# A SYNTHETIC SEX PHEROMONE FOR THE

# WESTERN HEMLOCK LOOPER, Lambdina fiscellaria lugubrosa (Hulst)

# (LEPIDOPTERA: GEOMETRIDAE)

by

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B.Sc.F. (Forestry), University of Toronto, 1979

## THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

# THE REQUIREMENTS OF THE DEGREE OF

## MASTER OF PEST MANAGEMENT

in the Department

of

**Biological Sciences** 

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# SIMON FRASER UNIVERSITY

July 1992

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Degree:

#### Master of Pest Management

Title of Thesis:

#### A SYNTHETIC SEX PHEROMONE OF THE WESTERN HEMLOCK LOOPER, LAMBDINA FISCELLARIA LUGUBROSA (HULST) (LEPIDOPTERA: GEOMETRIDAE)

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Title of Thesis/Project/Extended Essay A SYNTHETIC SEX PHERONOME FOR THE WESTERN TEMLOCIC Looper, Lambdina fiscellaria inqubroza (Hult) (LEPIDOPTERA: GEOMETRIDAE)

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#### ABSTRACT

Candidate sex pheromone components for the western hemlock looper, Lambdina fiscellaria lugubrosa (Hulst), were field tested in the interior of British Columbia to determine the blend most effective in attracting male moths. Plastic traps were baited with different combinations, doses and ratios of 5,11-dimethylheptadecane; 2,5-dimethylheptadecane; 7-methylheptadecane and 5-methylheptadecane, in a series of 15, randomised complete block experiments. The experiments showed the most effective lure to consist of 100  $\mu$ g of each of 5,11-dimethylheptadecane; 2,5dimethylheptadecane and 7-dimethylheptadecane. 5,11-Dimethylheptadecane was attractive alone and is the most important single pheromone component; 2,5dimethylheptadecane and 7-methylheptadecane are synergists, together raising the catches of males up to 7 times better than to 5,11-dimethylheptadecane alone. Hourly monitoring of traps baited with all four candidate pheromones, at a load of 100 µg per lure, showed daily flight to begin within less than one hour, and peak two to seven hours, after sunset, depending on local temperature and weather. The results indicate that developmental research can proceed on use of the 3-component synthetic pheromone for monitoring and control of the western hemlock looper.

# DEDICATION

To my husband and to my parents.

#### ACKNOWLEDGEMENTS

Work in Chemical Ecology requires a team approach and the efforts of many people have made this research possible. Throughout my involvement in this project, and the MPM program as a whole, Dr. John Borden has provided moral support. laughter, and an open and quite wonderful learning environment. I thank Drs. Keith Slessor and Roy Shepherd and, in particular, Gerhard Gries, for their guidance and support. I also thank Drs. B. Staffan Lindgren and Dan Miller, Phero Tech Inc., for advice, and Ms. Leslie Chong and, especially, Ms. Regine Gries for assistance. Mr. J. Li synthesised and provided the candidate pheromones. Dr. Alan Van Sickle, Messrs. Rod Turnquist, Alan Stewart and Archie McConnachie and Ms. Lorraine Maclauchlan provided information on western hemlock looper infestations. The interest and support of Ms. Heather Ward, manager, and the staff of the Frontier Motel, Revelstoke, and the hospitality of Mr. and Mrs. Wilf and Val Mourre, of the Blue River Motel, Blue River, made my visits to those towns memorable. I especially thank my employers, Mr. Peter Putland, C.E.O., and Phero Tech Inc., who encouraged and supported my participation in advanced studies in pest management. Without such support my involvement in this project would not have been possible. This research was also supported by Forestry Canada and the Natural Sciences and Engineering Research Council of Canada.

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#### **1.0 INTRODUCTION**

The western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst), is a periodically important insect pest of certain forest types of British Columbia. Outbreaks are typically shortlived and collapse within two to three years. They are intense, can quickly affect large areas, and cause considerable mortality and aesthetic damage (Kinghorn 1954). Forest managers need a system by which they can detect population trends before the appearance of major infestations. Such a predictive system would allow managers adequate lead time to consider and implement management tactics to prevent outbreaks or minimise their impact.

# 1.1 Lambdina fiscellaria IN NORTH AMERICA

Lambdina fiscellaria (Guen.) is considered to be a transcontinental species, comprised of three subspecies (Furniss and Carolin 1977; McGuffin 1987). Two of these, Lambdina fiscellaria lugubrosa (Hulst), the western hemlock looper, and L. fiscellaria fiscellaria (Guen.), the eastern hemlock looper, are capable of sizeable periodic outbreaks on a variable and as yet unpredictable basis.

The eastern hemlock looper is a major forest pest in the maritime regions of Eastern North America, but ranges west to Alberta. Its preferred host in Newfoundland, the Maritimes, Québec and Maine, is balsam fir, *Abies balsamea* (L.) Mill. In Ontario, Wisconsin and south of Maine, it prefers eastern hemlock, *Tsuga canadensis* (L.) Carr. During outbreaks, various other coniferous and deciduous tree and shrub species may also be attacked (McGuffin 1987).

The western hemlock looper is found in the coastal forests of Alaska, British Columbia, Washington, and Oregon, and the mountainous areas of south-central British Columbia, northern Idaho, western Montana and northeastern Oregon. Throughout much of its range its preferred host is western hemlock, *Tsuga heterophylla* (Raf.). True firs, *Abies* spp., are favoured in northern Idaho and western Montana. In south-central British Columbia, western red cedar, *Thuja plicata* Donn., is attacked as readily as western hemlock. During outbreaks the looper will feed, in all parts of its range, on: true firs; western red cedar; Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco; spruces, *Picea* spp.; western white pine, *Pinus monticola* Dougl.; western larch, *Larix occidentalis* Nutt; and associated deciduous trees and shrubs (Dewey et al. 1972; Prebble 1975; McGuffin 1987; Turnquist 1991).

*Lambdina fiscellaria somnaria* (Hulst), the third subspecies, is found on *Quercus* spp. in British Columbia and Oregon and is of minor consequence (Furniss and Carolin 1977; McGuffin 1987).

# **1.2 THE WESTERN HEMLOCK LOOPER**

#### 1.2.1 Biology and Life Cycle

The western hemlock looper overwinters as eggs that hatch in late May to mid-June; there are six larval instars (Erickson 1984). Early instars are attracted to sunshine and light and prefer to feed in the upper crowns of host trees. Feeding is light at this stage and apparently restricted to newly opened buds. The young larvae

are highly mobile within tree crowns, both vertically, via silken threads, and around their circumference. Older instars feed more heavily, on all ages of foliage, and defoliation increases in the lower crown as the upper crown foliage becomes depleted. The greatest volume of foliage is consumed by the fifth and sixth instars. Feeding damage is intensified by the fact that the larvae feed at the bases of the needles, causing the fall and waste of substantially higher volumes of foliage than they can consume (Thomson 1957; Erickson 1984; Turnquist 1991).

Pupation generally takes place from early August to early September and lasts 10-15 days. Pupae are found in lower branches, in moss and crevices on the lower boles, and under forest floor debris (Erickson 1984; Turnquist 1991). In outbreaks they are found on all available surfaces and may not be attached to anything (Hopping 1934).

Males outnumber females at the beginning of eclosion (Hopping 1934). Females can be found on lower tree trunks and tend to drop to the ground if disturbed. Males fly readily and walk up and down lower boles within 3 m of the ground, particularly within 1 m of the ground (Ostaff et al. 1974a).

Females in laboratory cages generally eclosed shortly after dark and were capable of calling males the same night. Virgin and mated females provided with 10% sucrose solution as a food source survived an average of 20.8 and 18.1 days, respectively. Mated females laid an average of 109 eggs, 90 % of them within 6 days of mating. Few males under 0.5 days old mated, but mating frequency increased after one day and peaked 2-4 days after eclosion (Ostaff et al. 1974b).

Females oviposit singly and in clusters of 2 to 10 on a variety of substrates (Hopping 1934; Meyers & Livingstone 1973). On the coast, mosses growing on the bole at mid-crown are the preferred substrate, while "witches hair", or "old man's beard" lichens (*Alectoria* spp. and *Usnia* spp.) are preferred in interior forests (Meyer & Livingstone 1973; Shore 1990; Turnquist 1991). Eggs also occur on needles, the bark of branches and trunks, foliose lichens and understorey species. Oviposition continues throughout adult flight, which usually occurs from mid-August to mid-October, but sometimes starts as early as mid-August and ends as late as November (Dewey et al. 1972; personal observation).

## 1.2.2 Outbreaks and Associated Forest Types

The western hemlock looper is found south of latitude 56°N in British Columbia, where outbreaks have historically been heavily concentrated in the Interior Cedar Hemlock and, Coastal Western Hemlock biogeoclimatic zones. Similar forest types are associated with past outbreaks in the susceptible interior areas of Idaho, Montana and Oregon, and the coastal Pacific Northwest. In British Columbia, outbreaks have occurred in five of the province's six forest regions, and have also included parts of the interior Douglas-fir and Sub-boreal Spruce biogeoclimatic zones (Turnquist 1991).

Outbreaks in British Columbia and elsewhere have been closely associated with mature to overmature, old growth forests; outbreaks in younger stands are rare (Dewey et al. 1972; Prebble 1975; Furniss & Carolin 1977; Harris et al. 1982).

#### 1.2.3 Outbreak Dynamics

Western hemlock looper population density can rise quickly to outbreak levels. Larval feeding can cause extensive mortality of trees after only one or two years of noticeable feeding. This is probably due to its wasteful feeding habits, the fact that it feeds throughout the crown, and its ability to feed on foliage of all ages. In the first year of an epidemic, defoliation may not become apparent until late summer, as the larger instars appear and feeding intensifies. Outbreaks normally last 3 to 4 years and crash quickly, due to natural causes (Thomson 1957; Prebble 1975; Dewey et al. 1972).

Infestations can be intense and localised or may occur over large areas involving tens of thousands of hectares. Because the western hemlock looper is generally confined to specific forest types and geographic areas, outbreaks tend to reappear in the same general locations. Fourteen outbreaks occurred in British Columbia between 1911 and 1990: three on Vancouver Island, between 1913 to 1947; six on the lower, south coast, between 1911 and 1988; and seven in the interior wet belt and upper Fraser areas, between 1937 to 1990 (Turnquist 1991). In the summer of 1991, outbreaks of the looper covered more than 86,235 ha in the Cariboo, Nelson and Kamloops Forest regions (Wood & Van Sickle 1992).

The outbreaks are short lived and collapse rapidly due, in part, to an extensive complex of natural enemies. Forty-seven parasitoid species have been recorded in western hemlock looper in British Columbia. Parasitoids of eggs and larvae appear to be the most important, with the diseases, *Entomophthora* spp., and a nuclear polyhedrosis virus, also playing significant roles (Turnquist 1991). Adverse

weather, such as heavy rain during the flight period, and competition for food, can also contribute to outbreak collapse (Dewey et al. 1972; Furniss & Carolin 1977).

## 1.2.4 Damage

During outbreaks the western hemlock looper kills trees over limited, welldefined areas (Harris et al. 1982). Studies conducted by Forestry Canada in 1929-30 and 1944-47, on trees affected in two coastal outbreaks, showed that completely defoliated trees died rapidly. Half the trees suffering over 80 % defoliation died; and 20-50 % of those suffering 50-75 % defoliation died. In both studies the weakening of significantly defoliated, but surviving trees, led to important infestations by secondary insects, such as *Tetropium velutinum* Lec. and *Pseudohylesinus tsugae* Sw., leading to further mortality in the second and third years after outbreak collapse (Turnquist 1991). Table 1 summarizes recorded western hemlock looper outbreaks and mortality in British Columbia and western United States forests, from 1889 to 1991.

#### 1.2.5 Controls

There are no proven forest management practices available to prevent or control western hemlock looper outbreaks. Aerially applied chemical insecticides have been used to control the insect. Calcium or lead arsenate were used in the first part of the century; DDT, applied in fuel oil, in the 1950's; and phosphamidon, operationally in Washington and Oregon, and experimentally in British Columbia, in the 1960's (Hopping 1934; Thomson 1957; Prebble 1975; Turnquist 1991).

# Table 1.Recorded western hemlock looper outbreaks and mortality, 1889-1991,In British Columbia, Washington, Oregon, Montana & Idaho

Compiled from: Prebble (1975), Furniss & Carolin (1977), Turnquist (1991), Wood & Van Sickle (1992).

Criterion	Year	Vancouver	Lower Mainland,	Interior Wet Belt,	B.C. Total	USDA, For. Ser.,Reg.6	USDA, For Ser.,Reg.1
assessed	rear	island,	•	•	TUIdi	(Wa./Or.)	(Mt./ld.)
		B.C.	B.C.	B.C.		(vva./OI.)	(1011,/10,)
Extent of	1889-91	-	-	-	-	No est.	-
defoliation	1911-14	extensive	Stan.Park	-	extensive	-	-
	1918-22	-	-	-	-	10,927	-
(area in ha, if	1925-26	small	•	-	small	-	-
data available)	1928-30	-	3,730	-	3,730	21,044	-
	1937-38	-	-	32,800	32,800	-	extensive
	1944-47	230,000	scattered	40,500	270,500	high	extensive
	1954-56	-	-	48,000	48,000	•	-
	1958-59	-	Stan.Park	-	Stan.Park	-	-
	1963-64	-	-	146	146	outbreaks	-
	1969-73	•	260	-	260	-	
	1972-76	-		38,745	38745	-	No est.
¥.	1982-84	-	•	46,055	46,055		<b>-</b> '
	1987-88	-	90	-	90	minor <sup>b</sup>	-
	1990-91	-	•	86,235	86,235	•	-
Volume losses	1889-91	•	-	-	•	no est.	•
to mortality	1911-14	no est.	no est.	-	no est.	-	-
	1918-22	-	-	-	•	1,180,000	-
(given in	1925-26	no est.	-	-	no est.	•	-
cubic metres	1928-30	•	no est.	-	no est. 🕤	472,000	-
if data	1937-38	-	•	high	high	•	extensive
available)	1944-47	1,180,000	no est.	high	>1,180,000	94,390	extensive
	1954-56	-	•	no est.	no est.	-	-
	1958-59	-	no est.	-	no est.		•
	1963-64	-	-	no est.	no est.	none <sup>C</sup>	-
	1969-73	-	no est.	-	no est.	-	-
	1972-76	-	-	no est.	no est.	-	nil
	1982-84	-	-	no est.	no est.	-	•
	1987-88	-	no est.	-	no est.	minor	•
	1990-91	•	•	no est.	no est.	-	-

a J.E. Dewey, USDA, Forest Service, Region 1, Missoula, Montana, pers. comm..

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b D. Bridgewater, USDA, Forest Service, Region 6, Portland, Oregon, pers. comm..

c Chemical controls applied: D. Bridgewater, USDA, Forest Service, Region 6, Portland, Oregon, pers. comm..

Timing of direct control is important since western hemlock looper outbreaks appear so quickly and are so short lived. In the absence of an accurate predictive monitoring system, considerable damage can occur before controls are applied. Awareness that the insect is present at problem levels usually arrives with the defoliation caused by the larger instars towards late summer, in the first year of an outbreak. Direct controls have been applied in the second or third year, to prevent further spread of infestations, but have often been applied too late to prevent significant mortality.

There have been no outbreaks requiring treatment in the western United States since the 1960's; although significant outbreaks have occurred in British Columbia during the last 30 years, none has been treated. In eastern maritime Canada, where the eastern hemlock looper is a major problem, fenitrothion, and the more environmentally acceptable *Bacillus thuringiensis* Berliner, have been used, alone and together, to treat outbreaks. Dimilin, an insect growth regulator, has also been successfully applied experimentally against larvae, but has not been registered for use against the eastern hemlock looper (Raske et al. 1986; R.J. West, Forestry Canada - Newfoundland & Labrador Region, St. John's, Nfld.,pers. comm.).

The cessation of control attempts in British Columbia reflects the changed socio-political climate with respect to the use of chemical pesticides, the absence of environmentally acceptable alternative means of control, the lack of an early warning system allowing time for planning and funding, and the absence of outbreaks in high value coastal forests. In the last 30 years, infestations have been concentrated in economically low value, old growth stands of the Interior Cedar Hemlock

biogeoclimatic zone. When outbreaks have occurred, salvage logging has been used to recover timber, reduce fire hazard and prepare the sites for reforestation.

#### 1.2.6 Current Survey and Monitoring Practices

A number of sampling techniques have been investigated in an attempt to develop a predictive index of rising western hemlock looper numbers and potential epidemics. Estimates of the quantities of frass produced by larvae, as well as egg, larval and pupal numbers, and egg viability, have been examined (Shore 1990).

The only technique currently available to detect rising western hemlock looper infestations is the standard tree beating method (Harris et al. 1972). This method is used to survey different species of trees for pest insects at permanent sample plots across the province. A 2.75 m -long pole is used to beat insects onto a 2.10 x 2.75 m cloth spread under the sample tree. These surveys have been run annually in British Columbia by Forestry Canada's Forest Insect and Disease Survey (FIDS) unit since 1911. Accumulated FIDS data show that defoliation by western hemlock loopers may be expected to occur at interior sites the year following one in which 64 % or more of the beating collections for a drainage are positive, with at least 8 larvae per collection. On the coast, defoliation may occur after a year producing 31% or more positive samples and 3 or more larvae per collection (Turnguist 1991). The tree beating method is laborious and requires that the collected insects be separated, taxonomically identified and tallied. In addition, the method is not always reliable. For example a 36,235 ha outbreak, in 1991, in the North Thomson River drainage, was not detected and anticipated by tree beating surveys in 1990.

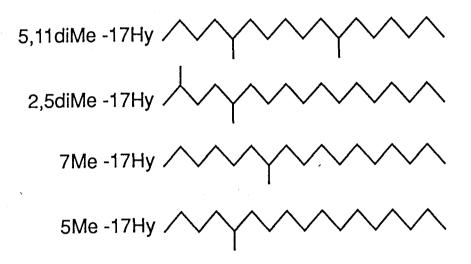
Aerial surveys are run annually by Forestry Canada to locate and map defoliation. Egg surveys are started once defoliation has been detected in an area. They are run to determine viable egg numbers, levels of egg parasitism, and the likelihood of defoliation in the subsequent year. Experience in British Columbia has shown that outbreaks are likely to collapse once a minimum of 30 % of the eggs are parasitised (Turnquist 1991). The method is tedious and laborious and involves the collection of an adequate volume of egg bearing substrate, i.e. lichens and mosses, from which the eggs are separated, collected, tallied and reared.

A trap developed for the eastern hemlock looper, has recently been investigated in an effort to replace the egg sampling method. It involves the "trapping" of western hemlock looper pupae in burlap strips, wrapped 2-3 times around the base of susceptible trees. The number of viable pupae per trap will be related to the number of healthy eggs subsequently laid on a sample tree, in an attempt to develop a dependable, predictable index (Shore 1989; Turnquist 1989).

#### 1.2.7 Sex Pheromones

Using coupled gas chromatographic-electroantennographic assays (GC-EAD), Gries et al. (1991) identified a series of candidate lepidopteran pheromone components for the eastern hemlock looper. These are: 5,11-dimethylheptadecane; 2,5-dimethylheptadecane; 7-monomethylheptadecane; and 5-monomethylheptadecane (5,11; 2,5; 7; and 5; respectively) (Fig. 1).

Figure 1 Mono- and dimethylheptadecanes present in female *L. fiscellaria lugubrosa* pheromone glands, as determined by Gries and associates (Simon Fraser University, Burnaby, B.C., pers. comm.). "Hy" refers to heptadecane.



In 1990, field testing in Newfoundland revealed that racemic 5,11 was attractive alone and was synergised by 2,5. Neither of the mono-methylheptadecanes affected the capture of males in traps (Gries et al. 1991). Ratio, dose and stereoisomer experiments involving 5,11 and 2,5, were carried out in 1991 by Forestry Canada, Newfoundland.

Employing GC-EAD analyses for the western hemlock looper, Gries and his associates (Simon Fraser University, Burnaby, B.C., pers. comm.) determined that the same four compounds identified in the eastern hemlock looper also occur in the western hemlock looper. As in the eastern hemlock looper (Gries et al. 1991), all four compounds were below the detection level of the gas chromatograph and were detected solely on the basis of electroantennagram responses. Therefore, the natural ratios of the compounds are unknown.

# 2.0 OBJECTIVES

My primary objective was to determine, through field bioassays, the attraction of the western hemlock looper to 5,11, 2,5, 5 and 7, alone and in combination and at different ratios of lure loading. The diel periodicity of field population response to pheromone sources was also studied.

## 3.0 MATERIALS AND METHODS

#### 3.1 Location

Field testing of candidate pheromone components of the western hemlock looper took place from mid-August to mid-October 1991, in the Interior Cedar Hemlock biogeoclimatic zone (Meidinger and Pojar 1991), of the interior wet belt of south-central British Columbia.

Experiments (Exp.) 1-8 were run in the Columbia River drainage, 70 km north of Revelstoke, along Highway 23. Forests of the area are comprised of old growth stands with a history of western hemlock looper outbreaks. FIDS surveys identified three small infestation centres covering 915 ha, in 1990. Downie Creek was chosen as the best of the three sites due to accessibility, a south-facing shoreline and an expected moderate to high population of moths. Cool wet weather through the early and mid-summer months raised concerns that the adult looper flight would be delayed and made the possibility of earlier emergence on the south-facing shoreline an asset.

Exp. 9-15 were run in the North Thomson River drainage, 26 km south of Blue River, along Highway 3. Forests of the area are comprised of old and second growth stands, and the area as a whole has a history of western hemlock looper infestations. A severe and unanticipated outbreak covering 36,235 ha erupted in the drainage in 1991. Finn Creek was chosen as a suitable experimental site, due to accessibility and the presence of a moderate looper population. Exp. 16 was run in Blue River, close to the centre of a severe local infestation.

## 3.2 Experimental Methods

High capacity, green, plastic Unitraps (Phero Tech Inc., Delta, B.C.) were used in all experiments. One dichlorvos cube (Green Cross Co., Mississauga, Ontario), approximately 1 cm<sup>3</sup>, was placed in each trap to ensure that moths were

killed quickly and kept in good condition for later identification and counting. Traps were suspended from tree branches, 1-2 m above ground, and 15-20 m apart. Traps were placed within 0-4 m of the south-facing forest edge at Downie Creek (Exp. 1-8); within 0-1 m of the west-facing forest edge at Finn Creek (Exp. 9-15); and were hung from three lodgepole pine trees, 20-25 m apart, in a residential area of Blue River in Exp. 16.

Guard traps were placed at the end of the trap lines for Exp. 1-6, in order to avoid bias in catches of experimental traps located at the upwind end of each trapping line (R.F. Shepherd, Forestry Canada - Pacific & Yukon Region, Victoria, B.C., pers. comm.). These were baited with 100  $\mu$ g of 5,11 and 100  $\mu$ g of 2,5. Guard traps were not used in Exp. 7-15 due to a limited supply of pheromone.

The traps were cleaned, before being transferred from Downie Creek to Finn Creek, to remove any pheromone possibly adhering to the traps. All parts were washed, scrubbed in a soap, ammonia and water solution, rinsed in water, air dried and rinsed three times in hexane.

Trap lures consisted of red rubber septa (Thomas Scientific, Swedesboro, N.J., 08085, U.S.A.) impregnated with candidate pheromone components in HPLCgrade (high pressure liquid chromatography) hexane. They were fastened to the underside of the trap lid with colour-coded pins to distinguish between treatments. Lures for different treatments were stored in separate glass jars, until their attachment to lure holders and placement in pre-hung traps. The septa were not touched as they were placed into the traps, to avoid contamination of trap surfaces and contamination between treatments. Exp. 1-15 followed a randomised complete block design (Table 2). Traps without lures were used as the control treatment in each experiment. Some experiments were repeated as new experiments after the treatments were completely rerandomised by relocating each lure-trap combination.

Exp. 1-11 tested the four candidate pheromone components alone and in various combinations. Exp. 1-3 duplicated a 1990 experiment for the eastern hemlock looper (Gries et al. 1991). All four candidate components were tested alone and as a quarternary blend. Exp. 4-6 duplicated a second 1990 Newfoundland experiment, testing the components in all possible binary, ternary and quarternary combinations. Exp. 7-8 were designed to evaluate interactions between 5,11 and the other three components. Apparent lack of treatment discrimination in Exp. 7-8, due to outbreak conditions, forced the move from Downie Creek to the more moderately infested Finn Creek. Exp. 9-11 repeated Exp. 7-8, but included a "contaminated control" treatment; this involved blank traps previously baited in Exp. 7-8 with lures containing 5,11.

Exp. 12 tested the attraction of increasing doses of 5,11, alone, relative to the increasing doses of the quarternary blend. Exp. 13-15 evaluated doses of the four components at a 1:1:1:1 ratio, relative to descending, equivalent doses of all but 5,11, for which the dose was held constant.

Moths collected from individual traps were emptied into prelabelled, plastic zip top bags, sorted by replicate block and stored in coolers in the field. Moths not counted at the end of the day, were frozen until counting could take place.

Statistical analyses were conducted as outlined by Zar (1984) and Day and

<b>F</b>	Treat		<u></u>		Oti	
Exp.	-ment				Stimulus	Thur a stat dealer
	No.	Ireatment	Components		load (ug)	Experimental design
1-3	4	5,11 2,	57	5	400	Randomised complete block, N=10,
1-3	1 2			5	100	at Downie Creek. Exp. 1 set up
	2 3	2,			100	24 Aug., 1991. Rerandomised and
	4		5 7		100	run as Exp. 2 and 3 on 25 and 26
	5			5	100	Aug., respectively.
	6	Unbaited o		5		Aug., Tespecitively.
		Chibaliou co				
4-6	1	5,11 2,	57	5	400	Randomised complete block, N=10,
40	2	5,11 2,			300	at Downie Creek. Exp. 4 set up
	3	5,11 2,		5	300	26 Aug., 1991. Rerandomised and
	4	_ · · ·	- 7	5	300	run as Exp. 5 and 6 on 27 and 28
	5	5,11 2,			200	Aug., respectively.
	6		7		200	
	7			5	200	
	8	2,		5	300	
	9	2,			200	
	10	2,		5	200	
	11		- 7	5	200	
	12	Unbaited o	ontrol			
7-8	1	5,11 2,	57	5	400	Randomised complete block at
	2	5,11 2,	57	-	300	Downie Creek. Exp. 7, N=12, set
	3	5,11 2,	5	5	300	up 5 Sept., 1991. Rerandomised,
	4	5,11	7	5	300	added to, and rerun as Exp. 8,
	5	5,11 2,	5		200	N=18, 6 Sept
	6	5,11	7		200	
	7	•,		5	100	
	8	5,11	•••••		100	
	9	Unbaited o	ontrol			·
	, I			_		Deadard
9-11	1	5,11 2,		5	400	Randomised complete block, N=10,
	2	5,11 2,		-	300	at Finn Creek. Exp. 9 set up 16
	3	5,11 2,		5	300	Sept., 1991. Rerandomised and
	4	5,11	7	5	300	run as Exp. 10 and 11 on 17 and
	5		5	( <b>1</b> -10)	200	18 Sept., respectively.
	6	5,11	7		200	
	7	5,11		5	200	
	8	5,11	••• •••		100	
	9 Unbaited control					
:_	10	"Contamin	ated" contro		<u> </u>	<u> </u>

# Table 2. Treatments and design of Exp. 1-15

# Table 2 - continued.

Exp.	Treat		Stimulus Loa	ad (ug)		Total	
	-ment			_		Stimulus	Experimental design
	No.	5,11	2,5	7	5	Load (ug)	
12	1	0.01	_	_		0.01	Randomised complete block,
	2	0.10				0.10	N=7, at Finn Creek. Set up
	3	1.00	-	-		1.00	28 Sept., 1991.
	4	10.00	-			10.00	
	5	100.00	· _			100.00	
	6	1000.00		-		1000.00	
	7	0.01	0.01	0.01	0.01	0.04	
	8	0.10	0.10	0.10	0.10	0.40	
	9	1.00	1.00	1.00	1.00	4.00	
	10	10.00	10.00	10.00	10.00	40.00	
	11	100.00	100.00	100.00	100.00	400.00	
	12	1000.00	1000.00	1000.00	1000.00	4000.00	
	13	Unbaited cor	ntrol				
13-15	1	100	100	100	100	400	Randomised complete block,
10-10	2	100	100	10	10	130	N=10, at Finn Creek. Exp.
	3	100	1	10	10	121	13 set up 20 Sept., 1991.
	4	100	10	1	10	121	Rerandomised and run as
	5	100	10	10	1	121	Exp. 14 and 15 on 21 and 22
	6	100	10	1		112	Sept., respectively.
	7	100	.0	10		112	
	8	100	1		10	112	
	9	100	1	1	1	103	
	10	Unbaited cor	ntrol	•	'		

Quinn (1989). Data for the majority of experiments were transformed by  $Log_{10}(x+1)$ , prior to analysis, to maximise homogeneity of variance. For Exp. 9-11, data were transformed by  $\sqrt{x+0.05}$ . A preliminary analysis was undertaken in which analysis of variance (ANOVA) was carried out on all data for each of Exp. 1-3, 4-6, 7-8, 9-11 and 13-15. There proved to be significant interaction between the variables "time" and "treatment" for Exp. 1-3, 4-6, 9-11 and 13-15. To ensure that treatment effects were not masked by variability created by collecting the moths over time, Exp. 1-15 were subsequently treated as separate experiments and individually subjected to analysis of variance and Tukey's multiple comparison test.

Exp. 16 used traps baited with 100 µg of each of the four components, to determine diel flight activity of male western hemlock loopers. Hourly collections were made from three traps over 24 h on 19-20 September, and from two traps, for periods of varying length, on 22-24 September. Ambient temperature was recorded from a thermometer suspended in a shaded location central to the three traps. Wind and cloud conditions were also recorded. Sunset and sunrise tables for the latitude and longitude of Blue River were obtained from the Canadian Atmospheric Environment Service (AES). These tables assume flat topography and zero elevation above sea level. Therefore, sunset may have occurred earlier than indicated by AES data.

#### 4.0 RESULTS

In Exp. 1-3 (Fig. 2) 5,11 was significantly more attractive than any other single candidate component. In two of the three experiments the quarternary blend

was significantly more attractive than any single component, including 5,11.

In Exp. 4-6 (Fig. 3) treatments lacking 5,11 were significantly less attractive than those in which it was present. The quarternary blend captured the greatest number of males, but was not significantly different from 5,11+2,5+7. Ternary or binary combinations of 5,11, with either 2,5+5, or 7+5, or 2,5, or 7, formed the second most attractive group of stimuli, and were never significantly different from each other. The least attractive binary combination containing 5,11 was 5,11+5. Responses to treatments containing 5,11+2,5 or 5,11+7 were not improved or diminished significantly when 5 was added.

There was no discrimination between treatments of Exp. 7-8 (Fig. 4), except that catches of all baited traps were significantly higher than those of unbaited controls. When Exp. 7-8 were repeated in another location, as Exp. 9-11, the quarternary blend and 5,11+2,5+7 once again proved to be the most attractive treatments and were never significantly different from each other (Fig. 5). As in Exp. 4-6, catches to 5,11+7 and 5,11+2,5 were never significantly different, suggesting that the two synergists may be redundant when combined in binary blends with 5,11. The addition of 5 to 5,11+2,5 or 5,11+7 again failed to increase or decrease catches, and again consistently produced the least attractive of the binary combinations involving 5,11.

Increasing pheromone quantities tested in Exp. 12 (Fig. 6) produced increasing trap catches. The attractiveness of 5,11 rose as its dose increased from 0.01 to 1000  $\mu$ g. The same pattern was seen with the quarternary blend. The quarternary blend at 100 and 1000  $\mu$ g was far more attractive than any other

stimulus and these treatments were not significantly different from each other.

In Exp. 13-15 (Fig. 7), the quarternary blend composed of 100  $\mu$ g per component was consistently more attractive than any other treatment. Attractiveness decreased when the dose of 2,5 or 7 or 5 was lowered.

Catches of male western hemlock loopers in Exp. 16 were negligible during daylight hours (Fig. 8). Catches started to rise within 1 h of sunset, by 1800-1900 h, peaked by 2000-2400 h, and dropped rapidly thereafter. Nocturnal air was generally still, with occasional light breezes. Peak catches occurred at different times and temperatures. On 20 September the peak occurred at 2400 h and 12°C. The evening of 21 September was clear and cloudless and temperatures fell to -1°C; a very small number of moths formed the peak catch at 2000 h and 5°C. The evening weather of 22 September was cool and overcast with occasional drizzle and light breezes; the peak catch appeared at 2000 h and 7°C. On 23 September the air was still and peak catch appeared at 2300 h and 9°C.

Figure 2 Mean catches of western hemlock looper males in Unitraps in Exp. 1 3, Downie Creek, B.C., 25-27 August, 1991. Means followed by the same letter are not significantly different, P<0.05, Tukey's test on data transformed by Log<sub>10</sub>(x+1). Untransformed data and their standard errors are presented. N=10 for each experiment.

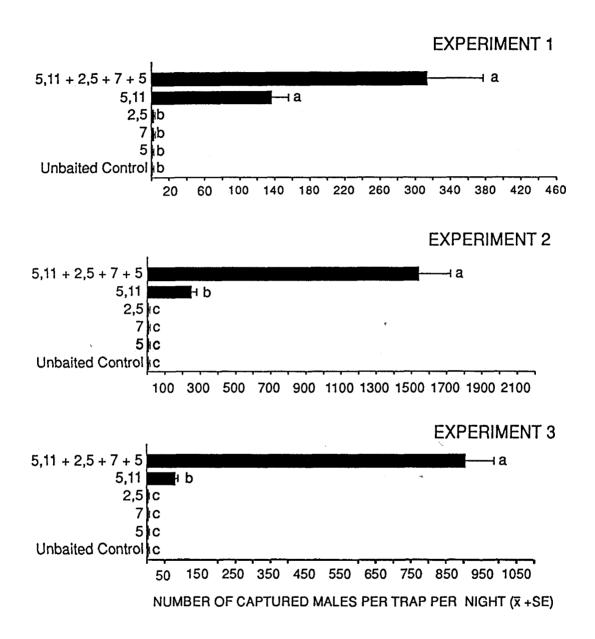


Figure 3 Mean catches of western hemlock looper males in Unitraps in Exp. 46, Downie Creek, B.C., 27-29 August, 1991. Means followed by the same letter are not significantly different, P<0.05, Tukey's test on data transformed by Log<sub>10</sub>(x+1). Untransformed data and their standard errors are presented. N=10 for each experiment.

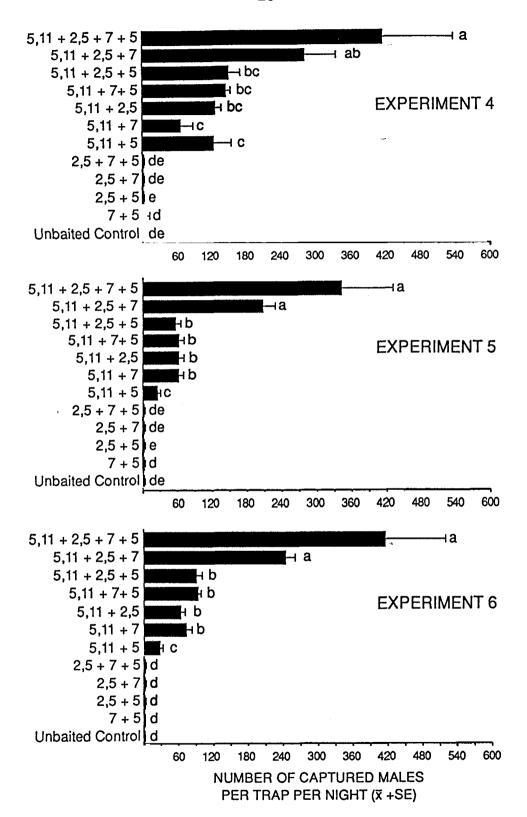


Figure 4

Mean catches of western hemlock looper males in Unitraps in Exp. 7-8, Downie Creek, B.C., 6-7 September, 1991. Means followed by the same letter are not significantly different, P<0.05, Tukey's test on data transformed by  $Log_{10}(x+1)$ . Untransformed data and their standard errors are presented. N=12 for Exp. 7; and 18 for Exp. 8.

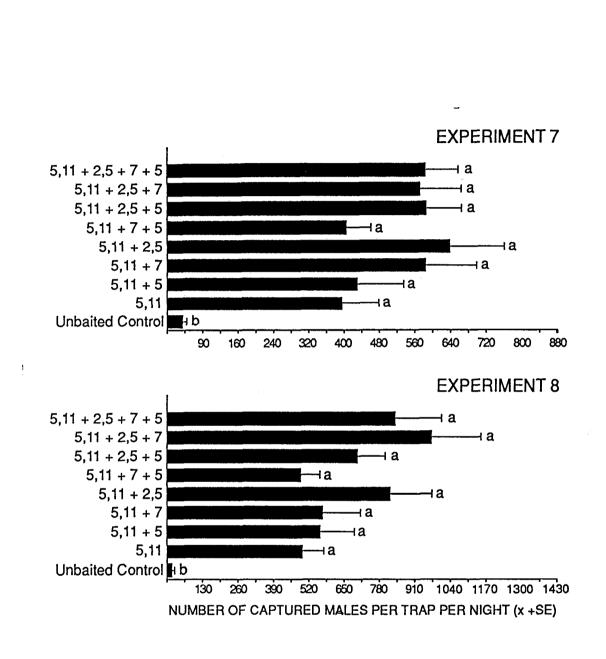


Figure 5 Mean catches of western hemlock looper males in Unitraps in Exp. 9-11, Finn Creek, B.C., 17-19 September, 1991. Means followed by the same letter are not significantly different, P<0.05, Tukey's test on data transformed by  $\sqrt{x+0.05}$ . Untransformed data and their standard errors are presented. N=10 for each experiment.

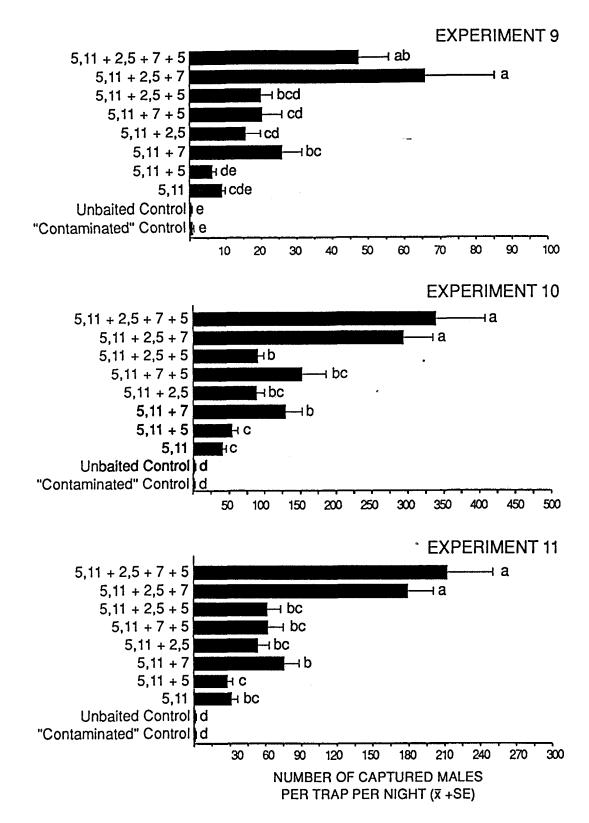


Figure 6 Mean catches of western hemlock looper males in Unitraps in Exp. 12, Finn Creek, B.C., 28-30 September, 1991. Means followed by the same letter are not significantly different, P<0.05, Tukey's test on data transformed by Log<sub>10</sub>(x+1). Untransformed data and their standard errors are presented. N=10 for each experiment.

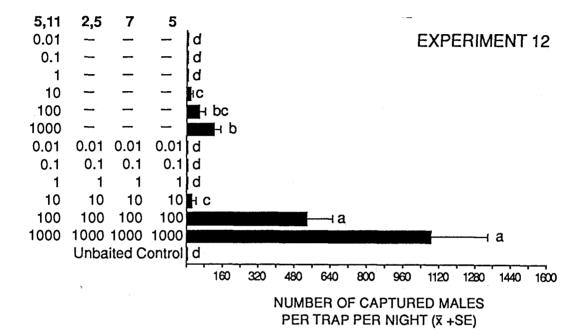


Figure 7 Mean catches of western hemlock looper males in Unitraps in Exp.13-15, Finn Creek, B.C., 21-23 September, 1991, Means followed by the same letter are not significantly different, P<0.05, Tukey's test on data transformed by Log<sub>10</sub>(x+1). Untransformed data and their standard errors are presented. N=10 for each experiment.

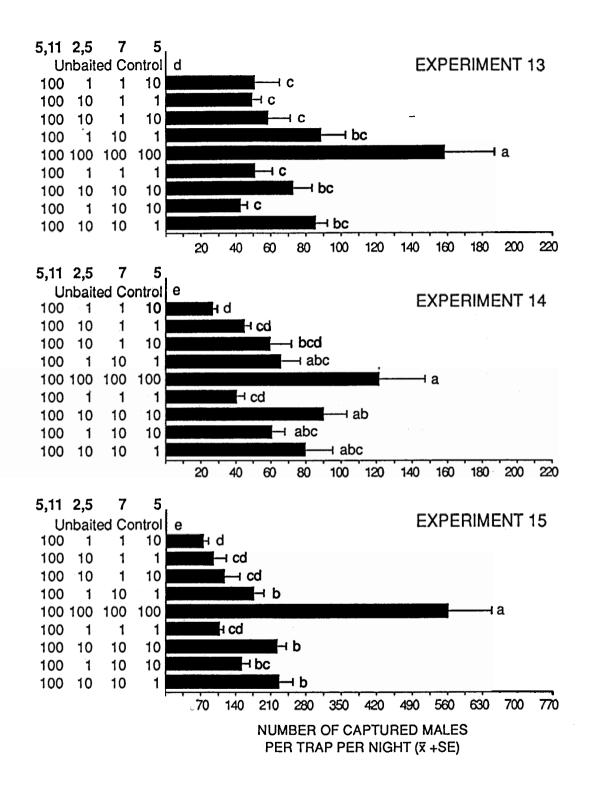
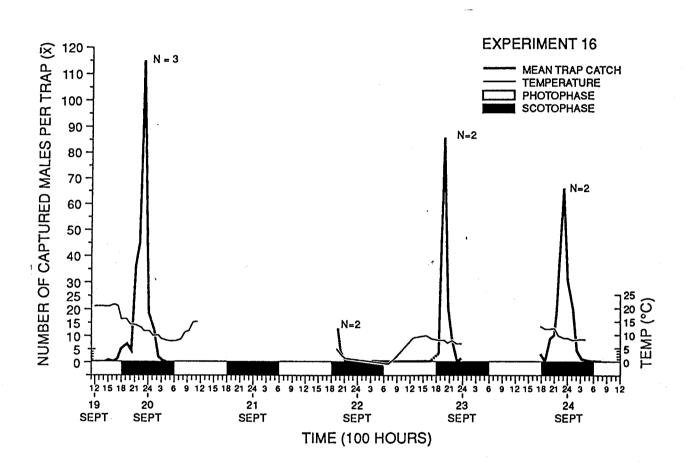


Figure 8 Hourly catches of male western hemlock loopers in Exp. 16, in Unitraps baited with the quarternary pheromone blend at 100 μg per component, Blue River, B.C., 19-23 September, 1991. Note sunset times.



#### 5.0 DISCUSSION

Exp. 1-6 and 9-11 proved the importance of 5,11 as the most biologically active individual component, with 2,5 and 7 as important and equal synergists. Highest catches were obtained with the quarternary blend and with 5,11+2,5+7. These results are different from those obtained for the eastern hemlock looper, which responds to 5,11+2,5 (Gries et al. 1991). They therefore support the division of the conifer feeding hemlock looper into two subspecies (McGuffin 1987).

The apparent lack of discrimination between treatments in Exp. 7-8 may have been due to the large numbers of moths in the Downie Creek outbreak. When moth density is high failure to discriminate between treatments may be expected (C.J. Sanders, Forestry Canada-Ontario Region, Sault Ste. Marie, Ont.; R.F. Shepherd, Forestry Canada-Pacific Region, Victoria, B.C.; K.N. Slessor, Dept. of Chemistry, Simon Fraser University, Burnaby, B.C.; pers. comm.). Western hemlock looper moths are predominantly nocturnal and are largely inactive in the day, resting on and under foliage and other surfaces (Hopping 1934). High day time flight activity has been observed under outbreak conditions (Shepherd 1979). During Exp.7-8 dense clouds of moths were freely observed in the forest and along the forest margins of Highway 23. Under such conditions, moths may overwhelm pheromone traps. In Exp. 7-8, many moths were alive upon collection, often filling 60 % or more of available trap volume, raising concerns about trap saturation, escape and inaccurate final catch numbers. A total of 149,681 males were captured in Exp. 7-8, in contrast to 24,681 males in preceding Exp. 4-6.

Exp. 12 and 13 indicate that, in the range of treatments tested, the highest

catches can be achieved with equal doses of 100  $\mu$ g of each of the four components. A 1:1 ratio between all components of a lepidopteran pheromone blend is unusual. Examples of species requiring the presence of minor components in small amounts are common (Arn et al. 1986).

The results of Exp. 16 verify the nocturnal nature of the western hemlock looper (Fig. 8). Temperatures reaching at least 9°C coincided with apparently normal flight activity. Shepherd (1979) captured males to female-baited sticky traps in peak numbers at 2400 h at temperatures slightly over 10°C, similar to the peak catches on 19 and 23 September at 2400 h and 12°C; and 2300 h at 9°C, respectively.

The peak catch periods observed at Blue River on 19 and 23 September also agree with observations relative to sunset times and female calling behaviour, made by Ostaff et al (1974a,b) and Shepherd (1979). The early decline of flight activity on 21 September is attributed to freezing temperatures, and that of 22 September to cool, drizzly conditions, and the impact of the previous night's weather. The results of Exp. 16, as well as those of other investigators, indicate that nocturnal flight of male western hemlock looper moths may begin within one hour of sunset and peak anywhere between 2 to 7 h after sunset, with 5 to 7 h being more common. Temperature and local weather conditions will influence both moth activity and flight patterns.

The identification of 5,11-dimethylheptadecane, 2,5-dimethylheptadecane and 7-methylheptadecane, as the pheromone components attractive to male western hemlock loopers, will allow the development of pheromone-based monitoring

procedures. Such procedures could replace the laborious surveys now used to monitor this insect (Harris et al. 1972; Shore 1990), as has been done for the western spruce budworm, *Choristoneura occidentalis* (Freeman) (R.F. Shepherd, Forestry Canada-Pacific & Yukon Region, Victoria, B.C., pers. comm), Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough) (Shepherd & Otvos 1986) and black army cutworm, *Actebia fennica* (Tauscher) (R.F. Shepherd, Forestry Canada-Pacific & Yukon Region, Victoria, B.C., pers. comm).

The optimal pheromone blend and dose required to sample field populations accurately over different infestation levels must be established and calibrated to current survey methods. The pheromone blend used in a monitoring trap need not be the most attractive. 5,11+2,5+7 and even 5,11+2,5 or 5,11+7, at 100 µg per component, are all highly attractive. Two Unitraps baited with 100 µg per component of 5,11+2,5, in second growth coastal western hemlock (Rolley Lake Provincial Park, Mission, British Columbia), at apparently low population levels, captured 274 and 437 moths over the course of the 1991 western hemlock looper flight. At endemic population levels lower trap catches would be desirable to reduce trap servicing labour requirements and avoid possible trap saturation problems at high population levels (Gray et al. 1991).

Long term monitoring of the insect at permanent sample sites, in different parts of its range, at low to high infestation levels, will allow identification of trap catch thresholds and patterns indicating rising populations and possible outbreaks. Ideally, such a system will give one to two years warning. It might therefore allow pre-outbreak control measures to be used, to prevent the massive outbreaks

periodically experienced in parts of British Columbia (Kinghorn 1954).

Direct control techniques are rarely applied against the western hemlock looper, but this insect is a candidate for successful control via pheromone-based mating disruption. Mating disruption provides an environmentally safe alternative to conventional chemical pesticides and specifically targets the problem insect. It is likely to be successful since western hemlock looper males are highly attracted to the pheromone, outbreaks are intense and concentrated, susceptible forests are themselves limited to distinct areas by topography and elevation, and the female is a weak flier. All these factors would minimise a major problem limiting mating disruption success, that of immigration of mated females into pheromone-treated infestation areas (Rothschild 1981). The one short, defined flight period per year would also reduce the number of treatments necessary to achieve control, should mating disruption prove to be effective for this insect. In this case, the complete pheromone would probably be required as secondary compounds, apparently less important in attraction, might affect mating behaviour.

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# ANOVA Tables for Experiments 1-3, 4-6, 9-11 & 13-15 analysed as four separate experiments

## EXPERIMENTS 1-3 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT	154.854	5	30.971	352.696	0.000
BLOCK	3.000	9	0.333	3.796	0.000
TIME	2.688	2	1.344	15.303	0.000
TREATTIME	3.190	10	0.319	3.633	0.000
ERROR	13.435	153	0.088		

#### EXPERIMENTS 4-6 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT	269.572	11	24.507	539.978	0.000
BLOCK	2.748	9	0.305	6.728	0.000
TIME	5.796	2	2.898	63.851	0.000
TREATTIME	3.796	22	0.173	3.802	0.000
ERROR	14.251	314	0.045		

#### EXPERIMENTS 9-11 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Ρ	
TREAT	4363.294	. 9	484.810	102.271	0.000	
BLOCK	429.228	9	47.699	10.062	0.000	
TIME	1404.962	2	702.481	148.189	0.000	
TREAT	577.680	18	32.093	6.770	0.000	
FRROR	1237 253	261	4 740			

#### EXPERIMENTS 13-15 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT	78.120	9	8.680	209.607	0.000
BLOCK	3.882	9	0.431	10.416	0.000
TIME	7.679	2	3.839	92.716	0.000
TREAT*TI	2.084	18	0.116	2.796	0.000
ERROR	9.442	228	0.041		

# APPENDIX II

# ANOVA Tables for Experiments 1-15 analysed as fifteen separate experiments

### EXPERIMENT 1 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Р
TREAT BLOCK	45.538 1.657	5 9	9.108 0.184	95.417 1.929	0.000 0.072
ERROR	4.295	45	0.095		

#### EXPERIMENT 2 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT BLOCK	66.113 1.030	5 9	13.223 0.114	149.515 1.294	0.000 0.267
ERROR	3.980	45	0.088		

#### EXPERIMENT 3 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT	46.393	5	9.279	123.752 3.111	0.000
BLOCK	2.099	9	0.233	3.111	0.005
ERROR	3.374	45	0.075		

#### EXPERIMENT 4 - ANOVA TABLE

1

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	P
TREAT BLOCK	85.022 2.069	11 9	7.729 0.230	134.659 4.005	0.000 0.000
ERROR	5.682	99	0.057		

#### EXPERIMENT 5 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	Р
TREAT BLOCK	83.764 0.700	11 9	7.615 0.078	184.270 1.881	0.000 0.063
ERROR	4.091	99	0.041		

#### EXPERIMENT 6 - ANOVA TABLE

SOURCE	SUM-OF-SQUAR	DF	MEAN-SQUARE	F-RATIO	P
TREAT BLOCK	106.773 0.727	11 9	9.707 0.081	274.781 2.288	0.000 0.022
ERROR	3.462	98	0.035		

# EXPERIMENT 7 - ANOVA TABLE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	Р
TREAT	18.618	. 8	2.327	33.259	0.000
BLOCK	1.742	11	0.158	2.263	0.018
ERROR	6.158	88	0.070		

#### EXPERIMENT 8 • ANOVA TABLE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P	
TREAT	47.086	. 8	5.888	19.628	0.000	
BLOCK	8.992	17	0.529	1.764	0.039	
ERROR	40.481	135	0.300			

#### EXPERIMENT 9 - ANOVA TABLE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	Р
TREAT	427.792	9	47.532	19.055	0.000
BLOCK	72.382	9	8.042	3.224	0.002
ERROR	202.055	81	2.495	<i>n</i> .	

#### EXPERIMENT 10 - ANOVA TABLE

1

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	Р
TREAT BLOCK	2835.703 287.885	9 9	315.078 31.987	41.910 4.255	0.000 0.000
ERROR	608.954	81	7.518		

#### EXPERIMENT 11 • ANOVA TABLE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT	1677.479	9	186.387	46.201	0.000
BLOCK	168.491	9	18.721	4.641	0.000
ERROR	326.774	81	4.034		

#### EXPERIMENT 12 - ANOVA TABLE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT	77.635	12	6.470	79.317	0.000
BLOCK	0.833	· 6	0.139	1.703	0.133
ERROR	5.873	72	0.082		

# EXPERIMENT 13 - ANOVA TABLE

SOURCE	SUM-OF-SQUARE	DF	MEAN-SQUARE	F-RATIO	Р
TREAT	17.406 1.470	9 6	1.934 0.245	59.425 7.530	0.000 0.000
ERROR	1.660	51	0.033		

## EXPERIMENT 14 - ANOVA TABLE

SOURCE	SUM-OF-SQUARE	DF	MEAN-SQUARE	F-RATIO	Ρ
TREAT BLOCK	24.484 2.157	9 9	2.720 0.240	60.532 5.332	0.000 0.000
ERROR	3.640	81	0.045		

#### EXPERIMENT 15 - ANOVA TABLE

SOURCE	SUM-OF-SQUARE	DF	MEAN-SQUARE	F-RATIO	Р
TREAT	43.121	9	4.791 <sup>*</sup>	192.841	0.000
BLOCK	2.384	9	0.265	10.661	0.000
ERROR	2.012	81	0.025		

}