neither the tallest nor the shortest but of moderate height. A similar result is found with respect to cone density.

The relationship between *L. occidentalis* and cone density is also unclear. For adults, cone density was significant in six orchards: Mt. Newton 1 - 1994 (Table 11), Mt. Newton 2 - 1992 (Table 12) and 1994 (Table 14), Kal 1 - 1993 (Table 15) and 1995 (Table 17) and Kal 2 - 1995 (Table 20). In all cases, cone density values of 3 or 4 had the higher relative risk values, indicating that more bugs are expected to be found on trees with a moderate cone crop than on trees with the highest cone rating. For Kal 2, where actual cone numbers were obtained, the relative risk value is >1.0, indicating that as the number of cones increases, so does the probability of finding *L. occidentalis*. For nymphs, cone density was significant in seven orchards: Mt. Newton 1 - 1992 (Table 10), Mt. Newton 2 - 1992 (Table 12), 1993 (Table 13) and 1994 (Table 14), Kal 1 - 1993 (Table 15) and 1995 (Table 17), and Kal 2 - 1995 (Table 20). As was found for adults, cone density ratings of 2, 3 or 4 all had the largest relative risk values. In only one instance did the highest cone density rating also have the highest relative risk value (Kal 1 - 1993, Table 15). In this case, the base case was the lowest cone density rating (1) with all other ratings being compared to it. The highest cone density rating was not significant (P=0.09) which was due to high coefficient and standard error values, indicating a great deal of variation in the data. For Kal 1 - 1995 and Kal 2 - 1995, actual cone numbers were obtained and the relative risks (1.00 and 1.00, respectively) indicate that cone numbers contribute to the explanatory power of the model but not in a linear

fashion. If the relative risks had been greater than 1, the relationship would be linear as increasing the number of cones would increase the probability of finding *L. occidentalis*. As this was not the case, the relationship is significant but not obviously linear. For both adults and nymphs, the cone density results support my hypothesis, proposed in Chapter 2, that *L. occidentalis* is choosing trees with moderate instead of heavy cone crops. This may be due to an increased seed quality and/or seed size as more resources can be distributed to each cone if the numbers are reduced (Wheeler and Jech 1990). That *L. occidentalis* is selecting those trees with moderate cone crops may give some insight into the choosing of moderate sized trees. Moderate sized trees may be under less stress than smaller trees and not infested by other pests as taller trees are (Borden 1993). The lack of competition for food and the altered volatile profile may make moderate sized trees more attractive to *L. occidentalis*.

Gibberellins were only used in Mt. Newton 1 and Mt. Newton 2. The relationship between gibberellin use and incidence of *L. occidentalis* is unexpected. Use of gibberellins was significant in two of five orchards for adults (Mt. Newton 1 - 1994, Table 11 and Mt. Newton 2 - 1994, Table 14) and in one of five orchards for nymphs (Mt. Newton 2 - 1992, Table 12). In all three cases, the relative risk was less than one, indicating that *L. occidentalis* was less likely to be on gibberellin-induced

trees. This result is counter-intuitive as gibberellins are used in many orchards to increase cone production (Ross *et al.* 1983) as are many other induction methods (Ross and Pharis 1985, Wheeler *et al.* 1985, Turgeon *et al.* 1994). However, it may be possible that injection of gibberellins may alter the volatile profile being emitted from a tree, or, by increasing the number of cones significantly, decrease the quality and size of seed being produced or, the increased gibberellin content in the tissues may be detrimental when ingested (Rhoades 1983). Examining the other orchards, the relative risk was either very small, or larger than 1. In one case (Kal 2 - 1993, nymphs, Table 18), the relative risk could not be estimated with any confidence due to insufficient data. As the data set was not well balanced (*i.e.* growers used gibberellins on trees in a non-systematic fashion), and results are conflicting, significant results, while significant, are not enough to draw any conclusions.

For Kal 1 and Kal 2, a few other variables were included in the model. Half-cone seed counts and yield data were found to be significant in some years. Seed count was significant for nymphs in both orchards (Kal 1 - 1994, Table 16 and Kal 2 - 1995, Table 20) which had seed counts included in the model. Relative risks in both cases were high, 1.52 and 1.48, Kal 1 and Kal 2, respectively, indicating that as the number of sound seed per cone increased, the probability of finding *L. occidentalis* on these trees increased as well. Seed count was not

significant for adult. Seed count is a measure of the quality of the cone crop as indicated by the number of sound seed. It would be advantageous for *L. occidentalis* to select those trees with a higher number of sound seed per cone to maximize their feeding efforts. *Leptoglossus occidentalis* invests a significant amount of time extracting the seed contents. Choosing trees with many filled seeds per cone reduces the search time between seeds. As results from nymphs was significant, it suggests that the females were choosing trees for oviposition which had high food resources available for their offspring. Adults not being significant could indicate the effect of male host selection which was not separated out during the data collection process.

Yield is another indicator of food resources. Yield data was available for only two orchards, Kal 2 - 1993 and 1994, Tables 18 and 19, respectively. The results from Kal 2 - 1993 are for adults only as the number of nymphs present in that orchard for that year was nine and not enough to obtain any valid statistics. Yield was not significant for adults in Kal 2 for either year. However, in 1994, yield was significant for nymphs, with a relative risk of 1.58. While adult male *L. occidentalis* may be compounding the effects of the adults, it appears that the females may be selecting trees for oviposition which have adequate food resources available. In Kalamalka orchards, not all trees are harvested. Only those trees with a half-cone seed count of 1 or greater are

considered harvestable. All other trees are harvested but only to have the cones destroyed so as to avoid contaminating the orchard with pests in the successive years. The effect of yield is tempered by the data collection method. It is unknown if the largest yields were from those trees with the highest number of cones. Trees bearing larger cone crops seem to have larger numbers of empty seed due to lack of pollination. Assuming that the majority of trees bearing higher cone numbers are not included in the yield data, the relationship between *L. occidentalis* and yield is consistent with *L. occidentalis* choosing trees with a moderate cone crop.

Predicted mean number of insects compared very well with the actual means obtained from the field for all years for both adults and nymphs in all orchards surveyed (Figures 12-15). As no statistic can be generated to describe the goodness of this fit, this is only a graphical aid. The similarity between the two curves indicates that the Poisson regression generates coefficient values which are accurate enough to reproduce, within the same year, the distribution of insects among the clones. These means were generated using the coefficients from a model which included both clone and tree characteristics. I also generated means using the coefficients from the clone-only and the tree-characteristics-only models. The clone-only model coefficients generated mean values which were equally as good as the whole model coefficients.

Figure 12: Estimated and actual mean numbers of *L. occidentalis* per tree for Mt. Newton 1 - 1992 and 1994, adults and nymphs. For clarity, not all clones included in the analysis are shown. Number of clones not shown for each year and life stage in brackets: 1992 - adults (26) and nymphs (26); 1994 - adults (19) and nymphs (22). Base case indicated by **.

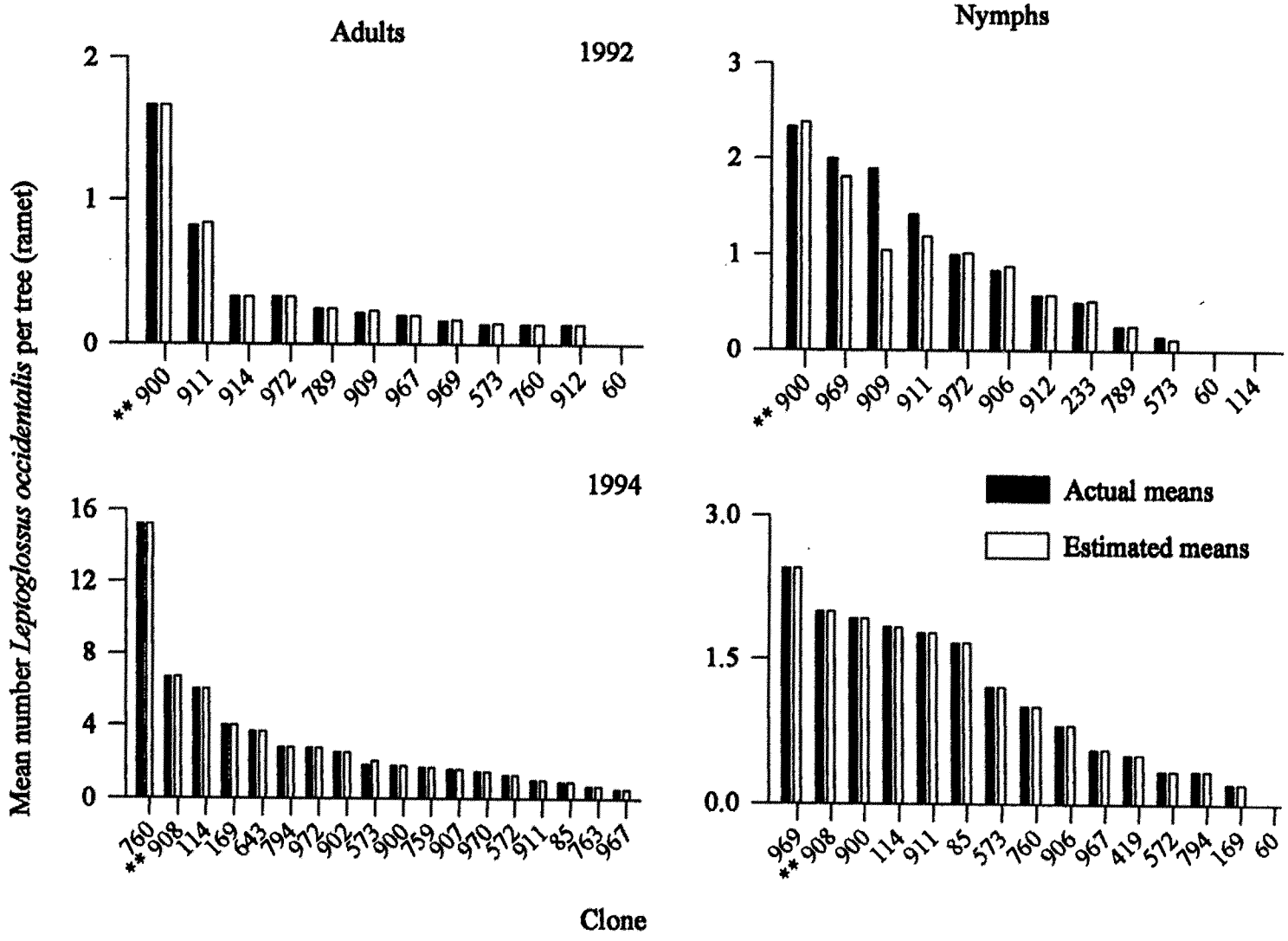

Adults
1992
Nymphs
1994
Actual means
Estimated means
Mean number Leptoglossus occidentalis per tree (ramet)
Clone
** 900 911 914 972 789 909 967 969 573 760 912 60
** 900 969 909 911 972 906 912 233 789 573 60 114
760 ** 908 114 169 643 794 972 902 573 900 759 907 970 572 911 85 763 967
969 ** 908 900 114 911 85 573 760 906 967 419 572 794 169 60

Figure 13: Estimated and actual mean numbers of *L. occidentalis* per tree for Mt. Newton 2 - 1992, 1993 and 1994, adults and nymphs. For clarity, not all clones included in the analysis are shown. Number of clones not shown for each year and life stage in brackets: 1992 - adults (14) and nymphs (14); 1993 - adults (34) and nymphs (34); 1994 - adults (20) and nymphs (20). Base case indicated by **.

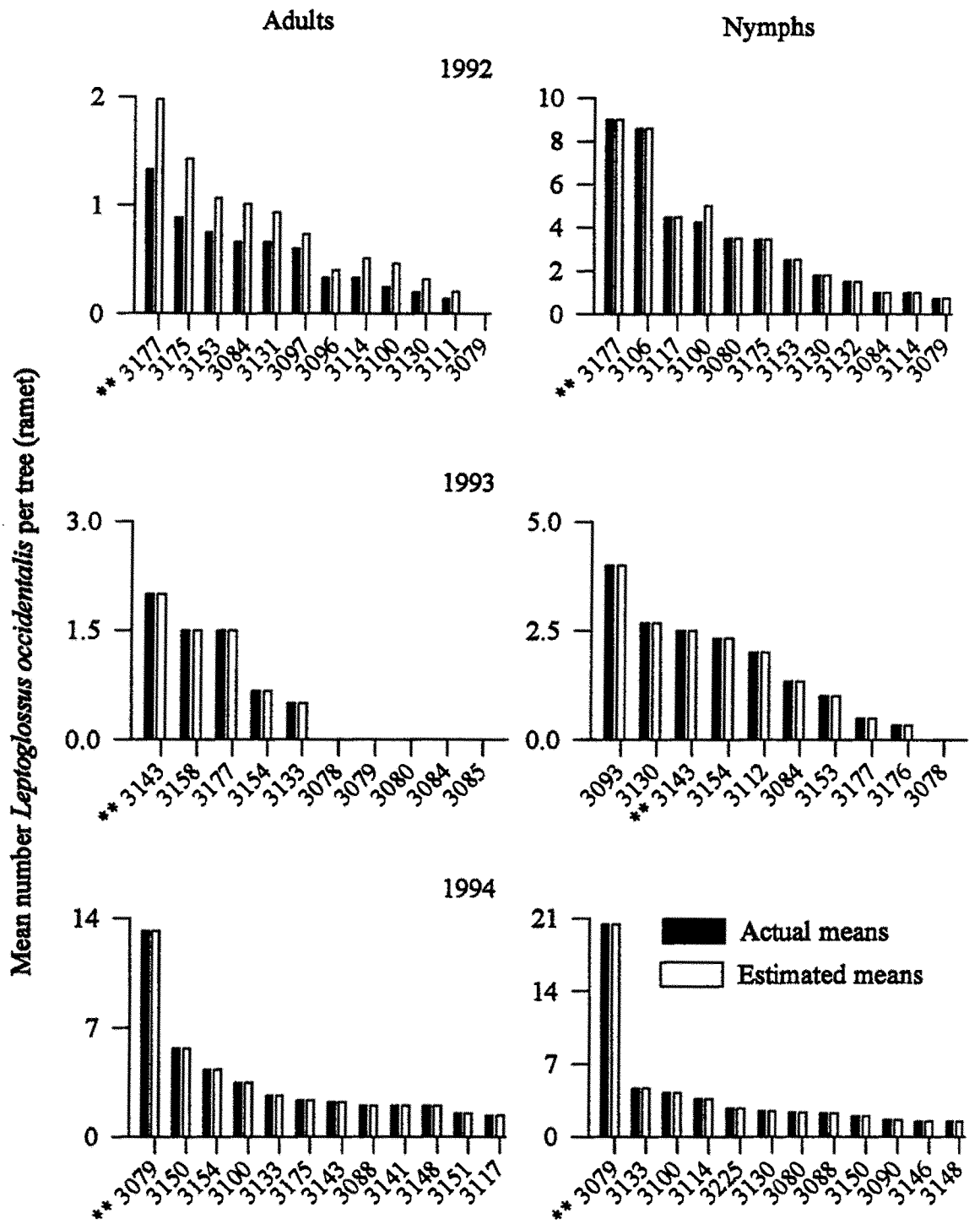

Adults
Nymphs
1992
2
1
0
** 3177 3175 3153 3084 3131 3097 3096 3114 3100 3130 3111 3079
10
8
6
4
2
0
** 3177 3106 3117 3100 3080 3175 3153 3130 3132 3084 3114 3079
1993
3.0
1.5
0.0
** 3143 3158 3177 3154 3133 3078 3079 3080 3084 3085
5.0
2.5
0.0
3093 3130 ** 3143 3154 3112 3084 3153 3177 3176 3078
1994
14
7
0
** 3079 3150 3154 3100 3133 3175 3143 3088 3141 3148 3151 3117
21
14
7
0
** 3079 3133 3100 3114 3225 3130 3080 3088 3150 3090 3146 3148
Actual means
Estimated means
Mean number Leptoglossus occidentalis per tree (ramet)
Clone

Figure 14: Estimated and actual mean numbers of *L. occidentalis* per tree for Kal 1 - 1993, 1994 and 1995, adults and nymphs. For clarity, not all clones included in the analysis are shown. Number of clones not shown for each year and life stage in brackets: 1993 - adults (50) and nymphs (50); 1994 - adults (46) and nymphs (46); 1995 - adults (83) and nymphs (83). Base case indicated by **.

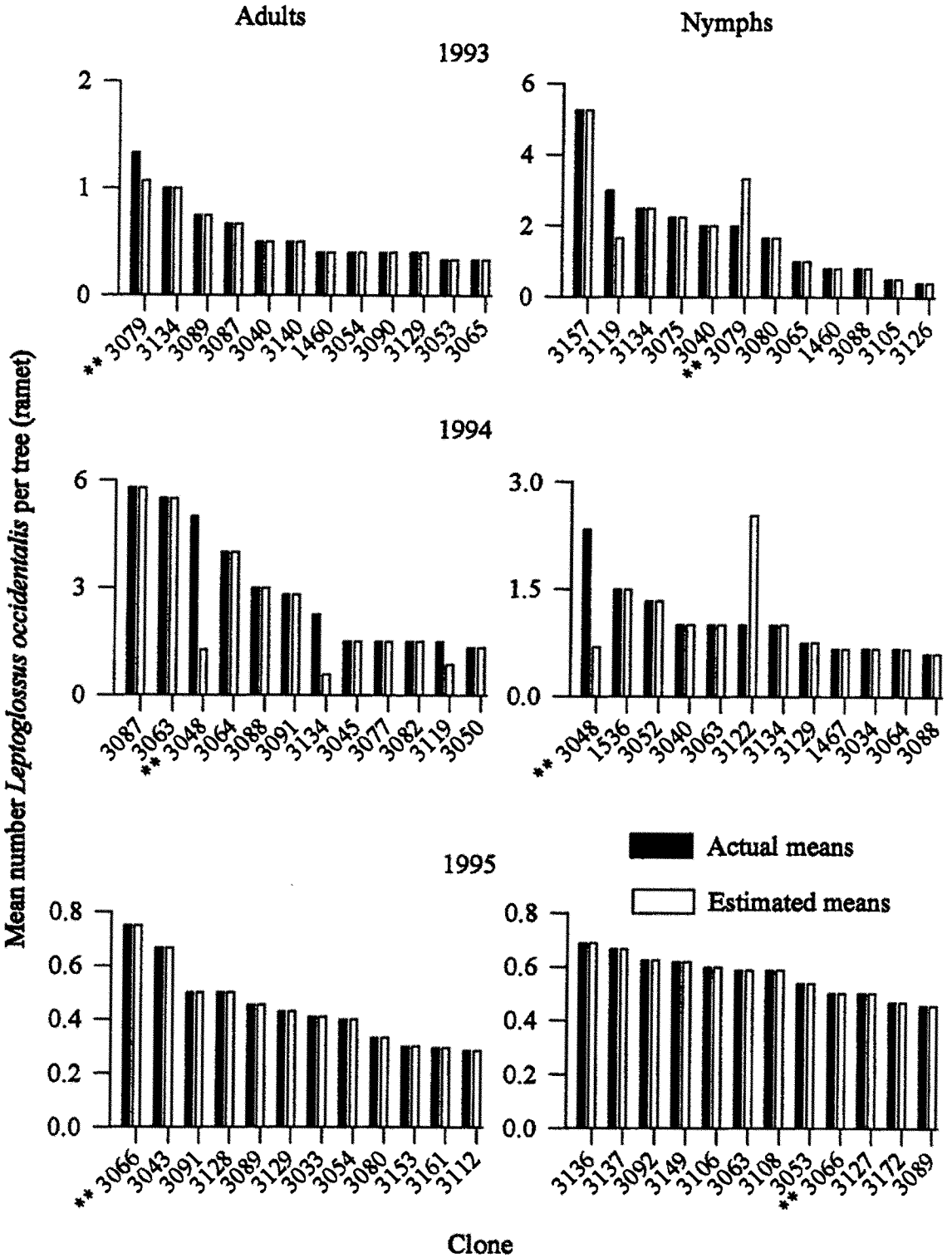
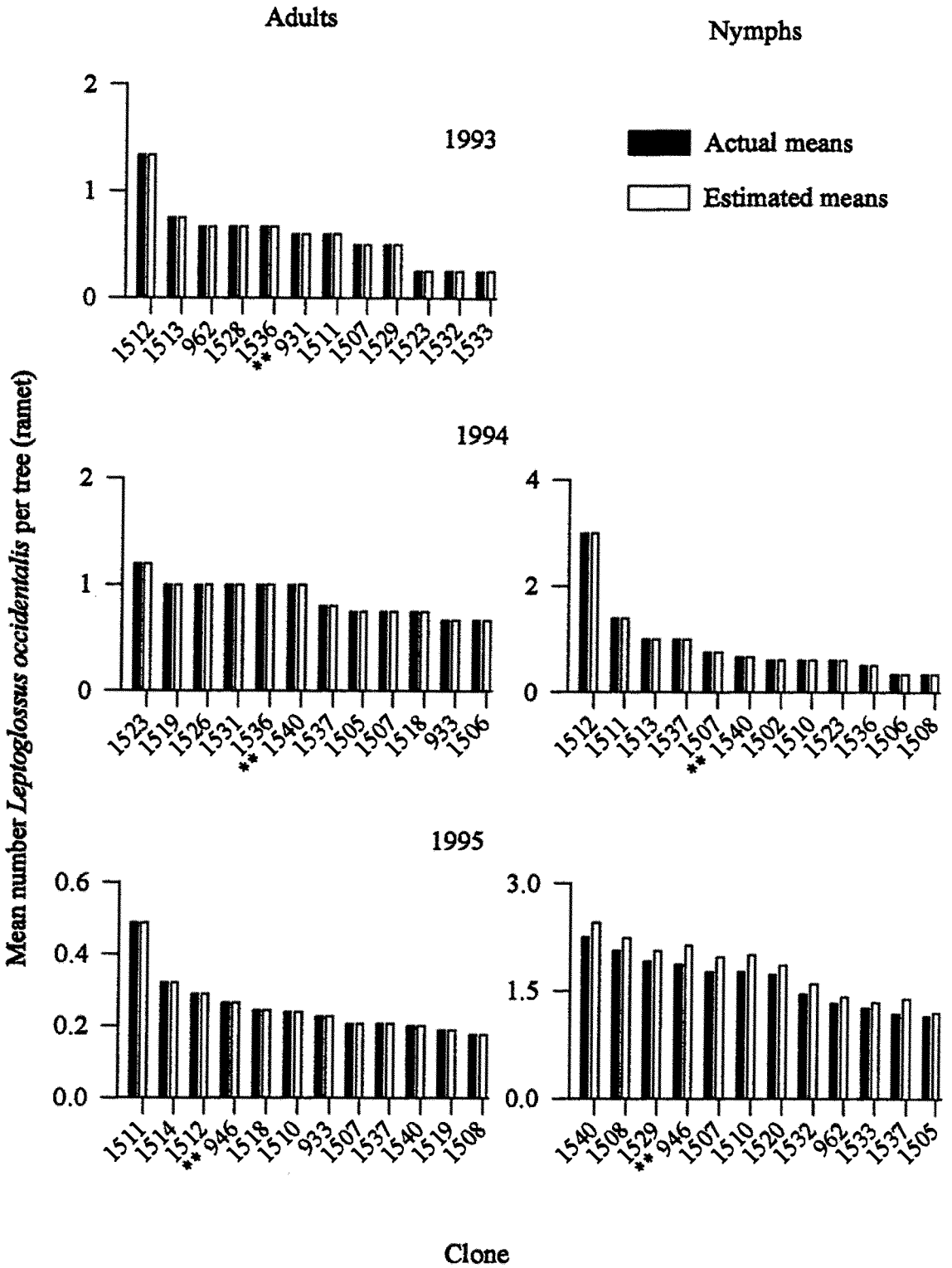

Adults
Nymphs
1993
1994
1995
Mean number *Leptoglossus occidentalis* per tree (ramet)
Actual means
Estimated means
Clone
** 3079 3134 3089 3087 3040 3140 1460 3054 3090 3129 3053 3065
3157 3119 3134 3075 3040 ** 3079 3080 3065 1460 3088 3105 3126
3087 3063 ** 3048 3064 3088 3091 3134 3045 3077 3082 3119 3050
** 3048 1536 3052 3040 3063 3122 3134 3129 1467 3034 3064 3088
** 3066 3043 3091 3128 3089 3129 3033 3054 3080 3153 3161 3112
3136 3137 3092 3149 3106 3063 3108 3053 ** 3066 3127 3172 3089

Figure 15: Estimated and actual mean numbers of *L. occidentalis* per tree for Kal 2 - 1993, 1994 and 1995, adults and nymphs. Data for nymphs in 1993 was not sufficient to obtain valid results and is not included. For clarity, not all clones included in the analysis are shown. Number of clones not shown for each year and life stage in brackets: 1993 - adults (21); 1994 - adults (18) and nymphs (18); 1995 - adults (26) and nymphs (26). Base case indicated by **.


Adults
Nymphs
Actual means
Estimated means
1993
1994
1995
Mean number Leptoglossus occidentalis per tree (ramet)
Clone
2
1
0
1512 1513 962 1528 1536 ** 931 1511 1507 1529 1523 1532 1533
1523 1519 1526 1531 1536 ** 1540 1537 1505 1507 1518 933 1506
4
2
0
1512 1511 1513 1537 1507 ** 1540 1502 1510 1523 1536 1506 1508
0.6
0.4
0.2
0.0
1511 1514 1512 ** 946 1518 1510 933 1507 1537 1540 1519 1508
3.0
1.5
0.0
1540 1508 1529 ** 946 1507 1510 1520 1532 962 1533 1537 1505

I did not present these graphs as only one set is required to get the point across. Means calculated from the tree-characteristics-only model were not similar to the actual mean values. These values were so poor that trees not harboring any *L. occidentalis* in reality were estimated as having bugs. The Log-Likelihood Ratio test (Tables 8, 9) indicate that tree characteristics are important to include in the analysis; however, that the coefficients from the tree-characteristics-only model does not accurately predict the actual insect values suggests that the clone variable is more significant to the model than the tree characteristics.

It could be argued that simply because there are a large number of clones being included in the model, that clone is a better predictor of the actual numbers should come as no surprise. To answer this argument, I chose two data sets and modified them in the following manner: the maximum number of clones could be no greater than ten which was twice the number of tree characteristics being included and the minimum number of trees per clone had to be 5. This resulted in a compact data set which was then analyzed like all the others. Coefficients were used to determine mean values and the results were compared for each model: unrestricted (whole model with clone and tree characteristics), clone only and tree characteristics only. The results from this experiment were no different than if the entire data set had been used. Estimated means from the whole model and clone-only

model were similar to the actual means, while those from the tree-characteristics-model were not. It appears that even with a smaller data set where the number of variables is reduced, the results are the same.

To see if the coefficients had any predictive power, I used coefficient values generated in one year to predict mean numbers of *L. occidentalis* for the next year. The results were very poor, indicating that the results from the Poisson regression analysis have no predictive power.

Unlike the analysis of variance, which was unable to integrate the many variables together into a single analysis without distorting the data itself, the Poisson regression was able to evaluate and generate estimations of the importance of each variable within a single model. This method will be useful for many other insect species which utilize a similar host selection behavior, such as the seed chalcid, *Megastigmus spermotrophus.* Being able to incorporate many different kinds of variables into a single model which theoretically fits the biology of the insect lends more credibility to the analysis being done and to the conclusions being generated. While some relationships, such as *L. occidentalis* numbers and tree height, will continue to be unclear it is with more certainty that I can conclude that for *L. occidentalis*, the single most important tree characteristic in its host selection criterion is clone.

As disclosed in Chapter 2 and further verified in Chapter 4, physical characteristics of the host do not fully explain the selection of preferred clones by *L. occidentalis*. Use of olfactory stimuli during host selection has been shown in particular among insects in these orders: 1) Lepidoptera [eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Städler 1974); pink bollworm, *Pectinophora gossypiella* (Saunders) (Wiessenborn and Baker 1990); black army cutworm, *Agrotis ipsilon* (Hufnagel) (Zhu *et al.* 1993); navel orangeworm, *Amyleois transitella* (Walker) (Phelan *et al.* 1991); corn earworm, *Heliothis virescens* (F.) and *H. subflexa* Gn. (Tingle *et al.* 1990, Mitchell *et al.* 1991); and the tortricid, *Lobesia botrana* Den. Et Schiff. (Gabel *et al.* 1992)], 2) Coleoptera [Douglas fir beetle, *Dendroctonus pseudotsugae* Hopkins (Rudinsky 1966), mountain pine beetle, *D. ponderosae* Hopkins (Hynum and Berryman 1980) and western pine beetle, *D. brevicomis* LeConte (Moeck *et al.* 1981); saw-toothed grain beetle, *Oryza%philus surinam%nsis* (L.) (White 1987); and driedfruit beetle, *Carpophilus* spp. (Blumberg *et al.* 1993)], and 3) Diptera [onion maggot, *Delia antiqua* J. (Harris and Miller 1991) and the cabbage maggot, *Erioischia brassicae* (Brouch%) (Hawkes and Coaker 1976)]. Herbivores are known to discriminate between suitable and unsuitable hosts based on smell, taste and, to a lesser degree, visual characteristics (Rhoades 1983). I hypothesize that attractive host kairomones associated with developing

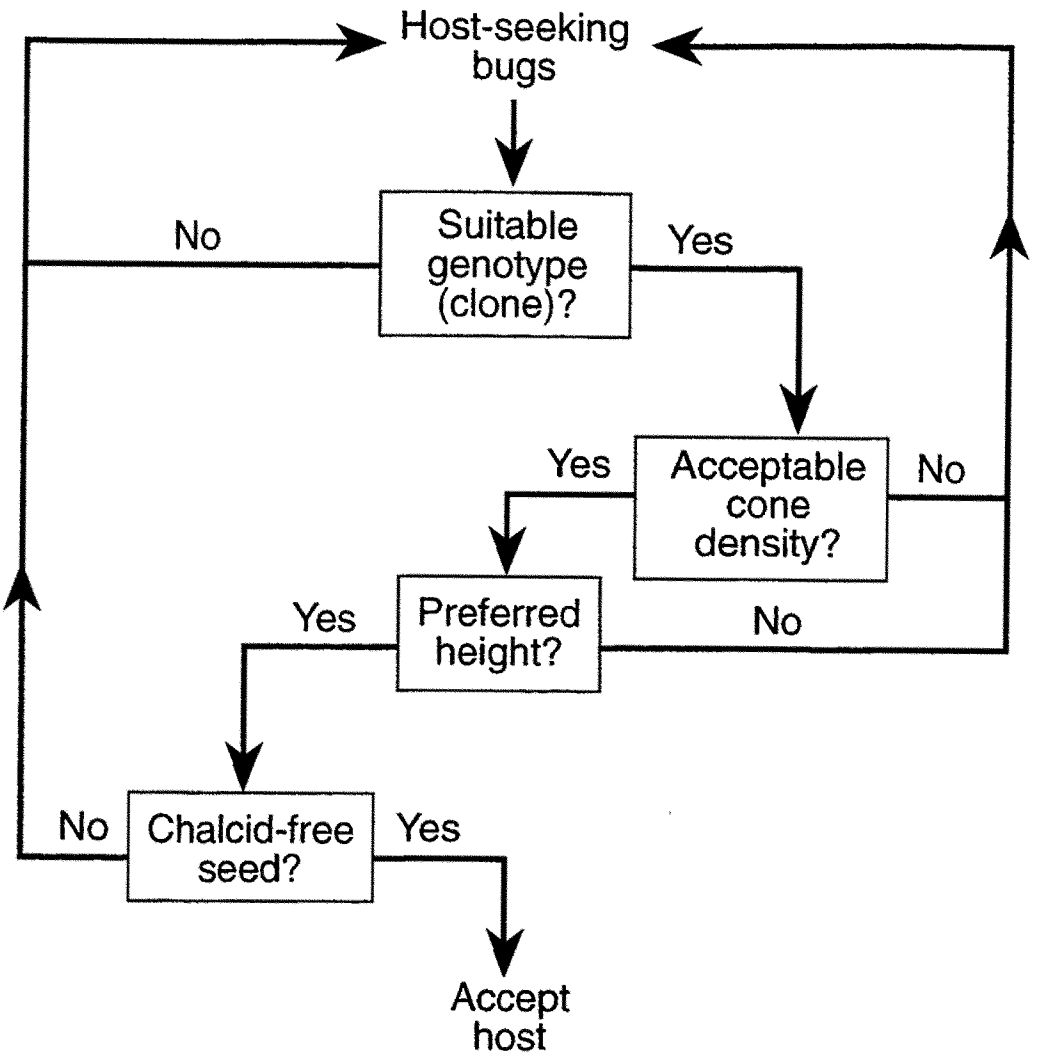
female strobili could be used in host selection by *L. occidentalis* and could account for discrimination between host clones. Attempts to verify this hypothesis are presented in Appendix 1.

Chapter 5

A Hypothetical Decision Tree Model for Host Selection by *Leptoglossus occidentalis*

My results indicate that host selection by female *L. occidentalis* can now be considered as a multi-step process, involving pre- and post-alighting assessment of potential hosts for four key characteristics: clone, cone density, height, and chalcid infestation. Host selection based on these characteristics, ranked in probable temporal order, as well as adaptive level of importance, can be conceptualized in a hypothetical decision tree (Fig. 16), similar to that proposed by Borden (1996) for bark beetles. This particular model is based on orchard research only and limited experimentation. A more complex and possibly different model would result when additional factors are considered and included, particularly with regards to natural stands. Of the three postulated pre-alighting criteria, clone is the most important factor (Chapters 1, 4), because trees in preferred clones are selected over others that apparently have suitable cone densities and height. Possibly preferred clones provide a food source that is low in secondary metabolites (Rosenthal and Janzen 1979, Berenbaum and Seigler 1992, Hobson 1995) that could be metabolic inhibitors or toxins for the seed bugs. Selecting trees with moderate cone density (Figs. 6, 7) will ensure a stable supply of

Figure 16: Hypothetical decision tree detailing four key choices made by *L. occidentalis* adults leading to the acceptance of a suitable host.


Host-seeking bugs
Suitable genotype (clone)?
No
Yes
Acceptable cone density?
Yes
No
Preferred height?
Yes
No
Chalcid-free seed?
No
Yes
Accept host

cones with substantial seeds. Given that trees of appropriate genotype and adequate cone density are found, selecting moderate-sized trees within that cohort (Figs. 8, 9) would increase the available food supply, and might also provide extra exposure that would promote rapid development. Finally, post-alighting selection of trees or cones without seed chalcid infestation (Tables 3, 4) would provide a food source compatible with the digestive capacity of the seed bug.

At any of the four critical evaluations (Fig. 16), an adult may elect to resume host seeking behavior and begin searching again. While four decisions that any adult female *L. occidentalis* must make are captured in the decision tree, a searching female might be expected to expend variable time and energy in the search, depending on its metabolic reserves. As postulated for bark beetles (Atkins 1969) and aphids (Kennedy and Booth 1963) a female with sufficient lipid reserves could continue searching in flight until the best host is located while an individual with fewer reserves could not. As well, a female bearing a full egg load might select a sub-optimal host which is within the gradient of acceptability (where < 100% of the offspring survive), while a female that has expended most of her egg load might be quite discriminating in selecting a host for the remainder of her egg load.

Part II: Chemical Ecology

Chapter 6
Alarm Pheromone

Introduction

Defensive secretions in some Homoptera and Heteroptera have been well documented. The secretions from: aphids (Wohlers 1981, Dawson *et al.* 1987); coreids, *Hotea gambiae* (Westwood) (Hamilton *et al.* 1985), *Leptoglossus zonatus* (Dallas) (Leal *et al.* 1993); alydids, *Megalotomus quinquespinosus* (Say), *Alydus eurinus* (Say) and *Alydus pilosulus* Herrich-Schaeffer (Oetting and Yonke 1978); and pentatomids, *Eurydema rugosa* Motschulsky (Ishiwatari 1974) and *Eurydema pulchra* Motschulsky (Ishiwatari 1976), *Nezara viridula* (L.) (Lockwood and Story 1987) and *Erthesina fullo* Thunberg (Kou *et al.* 1989) have been characterized as eliciting alarm behavior among conspecifics.

These species share common characteristics: 1) they are easily disturbed and readily emit their offensive odor, 2) they form aggregations, and 3) most possess hexanal as a component of their defensive secretion. Only one *Leptoglossus* sp. has had its alarm pheromone system

characterized (Leal *et al.* 1993). Given the variety of habitats and plant species used by this genus, and allopatric ranges of many species, I hypothesized that the alarm pheromone system would be fairly similar for related species. The propensity toward gregariousness may be a prerequisite to the evolution of alarm pheromones (Nault and Phelan 1984). Individuals in aggregations can benefit by perceiving volatile secretions used by other members of a group to deter predation, and can disperse to avoid being targets for predation.

Observations of the western conifer seed bug, *Leptoglossus occidentalis*, in the field and in the laboratory revealed that throughout the summer, groups of adults and nymphs were easily disturbed and emit an offensive odor. As hypothesized for other hemiptera, this scent apparently elicited dispersal behavior in adults and nymphs.

My objectives were: to capture, isolate, identify and bioassay the alarm pheromone of adults and nymphs of *L. occidentalis*.

Materials and Methods

Insects

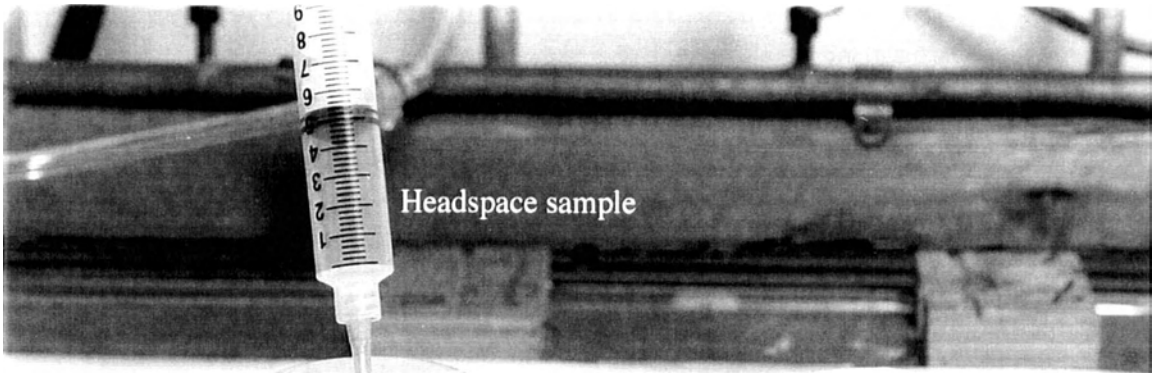
Adult males, females and nymphs of *L. occidentalis* for chemical analysis and most bioassay experiments were obtained from a laboratory colony, maintained at a 15:9 h L:D photoperiod regime, 32 and 20°C

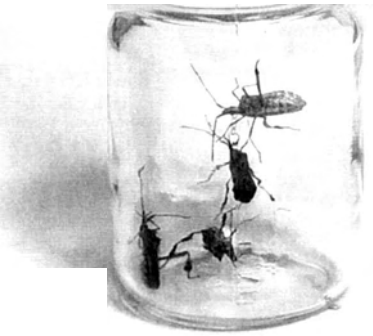
peak photophase and scotophase temperatures, respectively, and ~70% R.H. The colony was established in 1992 and revitalized using field collected bugs each summer. Adults were segregated by sex for all but one phase of the study but nymphs were not. One set of bioassay experiments was done with bugs collected in the field from Skimikin Seed Orchards, B.C. Forest Service, Tappen, B.C. and Kalamalka Seed Orchards, Kalamalka, B.C. in mid-September 1994.

Headspace bioassay for adult and nymphal volatiles.

Glass jars (130 ml) were washed and air dried prior to use and between replicates. Two sets each of five males or females were placed in separate jars, covered with Parafilm. One set of insects, designated 'non-agitated', were not disturbed. The jar containing the second set of 'agitated' insects was roughly handled and shaken until the putative alarm pheromone could be detected by the human nose. For bioassays (Fig. 17), a single non-agitated test insect was isolated in a Parafilm-covered jar and allowed to settle. Using a disposable syringe, 10 cc of headspace air, approx. 0.4 bug equivalents, was drawn from the jar containing the set of five non-agitated insects and injected into the jar containing the test insect, which was then observed for any change in behavior. In both laboratory and field bioassays, one bug equivalent is equal to the amount of volatiles given off by one agitated bug as determined by volatile capture. The test insects were then treated with

Figure 17: Apparatus used in headspace bioassays testing for response by adult and nymphs *L. occidentalis* to headspace samples taken from agitated bugs.





Agitated insects

Test insect

either 10 cc of air drawn from the headspace air in the jar containing the non-agitated insects (control) or the agitated insects (treatment) and their behavior was observed. In this way, any insect responding to air from non-agitated insects could be removed from the study group. An alarm response was recorded if the test insect exhibited agitated behavior, i.e. a sudden, rapid increase in movement or attempted flight. No change in behavior was deemed a negative response. Fifteen individuals (replicates) of females, males and nymphs were tested. Cross-over experiments were then conducted using the same bioassay and 15 replicates obtained for all combinations of adults exposed to volatiles from nymphs and nymphs exposed to volatiles from male or female adults. All results were analyzed using χ^2 analysis and Fisher's Exact test (Zar 1984).

Analysis of Volatiles

Volatiles from live adults or nymphs were collected on Porapak Q (Waters Associates Inc., Milford, Massachusetts). Twenty-five groups of 10 agitated males or females and five groups of 20 nymphs were placed in a glass chamber (6.5 cm high, radius 4.7 cm). Bugs were then agitated using a glass rod until alarm pheromone was detected by the human nose. Air was drawn by a water aspirator through a charcoal scrubber and over the insects for 10 min at 1.65 l/min. Volatiles were collected in a glass trap (6 mm O.D. x 30 mm) filled with Porapak Q (50-

80 mesh), extracted by eluting with 2-3 ml of double-distilled pentane and then concentrated under a nitrogen stream to 1 ml. A 1 μl sample of the extract was analyzed on a Hewlett Packard, 5830A gas chromatograph (GC) with a SP1000 column (30 m x 0.32 mm ID) (Supelco, Oakville, Ontario) and flame ionization detector, and on a Hewlett Packard 5890 gas chromatograph with a fused silica coated DB-5 column (30 m x 0.25 mm ID) (J&W Scientific, Folsom, California).

Volatiles were then analyzed by coupled gas chromatographic electroantennographic detection (GC-EAD) (*frn et al.* 1975, Gries *et al.* 1993), employing a Hewlett Packard 5890A gas chromatograph and a custom built amplifier with a passive low-pass filter and a cutoff frequency of 10 kHz. Antennae were gently pulled out of the insect's head; the exposed nerve endings were suspended in a saline solution which contained the indifferent electrode and the distal end of the antennae was pierced with a recording electrode. Antennally active compounds were analyzed by coupled GC-mass spectroscopy (MS) using a Hewlett Packard 5985B GC equipped with a fused silica (30 m x 0.25 mm ID) DB-5 column in full-scan and selection ion monitoring (SIM) mode. Compounds were identified by comparison with published spectra (Jennings and Shibamoto 1980) and identification was verified by GC using authentic standards. Quantities of components present in the

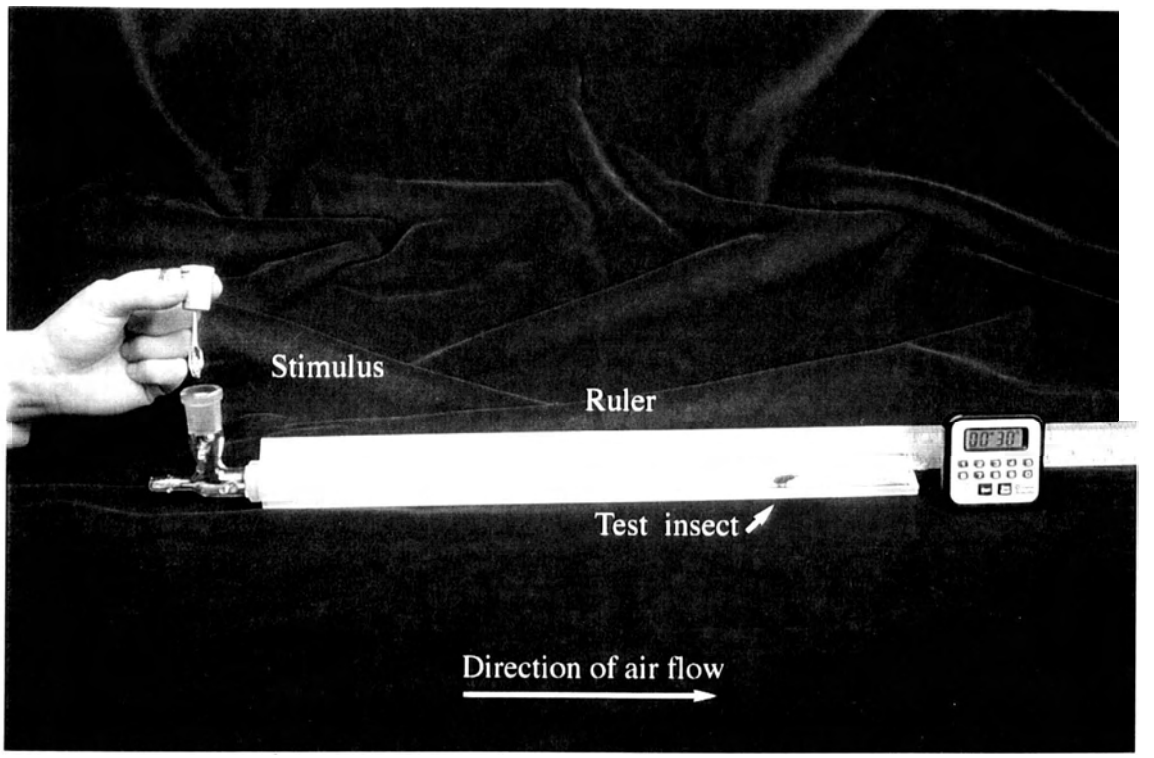
extracts were calculated by comparing area counts with those obtained from external standards of known concentration.

The contents of the metathoracic glands and reservoir from 20 adult males and 20 females and the contents of the dorsal abdominal glands of 51 third and fourth instar nymphs were also analyzed. Source insects were collected from the colony and held at -15°C for 20 min to facilitate handling and inhibit emission of pheromone. Insects were processed in batches of 5-10. The thoraxes were excised, immersed in pentane chilled on dry ice, and pulverized with a glass rod. The extract was transferred to a volumetric vial, concentrated to 300 μl with a forced nitrogen stream and placed in a glass tube containing glass wool attached to a Porapak Q trap (as described above). Volatiles were blown onto the trap by nitrogen gas for approximately 2 hr. Traps were then eluted with 1 ml of double-distilled pentane, and the extract was analyzed by GC and GC-MS and the amounts of components calculated as above.

Bioassay of extracted and synthetic volatiles.

A one-way forced air bioassay was developed to quantify the response of adult bugs to single and blended defensive secretion volatiles (Fig. 18). A 16/26 ground glass joint was welded to a glass tube resulting in a straight tube 48 cm long which was strapped horizontally

Figure 18: Apparatus used in forced air bioassays testing for response by adult *L. occidentalis* to synthetic alarm pheromone candidates.


Stimulus
Ruler
Test insect
Direction of air flow

to a board to prevent it from rolling. The ground glass joint fitted to a glass tube, 7 cm long, with a vertical open port, 3 cm long, which served as a receptacle for volatile stimuli. A ruler was taped to the board with 0 cm positioned at the end of the ground glass joint. An adult was placed in the tube, near the 0 cm mark, and its original position recorded. The component to be tested was applied to a glass ampoule, held by a cork, and inserted into the vertical port apparatus (Fig. 18). Room air, humidified by passing through water, was blown at 1.5 l/min into the horizontal tube, over the volatile stimulus and the test insect and then out through the open end of the tube.

All stimuli were evaluated at one bug equivalent diluted in 2 μl of pentane. Stimuli tested were: an extract from excised adult thoraxes, each of five antennally-active compounds found in the adult thoraxes, a five-component synthetic blend, and synthetic nymphal alarm pheromone. Each insect had 30 sec to respond to the stimulus after which its final position was recorded. Ten males and 10 females from the colony were tested in the spring and 10 field-collected individuals of each sex were tested in the fall. Ten control insects in each category were tested for their response to untreated air. Colony-reared 'summer' females and males did not differ in their response to any of the stimuli ($F=0.02$, $P=0.89$) and were pooled for analysis. Mean distances moved in

response to experimental stimuli were compared with distances moved in untreated air control tests by means of Dunnett's one-way test at α=0.05.

Field Bioassays.

Adults and nymphs resting and feeding on cone clusters of western white pine, *Pinus monticola* Dougl. ex. D. Don, were located at Skimikin Seed Orchards. They were counted and their position identified using plastic flagging. Using a small (150 mL volume) water atomizer, cone clusters were sprayed with a 4 mL pentane spray containing two or four bug equivalents of synthetic alarm pheromone (adult, nymph or a mixture of both). The four bug equivalent treatment of nymphal pheromone was lost by spillage in the field. Six clone clusters were sprayed for each treatment. Numbers of adults which responded by flying away from the cones, and numbers of nymphs responding by either dropping or walking away from the cones were recorded. Only six adults were found on treated cones. The numbers of nymphs were pooled for each treatment (heterogeneity chi-square values ranged from 3.14 to 14.82, *P* values ranged from 0.17 to 0.98), and percentage responses compared with the response to control sprays (pentane only) using a test of difference between proportions with Z scores as described by Zar (1984).

Due to the low number of adults in the field bioassay, a laboratory experiment for adults was designed. Potted Douglas-fir seedlings (approx. 25 cm high) were placed individually in a large mesh screened

cage measuring 76 x 61 x 36 cm. Ten adults (mixed sex) were placed on the seedling, allowed to settle, and then sprayed as above with two or four bug equivalents of synthetic (adult, nymph or a mixture) alarm pheromone. Ten replicates of 10 bugs each were tested for each treatment. Numbers of adults which left the seedling by flying and numbers which displayed agitated behavior were recorded. Mean percentage responses to experimental stimuli were compared with those to pentane control treatments using Dunnett's one-way test (α=0.05) following transformation using arcsin$\sqrt{x}$ to stabilize the variances.

Results and Discussion

Headspace bioassays.

Males, females and nymphs all showed a significant positive alarm response to the headspace volatiles from agitated males, females and nymphs (and all combinations thereof) (Table 21). Males and females did not differ significantly in their responses to volatiles from their own or from the opposite sex, χ^2=0.36, *P*=0.54. Volatiles from non-agitated individuals elicited little or no response.

Table 21: Alarm behavior responses of adult and nymphal *L. occidentalis* in headspace bioassay to headspace volatiles of agitated bugs and non-agitated (control) bugs. N=15 bugs per test.

Test Insect	Source of volatile stimulus	Percent positive response	χ^2 probability experimental *vs.* control
Females	Agitated females	100	
	Non-agitated females	0	0.001
Females	Agitated males	100	
	Non-agitated males	0	0.001
Males	Agitated females	87	
	Non-agitated females	0	0.001
Males	Agitated males	80	
	Non-agitated males	0	0.001
Females	Agitated nymphs	87	
	Non-agitated nymphs	13	0.001
Males	Agitated nymphs	93	
	Non-agitated nymphs	0	0.001
Nymphs	Agitated females	80	
	Non-agitated females	7	0.001
Nymphs	Agitated males	87	
	Non-agitated males	7	0.001
Nymphs	Agitated nymphs	93	
	Non-agitated nymphs	0	0.001

Analysis of volatiles.

GC, GC-MS, and GC-EAD analyses revealed that the antennally active volatiles emitted from agitated adults and contained within the metathoracic gland and reservoir were hexanal, hexyl acetate, hexanol, heptyl acetate and octyl acetate (Fig. 19). The single antennally active volatile emitted by agitated nymphs was identified as (*E*)-2-hexenal (Fig.20); which is also produced by other hemiptera nymphs (Lockwood and Story 1987). Male and female adults produced the same components in approximately the same quantity and ratio. Except for heptyl acetate and octyl acetate, these chemicals are also found in three other *Leptoglossus* species: *L. zonatus* (Dallas) (Leal *et al.* 1993), *L. oppositus* (Say) and *L. clypealis* Heidemann (Aldrich and Yonke 1975). *Leptoglossus zonatus* also produces hexanoic acid in small quantities (Leal *et al.* 1993); both *L. oppositus* and *L. clypealis* produce acetic acid (McCullough 1968 and 1969), *n*-hexyl hexanoate and (*E*)-2-octenyl acetate (Aldrich and Yonke 1975). These minor components have been argued as inconsequential for conspecific recognition (Aldrich and Yonke 1975). However, reanalysis of the volatiles from these bugs by GC-EAD may reveal other minor compounds of potential behavioral importance.

The utilization of different pheromone components by adults and nymphs may indicate that pheromones with different properties are

Figure 19: Flame ionization detector (FID) and corresponding electroantennographic (EAD) trace of adult female antenna to alarm pheromone collected *in vivo* from adult females and analyzed on a DB5 column using a program of 50° for 1 min, 2°/min to 65°, 5°/min to 120° then 20°/min to 240°.

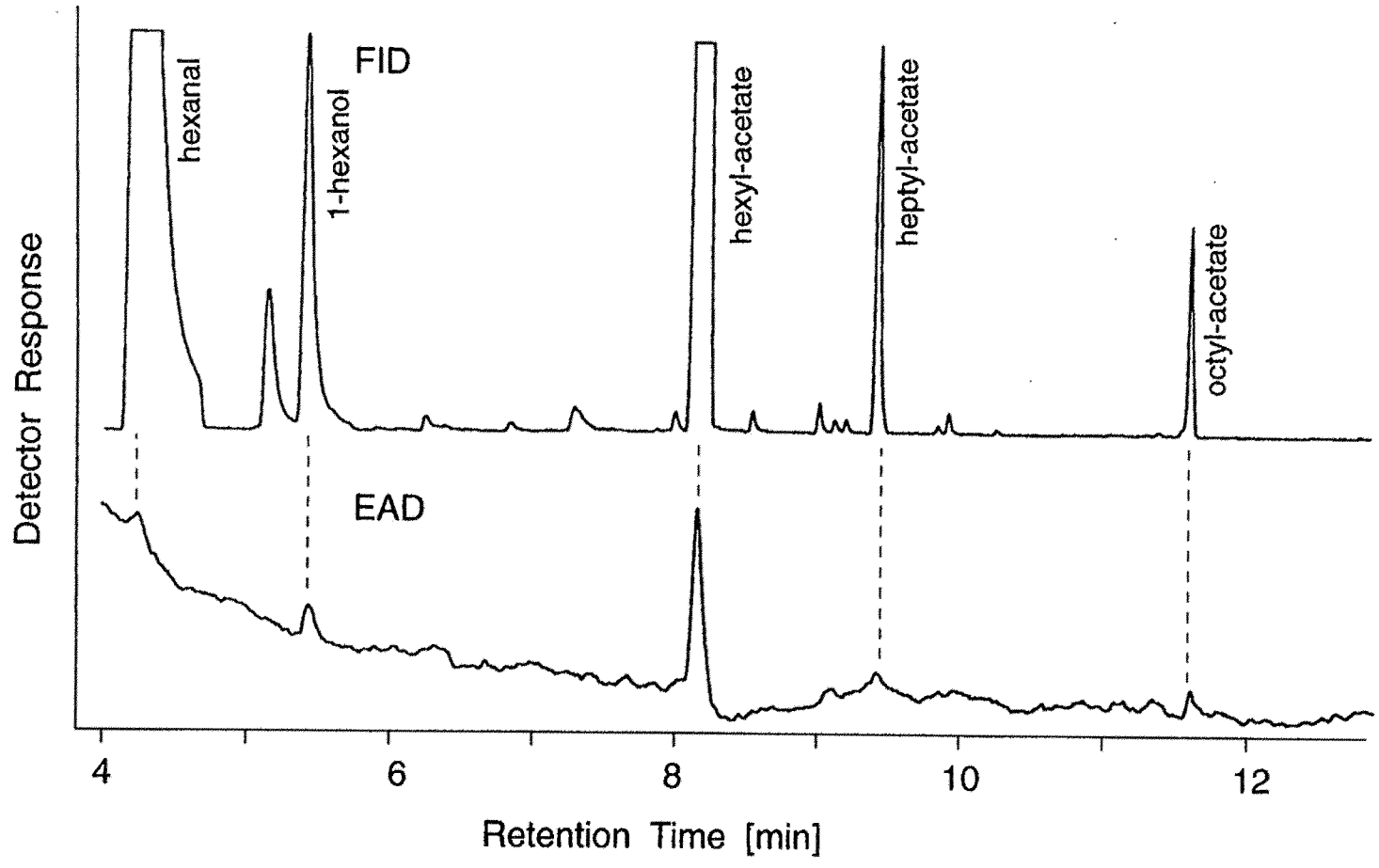
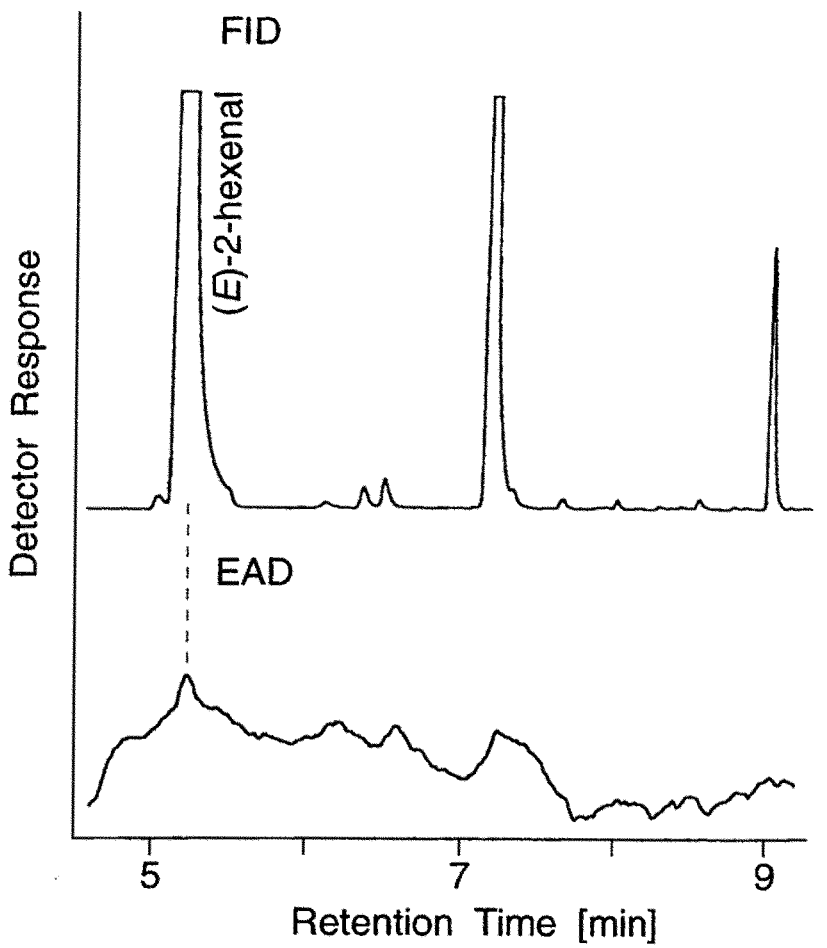

Detector Response
FID
EAD
hexanal
1-hexanol
hexyl-acetate
heptyl-acetate
octyl-acetate
4
6
8
10
12
Retention Time [min]

Figure 20: Flame ionization detector (FID) and corresponding electroantennographic (EAD) trace from nymphal antennae of alarm pheromone collected *in vivo* from 3rd and 4th instar nymphs and analyzed on a DB5 column using a program of 50° for 1 min, 2°/min to 65°, 5°/min to 120° then 20°/min to 240°.


FID
(E)-2-hexenal
Detector Response
EAD
5
7
9
Retention Time [min]

required to accommodate their differing release mechanisms. Adult *L. occidentalis* expel their pheromone through small openings in the thorax as a spray (pers. obs.) while nymphs release alarm pheromone through openings in their abdominal tergites. Hexanal produced by adults has a higher molecular weight than (*E*)-2-hexenal, and is more stable. Being less volatile, hexanal will disperse into the air or contact a predator prior to volatilization. (*E*)-2-hexenal, if released in a similar manner, would volatilize immediately on exposure to the air. As (*E*)-2-hexenal is probably released onto the surface of the tergites, rather than directly into the air, the large odor plume created would probably be as effective as a hexanal spray in warding off predators, particularly from a group of aggregated and alarmed nymphs. Aldrich and Yonke (1975) proposed that the difference in adult and nymphal alarm pheromones resulted from selection pressure by ants. This may be possible for species feeding on herbaceous plants with only a few layers of foliage, thus allowing the nymphs when alarmed to drop to the ground and easily climb back onto their hosts. For species feeding on conifers, which have many layers of foliage, the probability of the nymphs reaching the ground after being alarmed is smaller than if they were feeding on herbaceous plants, and the likelihood of their returning to their feeding sites without first coming in harms way is low. Furthermore, ants have frequently been observed on conifer boles and branches, but have not been noted as predators of *L. occidentalis* or any other *Leptoglossus* species.

Metathoracic glands and the associated reservoir of male and female adults contained several hundred micrograms of pheromone, while dorsal abdominal glands of nymphs contained < 10 μg (Table 22). Because only a portion of this amount, ~24% of the total for adults, ~33% for nymphs (Table 22) was emitted by agitated bugs, an individual insect should be capable of repeated emissions. However, approximately 15 and 45 min refractory periods occurred before adults and nymphs, respectively, could be provoked to re-emit alarm pheromone. In the nymphs, the long refractory period may indicate that (*E*)-2-hexenal is more useful as a deterrent against predation than as a conspecific alarm pheromone. Their aposematic coloring supports this hypothesis. Unlike adults, which can fly away from a host and return, departure from the host by nymphs in response to alarm pheromone could be maladaptive. For adults, only a small amount of material is needed to warn conspecifics of danger. Lockwood and Story (1987) hypothesized that alarming conspecifics evolved as a secondary function of the defensive secretion in *Nezara viridula* (Linnaeus). The reserve of pheromone remaining in the adults after one emission suggests that *L. occidentalis* could repeatedly defend itself against persistent attempts at predation, with alarm and dispersal by conspecifics being a secondary adaptation, as hypothesized for *N. viridula*.

Table 22: Comparison between quantities of alarm pheromone contained within metathoracic gland of adults and dorsal abdominal glands of nymphs and quantities given off when agitated.

Source of pheromone and number analyzed	Compound	Quantity (μg/bug) in gland (mean ±SE)	Quantity (μg/bug) given off when agitated (mean ± SE)	Percent of total content given off when agitated.
Adult	Hexanal	568.7 ± 170.4	17.1 ± 3.53	3.0
females	Hexanol	12.2 ± 7.1	1.29 ± 0.93	10.6
20	Hexyl Acetate	390.7 ± 156.9	21.7 ± 2.69	5.6
	Heptyl Acetate	0.26 ± 0.04	0.24 ± 0.10	92.3
	Octyl Acetate	3.06 ± 1.18	0.15 ± 0.02	4.9
Adult	Hexanal	339.7 ± 116.7	15.5 ± 4.46	4.6
males	Hexanol	4.6 ± 1.04	1.42 ± 0.87	30.8
20	Hexyl Acetate	337.1 ± 96.7	27.2 ± 5.25	8.1
	Heptyl Acetate	0.33 ± 0.14	0.21 ± 0.06	63.6
	Octyl Acetate	1.46 ± 0.48	0.17 ± 0.01	11.6
Nymphs	(*E*)-2-hexenal	3.64 ± 0.69	1.19 ± 0.35	32.7
51				

During the GC-EAD analysis at does of ~4 bug equivalents, some antennae ceased to respond following exposure to the hexyl acetate or hexanal in the extract. To solve this problem, extracts were diluted 20-fold enabling an antenna to respond for the duration of the analysis. A similar effect was observed in *Formica* spp. with *n*-undecane (Blum and Brand 1972), apparently an adaptive mechanism that allows the emitter to hide from aggressive conspecifics. In our analysis with *L. occidentalis*, the concentrations of hexyl acetate and hexanal were probably so high that there was a toxic inhibition of olfactory response.

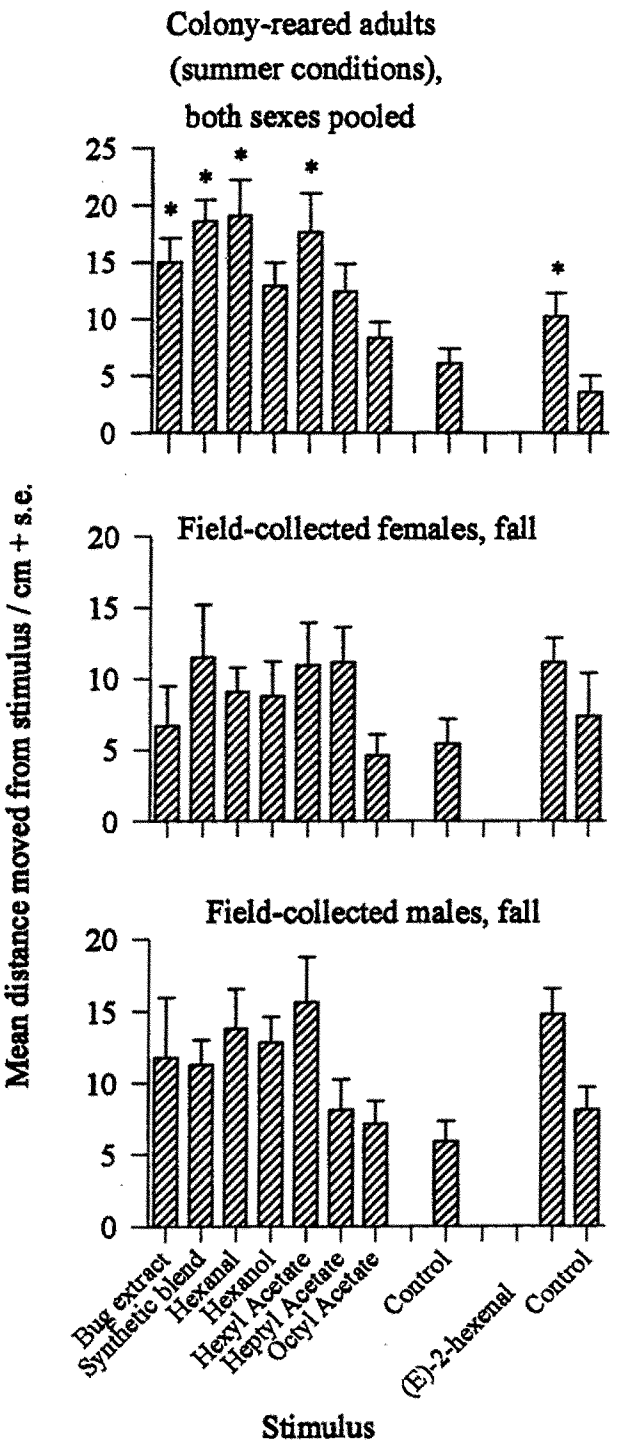
Bioassay of synthetic and extracted volatiles.

A synthetic blend containing all five components of adult pheromone elicited an alarm response by males, females and nymphs in the headspace bioassay (Table 23). Synthetic nymphal alarm pheromone elicited a similarly ubiquitous response (Table 23). Hexanal and hexyl acetate, the most abundant components in both glands and headspace volatiles from agitated adults, and (*E*)-2-hexenal from nymphs elicited a significant response from colony-reared 'summer' adults in the forced air bioassay (Fig. 21). Hexaldehydes are a common component of defensive secretions in numerous other hemiptera and homoptera (Waterhouse and Gilby 1964, McCullough 1970, 1973a and 1973b, Aldrich *et al.*

Table 23: Alarm behavior responses of adult and nymphal *L. occidentalis* in headspace bioassay to synthetic blend of adult alarm pheromone candidates and synthetic nymphal alarm pheromone, (*E*)-2-hexenal (approx. 0.4 bug equivalents per stimulus). N=15 bugs per test.

Test Insect	Source of stimulus	Percent positive response	χ^2 Probability experimental *vs.* control
Females	Synthetic adult blend	80	
	Pentane	13	0.005
Males	Synthetic adult blend	87	
	Pentane	6	0.001
Females	Synthetic nymph	93	
	Pentane	6	0.001
Males	Synthetic nymph	87	
	Pentane	6	0.001
Nymphs	Synthetic adult blend	73	
	Pentane	33	0.05
Nymphs	Synthetic nymph	80	
	Pentane	20	0.005

Figure 21 : Mean distance moved away from adult and nymphal alarm pheromone and its components by *L. occidentalis* reared in colony under 'summer' conditions or collected from the field in the fall. All components tested at concentration of one bug equivalent. Significant distance, Dunnett's one-way test, $P<0.05$, moved from stimulus as compared with control (pentane) indicated by an asterisk.


Colony-reared adults
(summer conditions),
both sexes pooled
Field-collected females, fall
Field-collected males, fall
Mean distance moved from stimulus / cm + s.e.
25
20
15
10
5
0
*
Bug extract
Synthetic blend
Hexanal
Hexanol
Hexyl Acetate
Heptyl Acetate
Octyl Acetate
Control
(E)-2-hexenal
Control
Stimulus

1978, Aldrich *et al.* 1979b, Everton *et al.* 1979, Aldrich *et al.* 1984b, Lockwood and Story 1987). The frequent occurrence of hexanal in such secretions suggests that it is an effective, broad spectrum irritant easily produced or acquired by insects and useful for defense. It is a common green leaf volatile in herbaceous plants (Visser *et al.* 1979), but its function may differ between insect species (Visser *et al.* 1979, Visser 1986 and Dickens *et al.* 1990, 1992). Although hexanol, heptyl acetate and octyl acetate were antennally active (Fig. 19), they did not elicit alarm behavior in these bioassays. I hypothesize that these compounds, less volatile than hexanal and hexyl acetate, may serve as a conspecific recognition signal. There was no evidence of an additive or synergistic interaction of components in the synthetic blend.

Adults collected in the fall did not respond to the synthetic alarm pheromone components ($F=1.16$, $P=0.27$ and $F=1.10$, $P=0.34$ for females and males, respectively) (Fig. 21). Males were more responsive than females ($F=4.66$, $P=0.03$). In many cases, the insects moved towards the stimulus. In the fall, both sexes of *L. occidentalis* seek cryptic overwintering sites, respond to an unknown male-produced aggregation pheromone, and are commonly aggregated in large numbers (Chapter 7). In these sites they are not easily accessible to predators, e.g. birds. Lockwood and Story (1985) demonstrated that in first-instar *N. viridula*,

n-tridecane serves as an alarm pheromone at 1.0 bug equivalent and as an aggregation pheromone at 0.1 bug equivalents. While this behavior is dose dependent, the differential response of summer and fall adult *L. occidentalis* to their defensive secretion is speculated to be primarily state dependent. During the fall when *L. occidentalis* forms stable aggregations, alarm responses followed by dispersal would be maladaptive. At this time the lesser produced components, hexanol, heptyl acetate and octyl acetate may serve as recognition cues of conspecifics.

Field Experiments.

Nymphs in the field showed a significant dispersal response to synthetic alarm pheromone sprays (Table 24). At a dose of 2 bug equivalents, synthetic nymphal pheromone and a mix of adult and nymphal pheromones were significantly more effective than the control (pentane only) at causing dispersal of nymphs from the cone clusters. Synthetic adult alarm pheromone at 4 bug equivalents caused a dispersal response similar to that caused by the nymphal-adult mix. At the 2 bug equivalent dose, the adult pheromone blend was no more effective than the control, suggesting that nymphs are more responsive to alarm pheromone from nymphs than from adults. While the pheromone treatments caused nymphs to walk away from the cones, the effect was short-lived and they would return to the cones within 5 minutes. For all

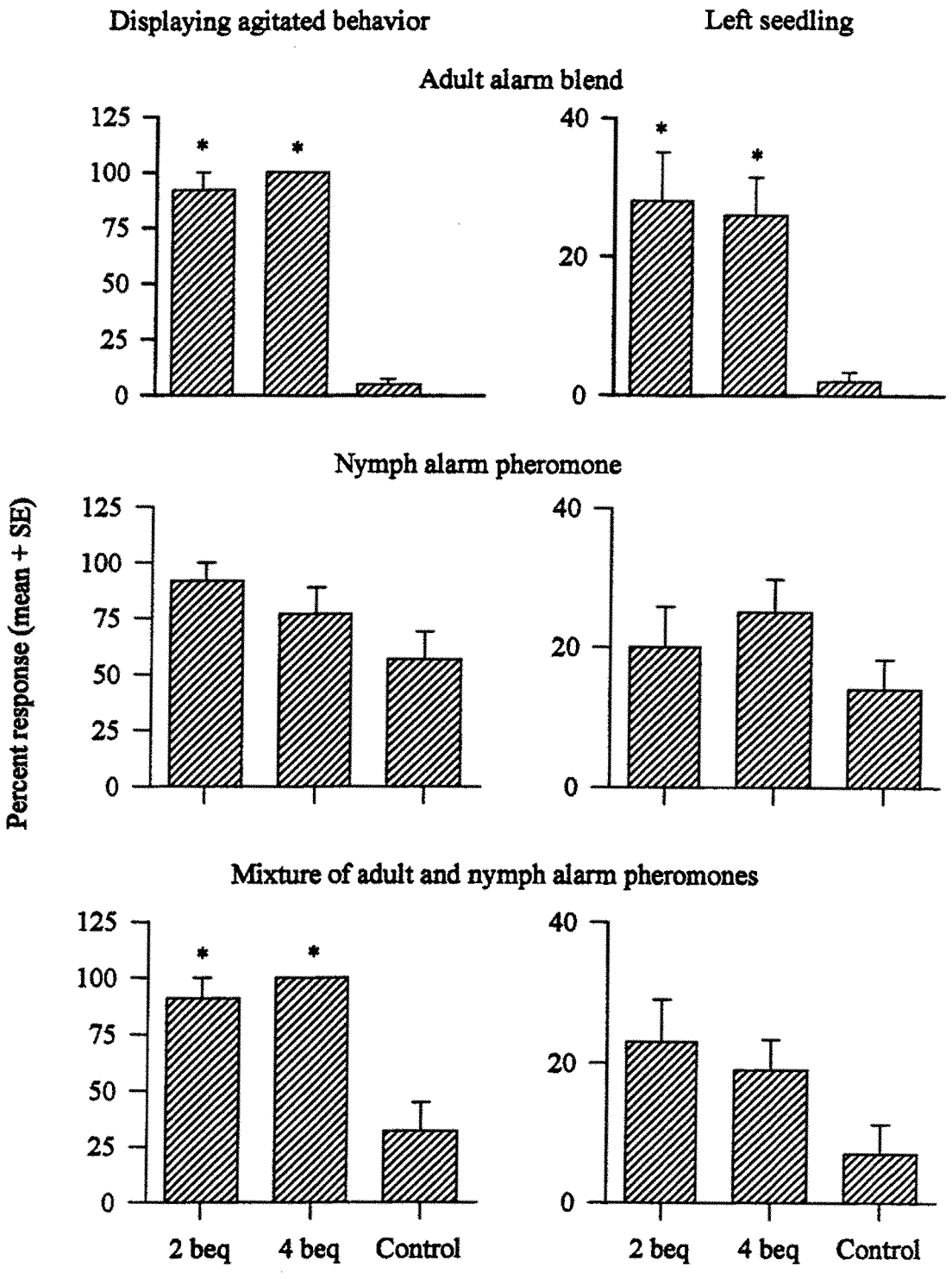
Table 24: Alarm behavior response of nymphal *L. occidentalis* in the field to synthetic blends of adult alarm pheromone, nymphal alarm pheromone or mixture of both applied as atomized sprays of 2 and 4 bug equivalents in 4 ml of pentane to western white pine cones in the field.

Source of pheromone	Bug equivalents	Number of insects tested	Percent dropping from cones	Z score probability experimental *vs.* control	Percent dispersing away from cones	Z score probability experimental *vs.* control
Control (pentane)	n/a	35	5.7	----	49	----
Adult	2	39	10.3	0.348	54	0.334
	4	48	8.3	0.405	77	0.005
Nymph	2	37	13.5	0.255	95	0.0001
Mix	2	44	18.2	0.133	73	0.017
	4	49	14.3	0.218	82	0.001

treatments tested, fewer than 20% of the nymphs present responded by dropping from the cone clusters (Table 24). Dropping from the cones would result in almost certain escape from an immediate threat of predation, but would potentially increase the chances of desiccation and predation (e.g. by spiders and rodents) while on the forest floor. Pea aphids, *Acyrthosiphon pisum* (Harris), from Kamloops, B.C., were less likely than aphids from Vancouver, to drop from their host plant in response to alarm pheromone (Roitberg and Myers 1978). This was postulated as a means to avoid desiccation in areas where ground conditions are harsh.

Adults tested on seedlings showed significant alarm responses (Fig. 22). The synthetic adult alarm pheromone at both 2 and 4 bug equivalents, either alone or in mixture with nymphal pheromone, caused significantly higher numbers of adults to become agitated than the pentane control treatment, but only the adult blend alone caused adults to leave the seedlings in significant numbers. Nymphal alarm pheromone had no significant effect. These results indicate that, like the nymphs (Table 24), adults are more responsive to their own pheromone than to the pheromone produced by bugs in a different life stage. The uniformly high responses to head space volatiles from any life stage (Tables 21 and 23) suggest that when stimuli are strong there is an adaptive advantage to respond to any conspecific alarm signal. The low

Figure 22: Percentage of adults responding to synthetic alarm pheromone sprays in a laboratory test on caged seedlings. Significant response, Dunnett's one-way test, $P<0.05$, compared with control (pentane) indicated by an asterisk.


Displaying agitated behavior
Left seedling
Adult alarm blend
Nymph alarm pheromone
Mixture of adult and nymph alarm pheromones
Percent response (mean + SE)
125
100
75
50
25
0
40
20
0
*
2 beq
4 beq
Control
Stimulus dose

percentage of adults leaving the seedling suggests that other indications of danger, e.g. rapid movement and shadows, would be necessary to cause adults to depart (Clegg and Barlow 1982). Of the six adults tested in the field, three flew away and the others dispersed from the cones by agitated walking. In both the field and the laboratory, adults that left the cones or seedlings returned within 10 minutes. Based on these results, I conclude that use of the alarm pheromone would not be operationally effective in reducing *L. occidentalis* populations.

Chapter 7
Aggregation Pheromone

Introduction

During the fall adult *L. occidentalis*, like other coreids, seek sheltered overwintering sites. When human habitation interfaces with forests, these sites can include garages, birds' nests (Hussey 1953) and houses (T.W. Koerber, Entomological Services Inc., Berkeley, California, pers. comm.). *Leptoglossus occidentalis* recently extended the eastern limits of its range and has become a common household pest in Michigan (Gall 1992), Ontario (Marshall 1991; McPherson *et al.* 1990), Wisconsin and Minnesota (Katovich and Kulman 1987). Numbers of insects found in or on the homes varied from less than a dozen to roughly a hundred individuals.

In October 1993, Richard Prebble, Manager of the Imperial Chemical Industries (ICI) Explosives Plant in Tappen, B.C., 78 km east of Kamloops, reported an infestation of *L. occidentalis* in and around the manufacturing plant. On inspecting the site, I found large numbers of *L. occidentalis* congregated around door jambs, windows and in cracks within the concrete walls. Hundreds of bugs were aggregated around

heating exhaust ports. I collected 1065 live bugs (608 males and 457 females) and estimated that there were at least 1000 more dead on the floors, window sills and in the door jambs, a result of chemical control by the plant staff. Several workers indicated that the infestation was 'manageable' compared with what it had been just two weeks before. For the previous three years, similar infestations had been observed at this plant; they reportedly lasted for roughly three weeks and then "disappeared". An aerial photograph obtained from the B.C. Ministry of Forests showed that *L. occidentalis* must have flown a considerable distance to reach the aggregation site. The plant is located in a meadow, at least 300 m from forest edges to the north and west, 900 m from a patch of conifers to the east and 750 m from a small patch of conifers to the south.

Small aggregations of *L. phyllopus* have been observed on Yucca plants in the southern USA (G.L. DeBarr, USDA Forest Service, Athens, Georgia, pers. comm.) and overwintering aggregations of other Hemiptera also occur. The first documented case of a *Leptoglossus* sp. forming a 'vast swarm' was in May 1912 (Hutson 1936), while *L. membranaceus* F., was observed aggregating in large numbers on the Island of Ceylon. Schowalter (1986) documented an aggregation of ca. 8,000 boxelder bugs, *Boisea rubrolineata* (Barber), in western Oregon. The aggregation was found 500-1,000 m away from feeding hosts. Aggregations of

>6,000 swallow bugs, *Oeciacus vicarius* Horvath, have been reported in Washington (Zack 1990). Chinch bugs, *Blissus leucopterus leucopterus* (Say), also aggregate to overwinter in groups of typically <200 individuals (Negron and Riley 1991). In all instances, pheromonal attraction was suggested as a causal factor of aggregations.

Many true bugs are known to use both sex and aggregation pheromones (Aldrich 1988). Species using aggregation pheromones include: milkweed bugs, *Oncopeltus fasciatus* (Dallas) (Aller and Caldwell 1979); *Eurydema rugosa* Motschulsky (Ishiwatari 1976); *Podisus maculiventris* (Say) (Aldrich *et al.* 1984b), southern green stink bugs, *Nezara viridula* (L.) (Lockwood and Story 1985) and *Leptoglossus australis* F. (Yasuda 1990). My objective was to determine if aggregations of *L. occidentalis* are pheromone-mediated.

Materials and Methods

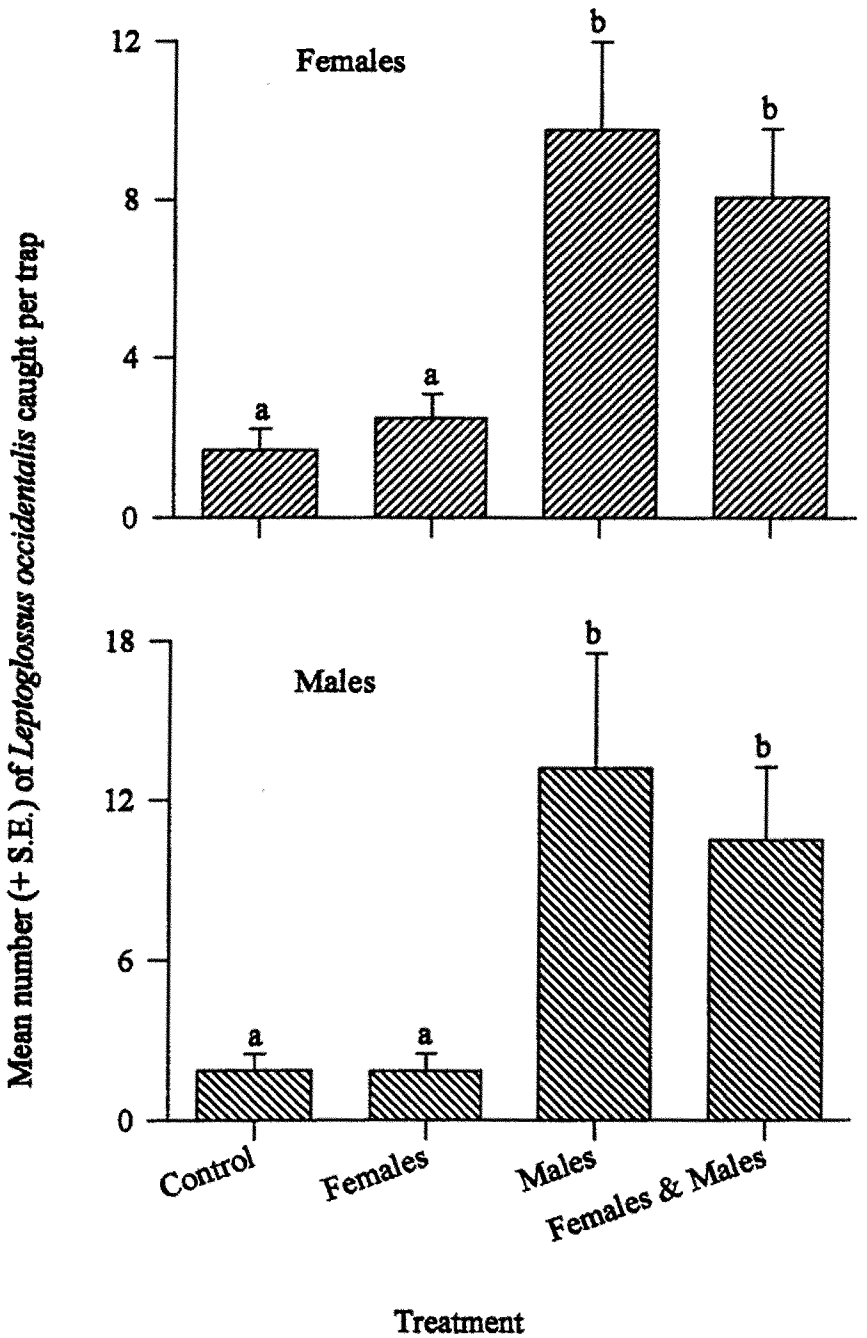
Live adult *L. occidentalis* were collected from the field in August 1994, maintained in the laboratory until 13 September, and then used to bait 12-unit, multiple funnel traps (Lindgren 1983). Ten adult insects were placed in a cylindrical wire cage (35 cm high, 14 cm diameter), provided with water and dried seed as food, and suspended in a white mesh bag alongside the trap. Fourteen replicates each of males, females, males with females and unbaited control traps, spaced 15 m apart, were

set up at Skimikin and eight replicates at the manufacturing plant. Traps were placed around the perimeter of the building complex at Skimikin, which is surrounded by forest, and in a line ca. 25 m north of the main buildings of the manufacturing plant, between the plant and the forest margin. In both locations, the dominant forest tree was Douglas-fir. After one week, captured bugs were sorted by sex and counted. Data were transformed by $\sqrt{x+0.5}$ to ensure normality, and treatment and site differences were analyzed at $\alpha=0.05$ using a General Linear Models analysis of variance and Scheffe's mean separation test (Day and Quinn 1989).

Results and Discussion

Traps baited with males alone or males with females attracted and captured significantly more *L. occidentalis* of both sexes than did female-baited traps or unbaited controls (Fig. 23). No difference between trap sites was noted ($F=1.18$, $P=0.28$). These results indicate that male *L. occidentalis* produce an aggregation pheromone and that females neither enhance nor inhibit the response by either sex to attractive males. This putative pheromone is hypothesized to initiate and maintain the large aggregations of *L. occidentalis* observed during the fall, when they are seeking overwintering sites. Volatiles specific to males are produced by other hemipterans (Aldrich *et al.* 1976, Aldrich *et al.* 1979a and 1979b,

Figure 23: Captures of male and female *Leptoglossus occidentalis* in 12-unit, multiple funnel traps baited with live males, females or both sexes of *L. occidentalis*, Tappen, B.C., n=22. Bars with same letter within sex are not significantly different, Scheffe's test, $P<0.05$.


Females
Males
12
8
4
0
18
12
6
0
a
a
b
b
Control
Females
Males
Females & Males
Mean number (+ S.E.) of Leptoglossus occidentalis caught per trap
Treatment

Aldrich *et al.* 1982, Aldrich *et al.* 1984b, Gough *et al.* 1985, Aldrich *et al.* 1986, Aldrich *et al.* 1993); however their biological function is unknown.

Male-specific attractants are produced by other true bugs (Mitchell and Mau 1971, Harris and Todd 1980, Aldrich *et al.* 1984a, Moriya and Shiga 1984 and James *et al.* 1994) including one other *Leptoglossus* sp. (Yasuda 1990). Five similar experiments, each consisting of six replicates each of live males, females, males and females and an unbaited control, conducted during June-September of 1993 and 1994 resulted in a cumulative catch for all experiments of 65 bugs (25 females and 40 males) with the insects being caught equally in all treatments (see Appendix 2). In these experiments, neither male nor female-baited traps were attractive, indicating that either aggregation pheromone is produced only in the fall, or that overwintered bugs lose the capacity to respond, or both. The results further suggest that if *L. occidentalis* utilizes a sex pheromone that it is relatively non volatile, unlike the aggregation pheromone. *Leptoglossus occidentalis* has a pronounced preference, possibly mediated by attractive host kairomones, for specific clones as hosts in seed orchards (Figs. 2-4). A strong attraction to preferred hosts would be consistent with a close range sex pheromone that was utilized once both sexes had reached preferred oviposition sites. If a synthetic aggregation pheromone were available, it might have

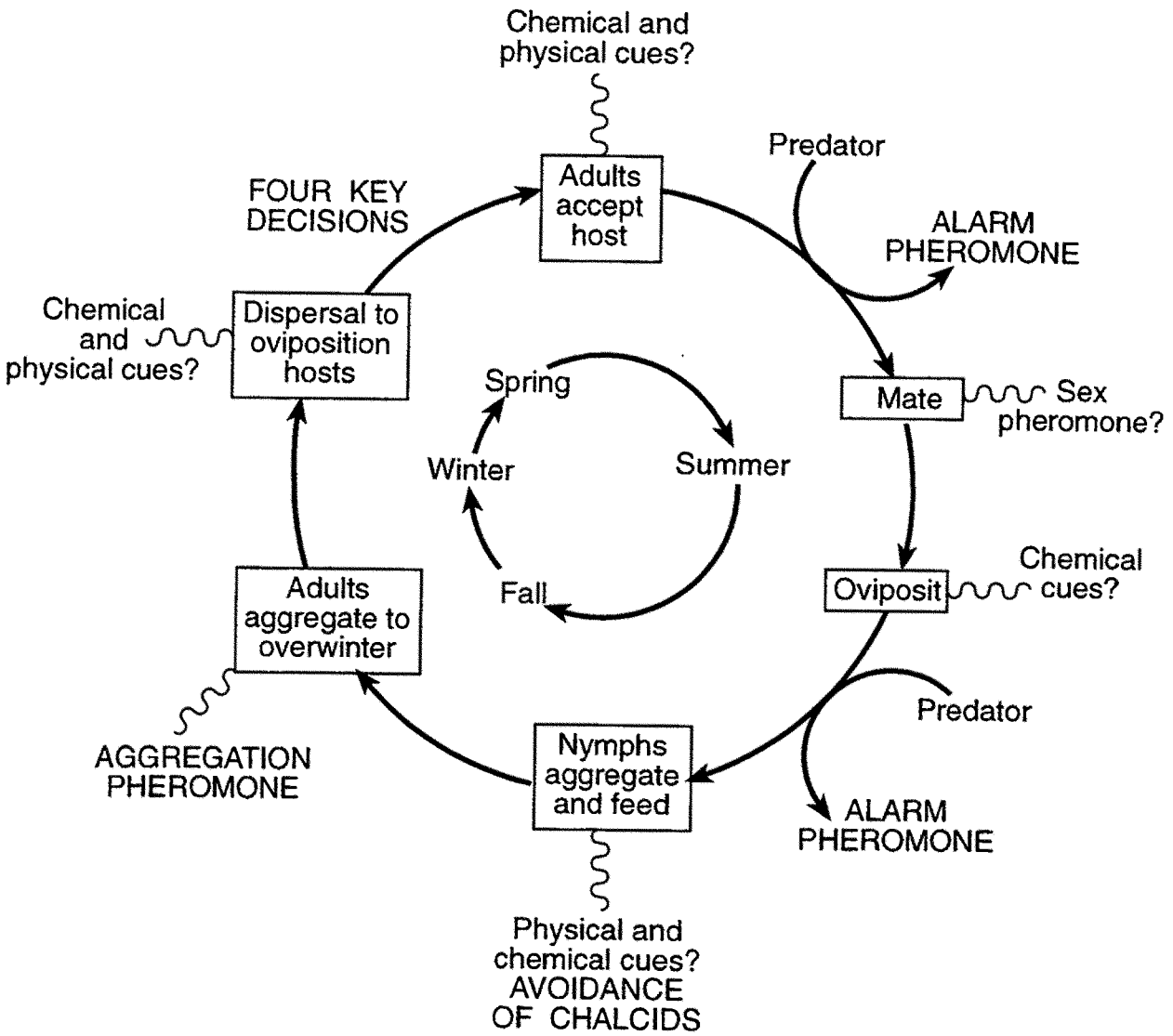
considerable potential for use in a mass-trapping tactic (Silverstein 1981, Borden 1993) that could replace or supplement chemical controls.

Concluding Discussion

Knowledge of the life cycle of *L. occidentalis* (Hedlin *et al.* 1981) has long remained at the natural history level. As temperatures rise in the spring, adult *L. occidentalis* emerge from overwintering sites and seek out suitable hosts. A choice of hosts is made and eggs are deposited on the needles in a row. The young hatch from the eggs approximately 10-14 days later and proceed through five instars. Toward the end of summer, new adults emerge and leave the brood host. An aggregation is formed prior to overwintering and the cycle repeats itself in the next year. My research allows many aspects of this life cycle to be understood in a much more profound manner than previously possible, particularly with respect to the role of *L. occidentalis* as a pest in seed orchards (Fig. 24).

A fundamental discovery that changed the intended course of my research is that like seed chalcids (Rappaport and Roques 1991) (Fig. 11), *L. occidentalis* exhibits a clonal preference in seed orchards (Figs. 2-4). I hypothesized that this preference is based on various physical characteristics of potential hosts, such as cone density and tree height, on host kairomones, and an interaction with seed chalcids that compete for the same resources as *L. occidentalis*. Analysis of empirical data

Figure 24: Life cycle of *Leptoglossus occidentalis* showing in capitals areas which are now better understood because of the research reported in this thesis.


Chemical and physical cues?
Predator
FOUR KEY DECISIONS
Adults accept host
ALARM PHEROMONE
Chemical and physical cues?
Dispersal to oviposition hosts
Spring
Mate
Sex pheromone?
Winter
Summer
Chemical cues?
Oviposit
Adults aggregate to overwinter
Fall
Predator
AGGREGATION PHEROMONE
Nymphs aggregate and feed
ALARM PHEROMONE
Physical and chemical cues? AVOIDANCE OF CHALCIDS

(Figs. 2-4, 6-10) and Poisson regression analysis (Tables 10-20) both disclosed that the most consistently significant factor in the host selection process of *L. occidentalis* is clone. Variables such as cone density and tree height are of lesser importance, with preference expressed in mid-range, rather than at high and low extremes (Figs. 6-9). There is only weak evidence that female *L. occidentalis* utilize host kairomones from the developing female strobili during host selection (Appendix 1). As females arrive first in the orchard in the spring and have the most investment in offspring, using cues emanating from female strobili as a means of evaluating the quality of the tree or its food resources would be highly adaptive. *Leptoglossus occidentalis* has been observed feeding on the staminate cones of Douglas-fir trees (T. Koerber, Entomological Services Inc., Berkeley, California, pers. comm.). This has previously been thought to be a source of supplemental protein. It is unknown if pollen-bearing staminate cones of Douglas-fir emit attractive volatiles, as has been shown for Rosaceae and Asteraceae species (Dobson *et al.* 1987, McNeil and Delisle 1992). The blends of antennally active volatiles emitted by the female strobili are highly complex (Appendix 1), and provide a potential basis for explaining shifts in clonal preference from year to year. An environment occupied by a given species has the capacity to change dramatically from year to year and place to place (Weis and Campbell 1992). Furthermore, abiotic factors such as water stress and light intensity can alter the volatile profile of a

plant, increasing or decreasing its attractiveness to herbivores (Takabayashi *et al.* 1994). As each tree within a clone is genetically identical, the within-clone responses to any given environmental stimulus, e.g. drought or nutrient deficiency, would be similar. Among the numerous clones in an orchard, a few would probably respond to changing environmental factors in a similar manner. So in any given year, there would be a few clones producing appropriate cues to indicate their suitability as oviposition sites for *L. occidentalis*. This ranking of hosts is consistent with the graded discrimination and host ranking process for phytophagous insects described by Rausher (1983). Clonal preferences would limit the capacity of *L. occidentalis* to damage seed throughout an orchard, a capacity that is already limited except in very high populations (Table 2). Such an interaction between environment and genotype has been shown for many insect species (Gerhold *et al.* 1966, Heybroek *et al.* 1982, Service 1984, McCrea and Abrahamson 1987, Maddox and Root 1987, Sacchi *et al.* 1988, Quiring and Butterworth 1994).

Post-alighting cues that indicate host suitability appear to be as important for *L. occidentalis* as they are for other species (Rausher 1983, Backus 1985, Todd *et al.* 1990). The primary reason for the importance of such cues is probably the adaptive significance of avoiding cones that are heavily infested by the Douglas-fir seed chalcid, *M. spermotrophus*

(Tables 3, 4). The presence of one herbivore in the same tissue that is required by another should have a negative effect on the second species (Rhoades 1983). Sampling by probing within the cone and the seeds therein for the presence of *M. spermotrophus* would be time consuming (possibly 20 min per cone), would expose adult *L. occidentalis* to potential predators such as vespid wasps (Sanchez and Genaro 1992) and spiders, would require a substantial output of energy to penetrate through a cone and into a seed, and would demand either a sensitive gustatory capability or some other means to detect chalcid larvae within the seeds. Less costly alternatives would be the detection of volatiles from infested tissues as is the case in other plants where herbivore-induced volatiles are reported (Takabayashi *et al.* 1991, Takabayashi and Dicke 1993, Dicke 1994, Takabayashi *et al.* 1994, Turgeon *et al.* 1994, Turlings *et al.* 1995) or the ability to detect chalcid larvae within infested seeds by infrared perception, just as the bark beetle parasitoid *Coeloides brunerl* Vireck detects host larvae of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, under the bark (Richerson and Borden 1972). In any case, assessment of the level of chalcid infestation would be essential for *L. occidentalis* adults if they lack the enzymatic capacity to digest animal tissue. If this hypothesis is upheld, *L. occidentalis* would be classed as an obligate rather than a facultative herbivore like some other hemiptera (Niemczyk 1978, Ruberson *et al.* 1986), and the impact of *L. occidentalis* and *M. spermotrophus* on seed production would be additive.

A hypothetical decision-tree in which *L. occidentalis* adults make four choices that lead to the rejection of unsuitable hosts has been proposed (Fig. 16). At each step, adult *L. occidentalis* must choose to accept or reject a host based on clone, cone density, height, or chalcid infestation. Should any of these characteristics not be acceptable, the adult would simply leave the host and continue searching. After encountering another potentially acceptable host, the four key decisions would again be faced, and the host would be accepted or rejected.

Leptoglossus occidentalis adults and nymphs have also been observed feeding on the foliage, presumably to supplement their diet with plant carbohydrates. Insects, having a high protein composition, have had to evolve mechanisms to enable them to feed on foliage and plant parts. It is hypothesized by Strong *et al.* (1984) that this evolution occurred in steps: first to pollen feeding and then to the leaves. As stated above, *L. occidentalis* does feed on the staminate flowers of conifers and it has been speculated that *Leptoglossus* spp. are responsible for conelet abortion (DeBarr and Ebel 1974, DeBarr and Kormanick 1975); however, I have not observed seed bugs feeding on conelets. The possibility that *Leptoglossus* spp. may cause conelet abortion and the observation of adults feeding on the staminate flowers

may indicate that in times of low cone crops seed bugs utilize pollen, a known source of protein, to sustain themselves (Strong *et al.* 1984).

Mating by *L. occidentalis* appears to occur in the spring and continue throughout the summer. There is a good argument for a female-produced sex pheromone but none of my experiments produced evidence to support this argument (Appendix 2). I hypothesize that female *L. occidentalis* produce a sex pheromone, but only after selecting a suitable host, and that this mechanism reduces the chances for females to be caught with a mature egg load but no suitable host on which to oviposit. I suggest that further research investigating the possibility of a sex pheromone in *L. occidentalis* should involve the host in all cases.

Once the eggs are laid on a suitable host, weather dictates the speed of development. Eggs will hatch within two weeks at >20°. However, females have been found in the field 4-6 weeks before the appearance of nymphs. Newly-emerged nymphs aggregate on the cones and begin to feed. Several experiments involving both visual, chemical and tactile stimuli were undertaken to explore the mechanism of nymphal aggregations with little success. Given full attention, this might be a fruitful area of investigation because nymphal aggregations are ubiquitous and as in other insects must be of adaptive significance

(Kiritani 1964, Wollerman 1965, Ishiwatari 1976, Harris and Todd 1981, Blum 1985, Lockwood and Story 1986, Pavis *et al.* 1994).

Both adults and nymphs of *L. occidentalis* are prone to predation. While there are no documented predators or parasites of this species, I have observed nymphs being fed on by spiders and adults being fed on by vespid wasps. Birds have been reported as major predators of *L. occidentalis* in seed orchards (L. Langois, Skimikin Seed Orchards, Tappen, B.C., pers. comm.). Other species of *Leptoglossus* have predators and an egg parasite (Sanchez and Genaro 1992, Mitchell and Mitchell 1986). Apparently to deter predators and warn conspecifics of danger, both adult and nymphal *L. occidentalis* release alarm pheromone from their metathoracic and dorsal abdominal glands, respectively. Adults produce a five-component blend (Fig. 19), which is similar to but not identical to that produced by *L. zonatus* (Leal *et al.* 1993). Nymphs produce a single component pheromone (Fig. 20). Adults and nymphs respond to their own and each other's pheromone (Tables 21, 23). It is unknown if predators, such as spiders or the vespid wasps, use the alarm pheromones as prey-finding kairomones, as do predators of other insects, e.g. thrips (Teerling *et al.* 1993).

In preparation for overwintering, adult *L. occidentalis* leave their brood hosts and the seed orchard. I have observed adults walking along

the ground and flying sporadically in the fall. In all cases, they were moving from higher to lower elevations and leaving their food source. In one situation, they were moving from the forest margin down into an open meadow and aggregating in and around a manufacturing plant in Tappen, B.C. In another instance adults moved out of the forest and into the marsh area of the Creston Valley. In both cases, the aggregations had become a nuisance. Departure from their food source by *L. occidentalis* adults is consistent with observations of the Colorado potato beetle (Hare 1983) and other species that are adapted to the seasonal availability of food and may use nutritional changes to trigger the induction of overwintering or to enhance the effect of other cues (Mansingh 1971).

The aggregations which are commonly seen in the fall are mediated by pheromones (Fig. 23), the first demonstration of an attractive pheromone in *L. occidentalis*. Aggregation pheromones commonly mediate the location of feeding and sheltering sites in insect populations (Borden 1993). Employment of such a pheromone in *L. occidentalis* would enable pheromone-producing insects to attract others as a source of metabolic heat and as physical barriers to the environment during the winter. Responding insects would be spared the necessity of finding their own overwintering site. *Leptoglossus occidentalis* enters a state of oligopause (Mansingh 1971). If they are similar to other coreids, this

condition may be required prior to reproduction in the spring (Nechols 1988). Mating has not been observed among *L. occidentalis* during the fall. When I dissected fall-collected females, I found more females without eggs in their ovarioles or spermatozoa in their spermathecae than those having either eggs and/or spermatozoa. Those females possessing either were dark in color, suggesting that they were mature and had probably mated sometime during the summer.

There are still many unanswered questions pertaining to the life history and biology of *L. occidentalis*. While my research has generated a few answers now, much more is yet to be discovered about this fascinating and somewhat perplexing insect.

Appendix 1
Potential Mechanism of Host Selection -
Chemical Characteristics

Materials and Methods

Bioassays.

Fresh conelets were field-collected from preferred clones no. 3143, and 3153 and non-preferred clones 3105, and 3188 (Figs. 2-4) from Mt. Newton 2 on 22 April 1994. They were tested within a few days using a static air olfactometer, a plexi-glass box (33 x 18 x 11 cm) with a removable lid. Two choice bioassays were conducted by placing live conelets, one each of preferred and non-preferred clones, in diagonally opposing corners of the box. The twig bearing a conelet was inserted into a small Parafilm-covered vial of water. Four groups of 10 male or 10 female adult *L. occidentalis*, obtained from the SFU colony, were given 15 min to select the conelet they preferred. Only bugs in contact with a conelet were scored as positive responders. The box was washed with hot soapy water and air dried between replicates.

Foliage and mature cones were field-collected from preferred clones no. 3143, 3153 and 3177 and non-preferred clones no. 3105, 3081 and

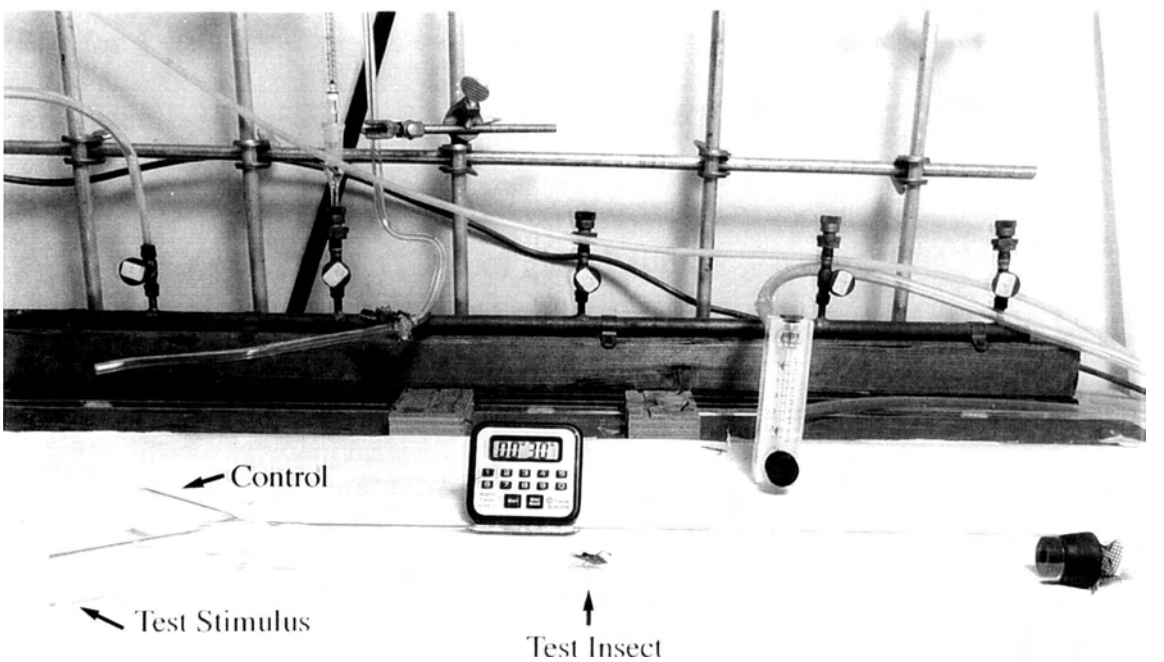
3188 from Mt. Newton 2 on 18 April 1994, stored at 4°C for up to 2 days, and tested in a two-choice Y-tube olfactometer (Fig. 1.1) held at 25°C and 60% RH under constant fluorescent lighting. Small cuttings of the foliage, ~ 5 cm long, were inserted into the short arms of the Y-tube. Room air was pulled at 2.5 L/min through the Y-tube. An individual adult or nymph *L. occidentalis* was given 10 min to select a foliage sample by fully walking into the arm of the Y-tube. Twenty males, females and nymphs (3^{rd}-5^{th} instars) were tested individually.

Four cones from a preferred tree were tested against four cones from a non-preferred tree. Each set of cones was placed in an open plastic Zip-Loc bag, 27 x 28 cm, fitted with a plastic tube leading from the bottom of the bag and into one fork of the Y-tube apparatus. Room air was pulled at 2.5 L/min as above through the bags, over the cones and into the Y-tube. Bugs were given 10 min to make a choice as above. Twenty males, females and nymphs (3^{rd}-5^{th} instar) were tested individually.

Instrumental Materials and Methods.

Volatile samples were analyzed on a Hewlett-Packard 5890 Series II gas chromatograph (GC) equipped with a capillary inlet system and a flame-ionization detector (FID). Fused silica columns (30 m x 0.25 mm I.D.) coated with DB-5 (J&W Scientific Inc., Folsom, California)

Figure 1.1: Y-tube olfactometer used for testing response of *L. occidentalis* to several stimuli. Here apparatus is modified to accommodate use of synthetic material on filter paper.


Control
Test Stimulus
Test Insect
Direction of air flow

were used. Injection port and detector temperatures were 260 and 270°C respectively. All GC spectra were obtained using the following program: 50° for 1 min, 5°/min to 240°, then isothermal for 5 min. Spectra were analyzed by comparing area counts of specific peaks for all clones.

For analysis by means of gas-chromatographic electroantennographic detection (GC-EAD) (*frn et al.* 1975, Gries *et al.* 1993), a Hewlett Packard 5890A gas chromatograph and a custom built amplifier with a passive low-pass filter and a cutoff frequency of 10 kHz. Antennae were gently pulled out of the insect's head; the exposed nerve endings were suspended in a saline solution which contained the indifferent electrode and the distal end of the antennae was pierced with a recording electrode. Antennally active compounds were analyzed by coupled GC-mass spectroscopy (MS) using a Hewlett Packard 5985B GC equipped with a fused silica (30 m x 0.25 mm ID) DB-5 column in full-scan and selection ion monitoring (SIM) mode. Compounds were identified by comparison with published spectra (Jennings and Shibamoto 1980) and identification was verified by GC using authentic standards. All GC-EAD analyses were conducted by R. Gries, Chemical Ecology Research Group, SFU.

Analysis of Steam-distilled Volatiles.

Conelets were field-collected in April, 1994 and 1995 from preferred and non-preferred clones as follows: preferred clones nos. 3143, 3153 and 3177 (April 22, 1994 and April 8, 25 and 8, 1995; respectively); non-preferred clones nos. 3105, 3081 and 3188 (April 22, 1994 and April 19, 21, and 18, 1995; respectively). Samples were ground with 200 mL distilled water in a blender, placed in a 1 L round bottomed flask and steam distilled with 20 mL hexane for 5 h. Distillates were dried with Na_2SO_4, transferred to pre-weighed, clean vials and evaporated down to the essential oil under nitrogen. Vials were then re-weighed to determine the quantity of oil extracted. Solutions of 1 mg oil/1 mL hexane and 1 flower equivalent/1 mL hexane were made and stored frozen until analyzed by GC, GC-MS and GC-EAD. Mean percent compositions for antennally-active volatiles in preferred and non-preferred clones were compared by t-tests, α=0.05.

Analysis of Volatiles Collected In vivo.

Headspace volatiles were collected from the conelets (Matile and Altenburger 1988, Loughrin *et al.* 1990, Robertson *et al.* 1993, Buchbauer *et al.* 1994 and Kamden *et al.* 1994). Plastic containers, 250 mL, were placed over the tips of branches bearing 4-6 conelets, with the lids used to secure the containers to the branches (Fig. 1.2). Air was

Figure 1.2: Apparatus used in the field to obtain the *in vivo* extracts from conelets at Mt. Newton in 1995.

pulled with a suction pump at 1 L/min through a chamber and a Porapak Q trap (8 cm long x 8 mm O.D.). Each trap was positioned beneath and in close proximity to the conelets being aerated. Volatiles were collected from enclosed conelets for 6-8 h beginning when the temperature within the chambers reached 15°C, the temperature at which *L. occidentalis* begins to fly both in the colony and in the field (pers. obs.), and the temperature at which volatiles are more likely to be secreted (Jakobsen and Olsen 1994). Temperatures fluctuated throughout the day, but were fairly consistent between days during the 4-25 April, 1995 collection period. Volatiles were collected from preferred clones nos. 3143, 3177, and 3153 on April 6, 8 and 23/25, respectively, and from non-preferred clones nos. 3188, 3105, and 3081 on April 4/5, 19 and 21, respectively. Three trees per clone were sampled, except for clones 3081 and 3105 which had only two trees bearing flowers that year. For comparison, one collection of foliage volatiles was made as above from each tree. Captured volatiles were eluted with pentane (2 mL), and analyzed by GC as above to evaluate within clone differences. Because within clone differences were not found, extracts from each clone were pooled, concentrated under nitrogen to 1 flower-h/ 2 μL, stored at 4°C until analyzed by GC as above.

Two-choice bioassays (20 bugs of each sex tested singly) were conducted as above using clones tested singly at a dose of 1 flower-h/2

μL against a pentane blank, or as paired preferred *vs.* non-preferred clones. Clones were paired based on quantity of oil contained within conelets as determined by steam distillation. Any insect not making a choice within 5 min. was deemed a non-responder and was discarded. Extracts from foliage volatiles collected *in vivo*, as described above, were also tested using the Y-tube bioassay. Two sets of bioassays were conducted: pairings of preferred and non-preferred clones and all clones tested singly against a blank of pentane. For each experiment 20 replicates of males and females were tested individually. Response data, compared to an expected 50:50 distribution, were analyzed by chi-square, α=0.05.

Results

Bioassays.

There was weak evidence in laboratory bioassays that female *L. occidentalis* were capable of distinguishing between clones that were preferred or not preferred in the field. Females consistently selected preferred clone no. 3153 over two non-preferred clones in the static-air bioassay (Table 1.1). In one bioassay, males also selected clone no. 3153 over a non-preferred clone. However; they selected two non-preferred clones (nos. 3105 and 3188) over preferred clone no. 3143. Clone 3143 did not significantly affect the preference of females. Given a four choice

Table 1.1: Response of *L. occidentalis* to freshly picked conelets in a static air bioassay.

Sex	Paired clones tested		Number of bugs	Percent response		χ^2 *P*, preferred
tested	Preferred	Non-preferred	responding	Preferred	Non-preferred	*vs.* non-preferred
Female	3153	3105	32	68.8	31.2	0.03
	3153	3188	30	66.7	35.0	0.049
	3143	3105	26	70.0	30.0	0.17
	3143	3188	27	63.0	37.0	0.67
Male	3153	3105	33	48.5	51.1	0.80
	3153	3188	35	31.4	68.6	0.03
	3143	3105	29	69.0	31.0	0.04
	3143	3188	26	23.1	76.9	0.006

situation, females chose the preferred clones while males showed no preference.

In the two-choice Y-tube olfactometer, neither males nor females were attracted to foliage or mature cones from either preferred or non-preferred clones (χ^2=0.69, P>0.05). Response of females to volatiles captured *in vivo* and tested in a similar bioassay were equivocal. Females more often chose the Y-tube arm treated with volatiles from preferred clones nos. 3153 and 3177, but this was not significant (n=17 and 18, χ^2 = 3.13, 3.56, P=0.07 and 0.06, clones 3153 and 3177, respectively). When paired volatiles (preferred vs. non-preferred clones) were tested, females responded by choosing the preferred clone no. 3143 significantly more than its paired non-preferred clone (n=20, χ^2 = 5, P=0.025). Clone 3153 was frequently chosen but not significantly so (n=20, χ^2=3.2, P=0.07). Males were unresponsive to captured volatiles from any clone, a finding consistent with other research (Evans and Allen-Williams 1992).

Chemical Analyses

Although the amount of oil extracted from the conelets varied between the clones, the variation and amounts were approximately the same for preferred and non-preferred clones (Table 1.2). The antennae of *L. occidentalis* responded to 35 compounds in the GC-EAD analysis of

Table 1.2: Comparison of the content of volatile oil in the distillates from conelets picked from preferred and non-preferred clones in two successive years. Weight not taken for clone no. 3105 in 1994.

Year	Status of clone	Clones ranked by weight of oil/flower	Number of flowers extracted	Fresh weight per flower (g)	Percent oil/gram fresh weight (%)	Weight of oil per flower (mg)
1994	Preferred	3143	50	1.35	0.08	1.08
		3177	48	1.29	0.05	0.60
		3153	57	0.59	0.06	0.33
	Non-preferred	3081	11	1.12	0.08	0.90
		3188	56	1.11	0.06	0.68
		3105	66	-------	-------	0.35
1995	Preferred	3143	85	0.74	0.1	1.07
		3177	70	0.88	0.03	0.28
		3153	75	0.65	0.05	0.38
	Non-preferred	3081	50	0.62	0.13	0.78
		3188	72	0.57	0.1	0.58
		3105	40	0.69	0.07	0.47

the distillates of Douglas-fir conelets (Table 1.3). A few compounds were present in significantly greater relative amounts in conelet distillates from preferred than non-preferred clones. Most notable among these were the monoterpenes: β-phellandrene and limonene (Table 1.3). Volatiles captured from conelets *in-vivo* differed quantitatively and qualitatively from those collected by steam distillation (Tatsuka *et al.* 1990), but contained no additional antennally-active compounds nor did they disclose any further differences between preferred and non-preferred clones.

Discussion

The ability of female *L. occidentalis* to choose conelets or captured volatiles from conelets of preferred over non-preferred clones in static air bioassays (Tables 1.1) suggests that discrimination between preferred and non-preferred clones occurs at least in part through olfaction. A possible basis for this discrimination could lie in differing ratios of a few compounds, e.g. β-phellandrene and limonene (Table 1.3), if not in the absolute amounts of volatiles produced (Table 1.2). Chemical differences between clones of Douglas-fir and between cones and foliage have previously been documented (Turgeon *et al.* 1994). Such a difference has also been demonstrated in hawthorn and raspberry cultivars (Robertson *et al.* 1993). Monoterpenes are good candidates for use in host selection

Table 1.3: Comparison of percent composition of antennally-active peaks disclosed in GC-EAD analyses of steam distillates of preferred and non-preferred clones (1 flower*hour dose) by *L. occidentalis* in the field.

Compound	Retention time (min.)	Percent composition (mean ± SE)[a] Preferred clones	Non-preferred clones
α-pinene	8.1	10.75 ± 0.81	11.87 ± 1.00
β-pinene	9.46	19.30 ± 0.98	24.13 ± 0.19
myrcene	9.69	2.29 ± 0.30	1.64 ± 0.53
α-phellandrene	10.36	4.21 ± 1.63	1.67 ± 0.21 *
p-cymene	10.82	0.42 ± 0.09	0.28 ± 0.05
limonene	11.01	6.17 ± 1.62	0.99 ± 0.24 *
Unknown 1	11.05	1.03 ± 0.39	0.85 ± 0.25
γ-terpinene	11.88	2.24 ± 0.32	1.38 ± 0.30 *
Unknown 2	12.76	6.31 ± 0.58	2.67 ± 0.45 *
linalool	13.14	0.37 ± 0.11	0.20 ± 0.11
nonanal	13.29	0.29 ± 0.09	0.19 ± 0.03
E-pinocarveol	14.55	0.38 ± 0.04	0.30 ± 0.06
Unknown 3	15.03	0.12 ± 0.02	1.25 ± 1.15
Unknown 4	15.39	0.26 ± 0.20	0.03 ± 0.01 *
Unknown 5	15.46	0.21 ± 0.02	0.16 ± 0.02
terpinen-4-ol	15.82	4.78 ± 0.88	3.87 ± 0.92
α-terpineol	16.21	2.31 ± 0.41	2.44 ± 0.18
bornyl acetate	18.9	0.73 ± 0.21	0.80 ± 0.14
Unknown 6	20.39	0.45 ± 0.21	0.05 ± 0.05 *
Unknown 7	20.65	0.22 ± 0.07	0.14 ± 0.05
(+)-α-longifolene	20.91	0.08 ± 0.02	0.02 ± 0.00 *
Unknown 8	21.46	0.07 ± 0.01	0.09 ± 0.04
Unknown 9	21.97	0.43 ± 0.09	0.20 ± 0.06

Table 1.3 (Continued)

Compound	Retention time (min.)	Percent composition (mean ± SE)[a]	
		Preferred clones	Non-preferred clones
α-gurjunene	22.38	0.09 ± 0.00	0.04 ± 0.01
longifolene	22.64	0.29 ± 0.10	0.10 ± 0.00
Unknown 10	22.87	0.40 ± 0.18	0.58 ± 0.54
Unknown 11	23.13	0.51 ± 0.08	0.32 ± 0.08
Unknown 12	23.57	0.26 ± 0.05	0.33 ± 0.03
Unknown 13	24.56	8.59 ± 1.20	13.10 ± 1.67
Unknown 14	25.94	2.03 ± 0.61	1.75 ± 0.88
Unknown 15	26.92	0.03 ± 0.02	0.08 ± 0.02
Unknown 16	31.52	0.56 ± 0.18	0.88 ± 0.67
α-farnesyl acetate	32.25	0.08 ± 0.03	0.10 ± 0.04
β-farnesyl acetate	32.82	0.11 ± 0.07	0.20 ± 0.21
Unknown 17	33.84	0.14 ± 0.05	0.28 ± 0.27

[a] Asterisks indicate significant differences between preferred and non-preferred clones, t-test, $P<0.05$.

by *L. occidentalis* because they are key stimuli in the selection of hosts by other conifer-infesting insects, e.g. spruce budworms (Muzika *et al.* 1993) and bark beetles (Wood 1972, Hynum and Berryman 1980, Moeck *et al.* 1981, Raffa and Berryman 1982, Schroeder and Eidmann 1987).

Neither mature cones nor foliage were attractive, a finding consistent with the hypothesis that *L. occidentalis* females use the odors from conelets at the time when they are open for pollination to select trees for oviposition. Terpene patterns from Douglas-fir foliage differ between provenance samples and have been shown to be genetically controlled and not influenced by ecological factors (von Rudloff 1971). As *L. occidentalis* was equally attracted to foliage volatiles from all clones tested, this may indicate that conelet instead of foliage volatiles are important indicators of host quality. Host quality may be manifested in either a quantity or ratio difference between volatiles from foliage and conelets. Using the conelets of preferred clones as indicators of adequate food resources would be an adaptive strategy. The conelets could be visually discernible from the foliage on the basis of their shape and reflectance spectra (Fig. 10). If the quality of the tree were poor, there might be a high rate of conelet abortion or fewer conelets produced. Orienting females would be able to determine the quality of the tree based on the quality of the conelets, and quickly decide whether to accept or reject that host.

Females were more responsive than males to the host volatiles. A similar differential response has also been shown for the cabbage seed weevil, *Ceutorhynchus assimilis*, (Evans and Allen-Williams 1992). As female *L. occidentalis* arrive in an orchard up to two weeks before males (pers. obs.), they are most likely to make the original host choice. This is evidently an adaptive strategy that ensures that females oviposit in the best potential habitat for their progeny. Because *L. occidentalis* is a strong flyer capable of traveling several kilometers (Schowalter 1984) females should have the capacity to survey a large section of an orchard and select the best possible hosts from the wide range of hosts available.

As neither physical characteristics nor olfactory cues could explain the host selection process of *L. occidentalis*, an integration of both is suggested. The number of documented cases of insects utilizing both olfactory and visual cues for host selection is growing and includes: *L. melania* (Roques 1987); apple maggots, *Ragoletis pomonella* (Aluja and Prokopy 1992 and 1993); tarnished plant bugs, *Lygus lineolaris* (P. de B.); European apple sawfly, *Hoplocampa testudinea* Klug. (Prokopy and Owens 1978); aphids (Pickett *et al.* 1992); and a leafhopper, *Dalbulus maidis* (DeLong and Wolcott) (Todd *et al.* 1990). That many other insect species utilize more than one criterion to evaluate a host indicates that

host selection by *L. occidentalis* and other insects may not always be based on a single stimulus, e.g. visual cues.

Appendix 2
Sex Pheromone

Introduction

Many insect species produce and utilize sex pheromones (Jacobson 1972, Silverstein 1981, Bailey 1991). In most documented cases females produce the sex pheromone and attract males (Jacobson 1972, Kuenen *et al.* 1994). Sex pheromones in the Hemiptera have proven difficult to demonstrate, challenging to isolate and identify, and inconvenient to utilize on a commercial level (Boivin and Stewart 1982, Campbell *et al.* 1990, Smith *et al.* 1991, Campbell *et al.* 1993, Smith *et al.* 1994). However, the synthetic sex pheromone for the mullein bug, *Campylomma verbasci* can be used effectively to monitor pest populations (McBrien *et al.* 1994), and shows promise for use in pheromone-based mating disruption (McBrien *et al.* 1996). There is no documented evidence for a sex pheromone in any *Leptoglossus* spp. Herein I argue, supported by some evidence, in favor of a female-produced sex pheromone.

Methods and Results

Field Studies

Live adult *L. occidentalis* were collected from the field in August 1992, maintained in the laboratory until the following spring, and then used to bait 12-unit, multiple funnel traps (Lindgren 1983). Ten adult insects were placed in a cylindrical wire cage (35 cm high, 14 cm diameter), provided with water and dried seed as food, and suspended in

a white mesh bag alongside a trap. Five experiments, each consisting of six replicates each of live males, females, males and females and an unbaited control, were conducted during June-September of 1993 and 1994 in two Douglas-fir orchards in B.C., and one each of sugar pine, *Pinus lambertiana* Dougl., western white pine, and ponderosa pine, *Pinus ponderosae* Dougl. ex. Laws, in the USA. Traps were checked every two weeks for the duration of the experiments and dead bugs in the bait bag replaced with live ones as required. Live insects were taken from the colony or caught from another field site. The cumulative catch for all five experiments was 65 bugs (25 females and 40 males) in 98 trap days, with the insects being caught equally in all treatments, including the controls.

Laboratory Studies

I utilized the Y-tube olfactometer to assess the response of adult males to live virgin females. Males and females were identified upon final molt from the fifth instar and placed in separate cages until utilized. A single female was placed in a small glass chamber with screened sides in one arm of the olfactometer, and the other arm was unbaited. One female served as the test stimulus for five males. Twenty-five individual virgin males were tested. Eleven males moved their antennae in apparent recognition of the female, and moved up the olfactometer. However, only five entered the arm of the Y-tube holding the female. In a reciprocal experiment, 13 of 25 individual virgin females moved up the tube to a virgin male, but only six entered the arm holding a male.

Video tape footage was obtained of the courtship behavior of six pairs of adults in a Petri dish. Virgin adult males were classed as 'excited' if they were observed extending their aedeagus in a holding cage containing only males. Previous attempts to observe courtship behavior utilizing males not exhibiting this behavior proved unsuccessful. A virgin adult female was placed in a plastic Petri dish (15 cm diam.). After a few minutes of walking around with her antennae waving the female would usually settle in a resting posture. An 'excited' male was then introduced into the Petri dish. Upon introduction, the male would turn toward the female and begin walking in her direction. Once within three-five centimeters of the female he would stop. If not already facing the male, the female would turn towards him and approach. On occasion either the male or female moved away at this point and returned in a few minutes. In some cases, a final approach followed by mounting was attempted, and the female would either allow it or push the male off her back. If the mount was successful, the pair would copulate for several minutes. Unsuccessful attempts resulted in the female moving away from the male. The male would then move towards the female again and stop about three-five centimeters away. After a few minutes, the female would then turn toward the male and approach. Mounting would again be attempted. If unsuccessful, the above sequence would be repeated. The female appeared to have the final choice of whether mounting would be successful or not.

Based on the behavior described above, I hypothesized that either sex could initiate courtship and that each was capable of eliciting the appropriate responses leading up to mounting and copulation. To test

this hypothesis, I used the Y-tube olfactometer again, only this time with a double-stimulus. If males were tested, I used a stimulus which consisted of males in a chamber upwind of females in another chamber and the air was drawn through both chambers and into the Y-tube arms. If the females were emitting a pheromone in response to male odor, then the test male would respond by choosing the arm with the double stimulus in it. To test females, I reversed the order of the stimuli so that females were upwind of males. Only one male and one female actually chose the stimulus; the remainder, 5 males and 5 females, chose the blank control. Some insects behaved in an excited manner possibly indicating recognition of another insect being present.

In the field, females were present in an orchard 2-3 weeks before males in 10 orchard-years of observations. This led to the hypothesis that females give off a pheromone only when in contact with an appropriate food source. I re-attempted the Y-tube bioassay using females on a cut Douglas-fir twig, suspended in a small water-filled vial, as a stimulus. Twenty-four of 30 males responded by orienting to the entrance of the arm but most eventually entered the unbaited control arm.

Discussion

Several observations suggest that it is most adaptive for female *L. occidentalis* to produce a sex pheromone. Females are the first to arrive in an orchard, are more responsive than males to preferred clones, and bear the greatest reproductive cost. Females thus would most likely release a sex pheromone after selecting a suitable host. Because males may not be able to select suitable oviposition hosts, it would be advantageous if mate finding were mediated by a female-produced sex pheromone. The failed field experiments suggest that if females do produce a pheromone, contact with the host is required prior to emitting it, similar to the release of aggregation pheromones following host selection by bark beetles (Raffa and Berryman 1983). All of these observations are consistent with a species in which females produce a sex pheromone.

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