

CHARACTERIZATION AND APPLICATION OF SEMIOCHEMICAL-BASED
ATTRACT-AND-KILL TO SUPPRESS CABBAGE LOOPER, *TRICHOPLUSIA NI*
(HÜBNER), POPULATIONS IN COMMERCIAL VEGETABLE GREENHOUSES

by

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Characterization and application of semiochemical-based attract-and-kill to suppress cabbage looper, *Trichoplusia ni* (Hübner), populations in commercial vegetable greenhouses.

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ABSTRACT

Objectives: 1. assess parameters of a paste (Last Call™ CL) containing insecticidal permethrin and pheromonal (*Z*)-7-dodecenyl acetate (*Z*7-12:OAc), and of modified paste, containing five floral semiochemicals and; 2. determine whether deployment of modified paste reduces crop damage inflicted by cabbage loopers, *Trichoplusia ni*, in commercial greenhouses.

In trapping experiments, droplets with 2 or 8% of *Z*7-12:OAc were equally effective, and more effective than droplets with 0.2%, in attracting male *T. ni*. Traps baited with a live female *T. ni* or with a droplet containing 8% of *Z*7-12:OAc captured similar numbers of males. Droplets with floral semiochemicals (16%) plus droplets with *Z*7-12:OAc (8%) as trap baits were more effective than floral semiochemical droplets alone in attracting males but not females. Floral semiochemical droplet baits attracted more females but not more males than *Z*7-12:OAc. Platforms carrying droplets with *Z*7-12:OAc (2%) and droplets with floral semiochemicals (16%) were contacted by 11 out of 42 male *T. ni* and 13 out of 39 female *T. ni* released into the compartment, whereas control platforms without droplets were not contacted. Honey bees, *Apis mellifera*, were observed not to contact droplets with or without floral semiochemicals.

To address the second research objective, treatment and control stimuli were randomly assigned to 16 greenhouse compartments (0.1-2 ha). The treatment consisted of 800 droplet pairs per hectare placed on platforms above pepper crop canopies, with one droplet per pair containing *Z*7-12:OAc and the other containing floral semiochemicals. Droplet pairs in control compartments did not contain attractants. Floral semiochemical and *Z*7-12:OAc droplets were replaced every 4 and 8 weeks,

respectively. Every 4 weeks for 20 weeks, five criteria were assessed. Mean numbers of *T. ni* eggs, larvae, and pupae were significantly lower in treatment than in control compartments. Plant damage in control unlike treatment compartments continuously rose. The treatment effect is significant as control compartments were not true controls, with growers applying tactics against *T. ni* eggs and larvae. The attract and kill tactic targets *T. ni* adults and is complementary with tactics aimed at eggs or larvae. It may become part of *T. ni* control in commercial greenhouses.

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TABLE OF CONTENTS

SECTION	PAGE
Approval	ii
Abstract	iii
Acknowledgements	v
Table of Contents	viii
List of Tables	ix
List of Figures	x
1.0 Introduction.....	1
1.1 Background.....	1
1.2 Biology and damage of <i>T. ni</i>	2
1.3 Tactics to control <i>T. ni</i>	6
1.2.1 Chemical control	6
1.2.2 Biological control	8
1.2.3 Semiochemical-based tactics.....	10
1.4 Research objectives	15
2.0 Assessment of "Attract and kill" parameters	16
2.1 Introduction	16
2.2 Materials and Methods	17
2.2.1. Experimental Insects	17
2.2.2. Last Call™ CL	19
2.2.2.1. Release rates of pheromone & floral semiochemicals from Last Call™ CL	19
2.2.2.2. Optimal pheromone dose and placement of pheromone-baited traps	22
2.2.2.3. Relative attractiveness of synthetic pheromone and virgin female <i>T. ni</i>	23
2.2.2.4. Dose of floral volatiles and comparative attractiveness with synthetic pheromone.....	23
2.2.2.5. Contact with paste droplets by <i>T. ni</i> and by <i>Apis mellifera</i>	24
2.2.3. Statistical Analyses.....	25
2.4. Results	25
2.5. Discussion.....	41
3.0 Operational Trial	43
3.1 Materials and Methods	43
3.1.1 Justification of Methodology	43
3.1.2 Experimental Methodology	44
3.3 Statistical analyses	46
3.4 Results	49
Assessment	62
3.5 Discussion.....	64
4.0 Concluding Discussion	67
5.0 References.....	70
Appendix A: Control Measures Applied to Suppress <i>T. ni</i> populations in commercial pepper greenhouses during the operational trial. T=Treatment and C=control	79

List of Tables

Table	Page
Table 1.1. Pheromone components identified in male and female <i>Trichoplusia ni</i>	5
Table 2.1. Summary of experiments (Exp.) conducted to determine release rates of pheromonal and floral volatile components from Last Call™ CL, and to optimize lure attractiveness and trap placement. n=number of replicates. AAFC=Agriculture and Agri-Food Canada.	18
Table 3.1. Location and size of greenhouse compartments with pepper crops in which replicates of the operational Last Call™ CL experiment were conducted.	45
Table 3.2. Repeated measures ANOVA results for all criteria used to assess the “Attract and kill” tactic for reducing incidence and damage of <i>Trichoplusia ni</i> populations in commercial greenhouses.	62
Table 3.3. Grid of Pearson correlation coefficients (r) and p-values for all criteria used to assess the “Attract and kill” tactic (Table 3.2) for reducing incidence and damage of <i>Trichoplusia ni</i> populations in commercial greenhouses. n=number of observations.	63
Table 3.4. Summary of control measures employed by growers to reduce <i>Trichoplusia ni</i> populations in greenhouses.	66

List of Figures

Figures	Page
Figure 2.1. Delivery of a Last Call™ CL droplet onto a Templast platform in a commercial greenhouse.	20
Figure 2.2. Mean amount (ng/h) of (<i>Z</i>)-7-dodecenyl acetate (<i>Z</i> 7-12:OAc) Porapak-Q-captured from Last Call™ CL paste droplets during each of nine discrete 24 h periods at weeks 0-8. Each of 3 replicates consisted of 10 droplets (50 mg each), each containing 2.0 % by weight of <i>Z</i> 7-12:OAc, placed on a Templast platform, and stored in the experimental greenhouse at SFU in between periods of volatile capture.	26
Figure 2.3. Mean amount (ng/h) of five floral volatile components Porapak Q-captured from Last Call™ CL paste droplets during each of nine discrete 24 h periods at weeks 0-8. Each of 3 replicates consisted of 10 droplets (50 mg each), each containing 16.0 % (by weight) of the floral volatile blend, placed on a Templast platform and stored in the experimental greenhouse at SFU in between periods of volatile capture. Asterisks indicate that data from only one replicate were available.	28
Figure 2.4. Mean number of male <i>Trichoplusia ni</i> captured per day in delta traps baited with a Last Call™ CL paste droplet containing 0.0, 0.2, 2.0, or 8.0% of (<i>Z</i>)-7-dodecenyl acetate. Commercial greenhouse with pepper crop, May 1999, Abbotsford, B.C. Bars with the same letter are not significantly different, Tukey-Kramer HSD test, $F = 10.6129$, $df = 3, 28$, $P < 0.0001$, $n = 8$	31
Figure 2.5. Mean number of male <i>Trichoplusia ni</i> captured per day in delta traps baited with a Last Call™ CL paste droplet and suspended at 0, 1, or 2 m above ground in a commercial greenhouse with pepper crop, May 1999, Abbotsford, B.C. Catches of traps above ground (both heights combined) (A) are significantly greater than at ground level (B), orthogonal contrast ANOVA ($F = 6.3877$, $df = 1, 25$, $P = 0.0182$). ..	33
Figure 2.6. Mean number of male <i>Trichoplusia ni</i> captured per day in delta traps baited with a Last Call™ CL paste droplet (~50 mg) containing 8.0 % (by weight) of (<i>Z</i>)-7-12:OAc, or a live caged virgin female <i>T. ni</i> in a commercial greenhouse with pepper crop, June 1999, Abbotsford, B.C. Bars with the same letter are not significantly different, Tukey-Kramer HSD test ($F = 15.9075$, $df = 2, 34$, $P = < 0.0001$).	35

Figure 2.7. Mean (+SE) number of male *Trichoplusia ni* captured in delta traps baited with 1, 10 or 20 Last Call™ paste droplets (50 mg) containing 16.0% (by weight) of a floral volatile blend (phenylacetaldehyde, (+/-)-limonene, methyl salicylate, phenyl ethanol, and methyl-2-methoxybenzoate) in a commercial greenhouse with pepper crop, November 1999, Abbotsford, B.C., Dunnet's test, $F=3.1910$, $df=3, 36$, $P=0.0351$, $n=10$. Data were square root transformed to ensure conditions of normality. Bars with the same letter superscript are not significantly different. 37

Figure 2.8. Mean (+SE) number of male and female *Trichoplusia ni* captured in delta traps baited with either one droplet (50 mg) of Last Call™ CL containing 8.0% (Z)-7-12:OAc, one droplet containing 16.0% of a floral volatile blend (phenylacetaldehyde, (+/-)-limonene, methyl salicylate, phenyl ethanol, and methyl-2-methoxybenzoate) or both in a commercial greenhouse with pepper crop, November 1999, Langley, B.C. Bars with the same letter superscript (lower case males, uppercase females) are not significantly different. Tukey-Kramer HSD test, $P < 0.0001$, $n=10$. Note: data for males and females were analyzed separately.... 39

Figure 3.1. Graphical illustration of the five-point scale employed to assess the degree of damage to pepper plant leaves inflicted by *Trichoplusia ni* larvae. 47

Figure 3.2. Mean number of *Trichoplusia ni* eggs per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an "attract and kill" tactic. Repeated Measures ANOVA, $F=6.28$, $df=1, 10.7$, $P=0.0298$, $n=8$. Treatment compartments received 400 pairs of paste droplets per ha, with one droplet (50 mg) Last Call™ CL containing (Z)-7-12:OAc (2.0% by weight) and one containing a floral volatile blend (16.0% by weight). Control compartments received 400 pairs of paste droplets containing only permethrin; growers were not told which compartments had received treatment or control stimuli, and were allowed to apply other means of control as deemed necessary. 50

Figure 3.3. Mean number of *Trichoplusia ni* larvae per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an "attract and kill" tactic. Repeated Measures ANOVA, $F=8.06$, $df=1, 13.3$, $P=0.0137$, $n=8$. Additional information in caption of Figure 3.2. 52

Figure 3.4. Mean number of *Trichoplusia ni* pupae per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an "attract and kill" tactic. Repeated Measures ANOVA, $F=10.19$, $df=1, 14.4$, $P=0.0063$, $n=8$. Additional information in caption of Figure 3.2. 54

- Figure 3.5. Mean number of *Trichoplusia ni* moths per randomly selected row of peppers (100 per ha) in treatment and control compartments of commercial green houses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 3.55$, $df=1, 14.8$, $P=0.0794$, $n=8$. Additional information in caption of Figure 3.2..... 56
- Figure 3.6. Mean degree of damage on a five-point scale (Figure 3.1) per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Likelihood-ratio Chi-square test, $\chi^2=16.636$, $df= 11$, $P=0.1191$, $n=8$. Additional information in caption of Figure 3.2..... 58
- Figure 3.7. Mean weight of *Trichoplusia ni* larval feces beneath 100 randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 0.89$, $df= 1, 22.7$, $P=0.3567$, $n=8$. Additional information in caption of Figure 3.2..... 60

1.0 Introduction

1.1 Background

In the agricultural plant production industry, large-scale monocultures, selection of varieties with rapid growth, constant production demand on the area's resources, and disruption of natural population "checks" have led to increasing pest problems.

Historically, insect pest control in this industry has had a heavy reliance on persistent insecticides. However, their repetitive use has led to development of resistance as well as concerns for human health and the environment. With increasing numbers of insecticides undergoing de-registration and decreasing numbers of new pesticides registered, the industry must seek alternative insect controls, such as those based on semiochemicals.

In British Columbia's greenhouse vegetable industry, 2 million m² of space are devoted to vegetable production of which ca. 1.87 million m² are allocated to the production of tomato and sweet bell pepper (Kurschner, 2001). New greenhouses are up to ten times larger than older houses with little separation between compartments. This increase in the scale of production coupled with changes in daily operations required to achieve this increase may have led to new problems in the industry.

The cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), has increased from minor to foremost lepidopteran pest in the industry in the past few years, causing \$2 million in lost revenue yearly in North America (Peter Isaacson, BC HotHouse, pers. comm.). Larvae of *Trichoplusia ni* attack all three major vegetables in greenhouse production: tomatoes, sweet bell peppers, and long English cucumbers. They cause both direct crop damage and indirect production losses. With the industry's determination to minimize pesticide use, a heavy reliance has been placed on biological

control programs that target egg and larval stages of *T. ni*. These programs on their own, however, may not provide adequate control and may need to be supplemented with semiochemical-based control tactics that target adults.

1.2 Biology and damage of *T. ni*

Trichoplusia ni originated in tropical Africa and radiated to Europe, Asia, and North and South America (Sutherland, 1965). Presently, *T. ni* are found wherever crucifers are cultivated. Like most noctuids, adult moths are primarily nocturnal. They have brownish-gray wings (35-40 mm wingspan) (Borror *et al.*, 1989), with an “8”-shaped silver spot in the middle of the forewing. Larvae are pale green with a white line along the side of the body, with three pairs of prolegs. Looper larvae move like “measuring worms” in the family Geometridae.

Trichoplusia ni complete their lifecycle in 18-25 days at 32-21°C (Toba *et al.*, 1973). Eggs hatch ca 3 days after oviposition, and larvae feed for ca 2 weeks at 27°C (Guy *et al.*, 1985). Fifth instars construct a silken chamber for pupation, which lasts one week. Three days after eclosion adults are sexually mature. Females produce 50 to 1,400 eggs (Landolt, 1997; McEwen & Harvey, 1960). Both males and females may have multiple matings (Ward & Landolt, 1995). Although one mating can fertilize nearly all of a female’s eggs, females that mate more than once oviposit more and live longer than singly-mated females (Ward & Landolt, 1995).

Under favourable environmental conditions *T. ni* is multivoltine (Grant *et al.*, 1996), resulting in overlapping life stages as the growing season progresses, and in four-fold increases in population density between generations (Ehler & Bosch, 1974). In greenhouses, *T. ni* are active almost year round, with early infestations stemming from residual populations that have not been eliminated in year-end clean up (D. Gillespie, Pacific Agri-Food Research Centre AAFC, pers. comm.).

Trichoplusia ni exhibit distinct diel activity patterns. Larvae are active during the photophase (Johnson *et al.*, 1987), whereas adults become active at and sexually communicate after sunset (Sharp *et al.*, 1975). Females call between the fourth and ninth hours of a 10-hour night (Landolt & Heath, 1990).

Larvae are polyphagous with late instars feeding and completing their development on many plant species including broccoli, cabbage, cauliflower, Chinese broccoli, Chinese cabbage, daikon, flowering white cabbage, head cabbage, mustard cabbage, lettuce, beet, peas, celery, tomato, cotton, tobacco, potato, spinach and certain ornamental plants (Soo Hoo *et al.*, 1984).

Adult moths feed on floral nectar at night (Grant, 1971a), locating it by responding to floral volatiles such as phenylacetaldehyde, benzaldehyde, 2-phenyl ethanol, benzyl alcohol, and benzyl acetate (Cantelo & Jacobson, 1979; Haynes *et al.*, 1991; Heath *et al.*, 1992a). A blend of these semiochemicals attracted both male and female *T. ni*, with phenylacetaldehyde being primarily responsible for attraction of females (Heath *et al.*, 1992b). Liu *et al.* (1988) identified 4-hexen-1-ol acetate, 2,2-dimethyl hexanal and 2-hexenal as attractive nonfloral plant volatiles.

Pheromonal communication of *T. ni* is well documented (Table 1.1). (*Z*)-7-dodecenyl acetate (*Z*7-12:OAc), the major pheromone component, was isolated from 2500 females (Berger, 1966). Five additional components were subsequently identified (Table 1.1). In field traps, (*Z*)-7-dodecenyl acetate was as attractive as the multicomponent blend (Haynes *et al.*, 1995). (*Z*)-7-dodecenyl acetate alone or in combination with dodecyl acetate was also as effective as the complete blend in windtunnel bioassays (Bjostad *et al.* 1984). Linn *et al.* (1984) demonstrated redundancy of components by showing that blends of “any four components can compensate for the lack of the fifth”. Linn *et al.* (1986) also concluded that partial and complete blends provoked more males to initiate upwind flight than did *Z*7-12:OAc alone, particularly at low doses. Secondary components may enhance specificity of the pheromone signal (Dunkelblum & Mazor, 1993), play a role in close-range attraction, and elicit contact and copulation attempts (Linn & Gaston, 1981). Three-day-old males are receptive to pheromone (Toba *et al.*, 1968). Interestingly, females were caught in traps baited with *Z*7-12:OAc (Birch, 1977).

Male *T. ni* release a 3-component pheromone from hair pencils at the eighth abdominal segment (Grant, 1970, 1971b) (Table 1.1). This blend attracts females at short range (Heath *et al.*, 1992b) but is not required for successful mating (Gothilf & Shorey, 1976). Male *T. ni* as trap baits attract males, and mated and unmated females (Landolt, 1995) mostly during the first three hours of the night. Attraction of females is enhanced in the presence of host plant volatiles (Landolt & Heath, 1990).

Table 1.1. Pheromone components identified in male and female *Trichoplusia ni*.

Sex	Pheromone Component	Proportion (% of major component)	Reference
Female	(Z)-7-dodecenyl acetate (Z7-12:OAc)	100	Berger, 1966
	dodecyl acetate (12:OAc)	6.8	Bjostad <i>et al.</i> , 1980
	(Z)-5-dodecenyl acetate (Z5-12:OAc)	7.6	Bjostad <i>et al.</i> , 1984
	(Z)-9-tetradecenyl acetate (Z9-14:OAc)	0.6	Bjostad <i>et al.</i> , 1984
	11-dodecenyl acetate (11-12:OAc)	2.3	Bjostad <i>et al.</i> , 1984
	(Z)-7-tetradecenyl acetate (Z7-14:OAc)	0.9	Bjostad <i>et al.</i> , 1984
	Male	(S)-(+)-linalool	100
<i>p</i> -cresol		20.8	Grant 1970 &1971b
<i>m</i> -cresol		2.9	Grant 1970 &1971b

Plant damage is caused by larvae of *T. ni* even at low population density. As few as 0.05 *T. ni* larvae per lettuce plant can cause yield loss (Johnson *et al.*, 1987), and 0.5 larvae per cabbage plant exceed an action threshold (Shelton *et al.*, 1982). Damage occurs through larval feeding on the lower side of leaves, resulting in “windows” in the leaf. Older larvae tend to chew large holes in leaves. Occasionally, larvae feed on fruit, such as watermelon, flowers of various host plants, vegetative tip tissues in celery, tomato fruit surfaces, and on cotton squares and flower buds. Defoliation of plants in greenhouses reduces productivity through reduced photosynthesis, and unshaded fruit can “sun burn”, making it unmarketable. At high population density, larvae may attack the calyx on sweet peppers and the embryo of cucumbers. Accumulation of larval frass around the calyx renders even high quality fruit non-gradable according to food safety regulation (Peter Isaacson, BC HotHouse, pers. comm.). Such fruit is returned at the grower’s expense, resulting in lost revenue, or requiring cleaning that increases production costs and reduces productivity.

1.3 Tactics to control *T. ni*

1.2.1 Chemical control

Traditionally, *T. ni* larvae have been controlled in the field through widespread use of chemical insecticides, including acephate, methomyl, fenvalerate, permethrin (Workman 1981) and fipronil (Ebert *et al.*, 1999). Most effective insecticides in a trial included tefluthrin, esfenvalerate, permethrin, cypermethrin, chlorfenapyr, flucycloxuron and abamectin (Chalfant, 1997).

Organophosphates and Thiodan were as effective as *Bacillus thuringiensis* (Bt)-based insecticides (Chalfant, 1997), and azadirachtin killed *T. ni* as well as other lepidopterous larvae (Leskovar & Boales, 1996). Various pyrethroids controlled *T. ni* larvae on broccoli (Paulumbo, 1997). Imidates with similar modes of action as pyrethroids also controlled larvae (Fisher *et al.*, 1996).

Sublethal-doses of pyrethroids can affect pheromonal communication by adults. A cypermethrin treatment significantly reduced calling behaviour by female *T. ni* resulting in reduced mating success, or reduced ability of males to detect and/or respond to female-produced pheromone in wind tunnel experiments (Clark & Haynes, 1992a). This effect may, however, last for only 24 h.

Methoprene, a juvenile hormone analogue, had “no detrimental effect on the probability of successful courtship” (Campero & Haynes, 1990), whereas Chlordimeform, a formamidine insecticide, interfered with “pheromone emission, increased calling behaviour of females, and decreased both oviposition by mated females and egg hatch at a dose yielding 1% mortality” (Clark & Haynes, 1992b). It also significantly reduced the probability of successful courtship.

Repetitive applications of insecticides, such as permethrin or methomyl, led to development of resistance (Shelton *et al.*, 1996). General concerns over reduced efficacy, and environmental and human health have provoked governments to tighten regulations for registration and application of insecticides. Registration of new insecticides is now prohibitively expensive in many crops. With a reduction in the number of effective pesticides available for rotation throughout a growing season, the lifespan of insecticides is further decreased. In addition, strong food safety regulations

regarding use and residues of pesticides have reduced the use of some and eliminated many chemicals from use. Employee resistance to application of pesticides, and worker safety regulations regarding re-entry and handling of treated crops, also reduce the available and practical choices to the grower. Chemical controls interfere with biocontrol programs and the use of bumblebees for pollination (Ehler & van den Bosch, 1974). Innovative growers have capitalized on the insecticide shortage by marketing their crops as “almost organic”, and charging premium prices to recoup their increased production costs or reduced yields.

1.2.2 Biological control

Biological control (biocontrol) has become a standard tool to manage *T. ni* populations, e.g. in California, where a complex of general predators of eggs and larvae maintains *T. ni* population densities at innocuous levels in cotton (Ehler & Bosch, 1974). These natural predators include larval green lacewings, *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae), nymphal and adult pirate bugs, *Orius tristicolour* (White) (Hemiptera: Anthocoridae), big-eyed bugs, *Geocoris pallens* Stål (Hemiptera: Lygaeidae), and damsel bugs, *Nabis americanoferus* Carayon (Hemiptera: Nabidae). Spiders and reduviid bugs (Hemiptera: Reduviidae) are less abundant and effective. Parasitoids include *Trichogramma semifumatum* (Perkins) (Hymenoptera: Trichogrammatidae), *Microplitis brassicae* Muesebeck (Hymenoptera: Braconidae), *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae), *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), *Copidosoma truncatellum* (Dalman) (Hymenoptera: Encyrtidae), *Chelonus texanus* Cresson (Hymenoptera: Braconidae),

Voria ruralis (Fällen) (Diptera: Tachinidae), and *Patrocloides montanus* (Cesson) (Hymenoptera: Ichneumonidae). The predators exert more pressure on *T. ni* populations than parasitoids, perhaps due to their “activity” in early *T. ni* generations (Ehler & Bosch, 1974). In the B.C. greenhouse vegetable industry, biocontrol agents commonly and commercially used to control *T. ni* are *Trichogramma* spp., larval green lacewings, *Chrysoperla rufilabris*, spined soldier bugs, *Podisus maculiventris* (Hemiptera: Pentatomidae), minute pirate bugs, *Orius* spp., and *Cotesia marginiventris* (Cresson). The pathogen *Bacillus thuringiensis* var. *kurstaki* (Btk) is commercially available as Dipel (oil-based suspension) and Foray (aqueous solution). To be effective, it must be ingested by larvae and may require up to 12 days to cause 90% mortality (Gharib & Wyman, 1991), during which time larvae continue to feed. Repetitive and high-dose applications of Dipel WP against greenhouse *T. ni* populations have led to Btk resistance 68-fold higher than that of a laboratory-reared *T. ni* colony (Janmaat, 2001).

The entomopathogenic nuclear-polyhydrosis virus (NPV) causes up to 60% mortality in medium-sized and large *T. ni* larvae (Ehler & van den Bosch, 1974) late in the season. It maintained 25% of its pathogenic activity five years after being applied to the soil (Jaques & Harcourt, 1971), and is highly specific to the host insect. NPV strains have been developed as viral insecticides against *T. ni* (Ignoffo & Garcia, 1997), but proper application to leaf surface is required for efficacy (Biever & Hostetter, 1985). NPV is more expensive to produce and less effective than chemical insecticides (Milks *et al.*, 1998).

Except for pathogens, biocontrol agents generally cause density-dependent mortality in target pests and need to multiply before they are effective (Ehler & van den Bosch, 1974). During this time, significant crop damage may still occur. Biocontrols do not act like chemical pesticides that express almost 100% knockdown in pest populations immediately following application (Ehler & van den Bosch, 1974). However, this may not necessarily apply to all inundative programs. Furthermore, a successful biocontrol program requires diligence in monitoring, pest identification, releases of insect biocontrol and inspection of shipments from suppliers. Due to these cost contributors, a successful biocontrol program may cost more than chemical control alternatives.

1.2.3 Semiochemical-based tactics

Pheromones may be used in two ways to manage *T. ni* populations: mating disruption and mass trapping, including attract-and-kill (Shorey, 1977). Mating disruption interferes with communication during mate location, potentially through sensory adaptation, central nervous system habituation, camouflage, and false-trail following (Bartell, 1982). It is typically accomplished through release of large amounts of pheromone, e.g. Z7-12:OAc, from many point sources (Shorey *et al.*, 1967, 1972; Gaston *et al.*, 1967; Kaae *et al.*, 1974; Farkas *et al.*, 1974). Continuous exposure to, unlike pulses of, Z7-12:OAc reduced the males' response to a pheromone source (Farkas *et al.*, 1975). In laboratory studies, response of males to high pheromone concentration ceased after exposure to low concentrations for 25 min (Shorey *et al.*, 1967). Although mating of *T. ni* was successfully disrupted by releasing pheromone at 406.5 g per ha for approximately one week (Mitchell *et al.*, 1997), mating disruption may fail if strong

flyers such as mated *T. ni* females immigrate into treated areas and oviposit. Mating disruption most likely affects only males and may not be effective in high-density infestations (Mitchell *et al.*, 1997). Although mating disruption of *T. ni* was demonstrated in a greenhouse environment (McGregor *et al.*, 2000), it is not practiced commercially in B.C. vegetable greenhouses (D. Gillespie, pers. comm.)

If synthetic pheromone is as attractive as virgin females, mass trapping may have potential for suppressing adult moth populations (Plimmer, 1981). However, reductions in trap catches may not correlate with fewer matings, and reduced pest damage could rarely be attributed to mass trapping (Kirsch, 1988). Reusable traps are costly, and at high population densities trapping surfaces of disposable sticky traps become clogged with bodies and scales (Kaae & Shorey, 1972). Mass trapping of *T. ni* is not used in B.C. vegetable greenhouses.

The attract-and-kill (A&K) tactic combines an attractant and an agent that kills or subdues the attracted insect (Downham *et al.*, 1995). The attractant may consist of visual, auditory and/or semiochemical cues (Lanier, 1990), and the killing agent may be a sticky surface, electrical current, or a pesticide. Any combination of attracting and killing agents can be used in an A&K tactic.

The A&K tactic is generally species-specific, requires little insecticide and greatly reduces exposure of workers and the crop to insecticides. For example, only 3.1-32.2 g of permethrin per ha in Sirene CM was necessary to control codling moth, *Cydia pomonella* L., populations below the 1% economic threshold (Charmillot *et al.*, 2000). The insecticides in this A&K approach do not contact the fruit and leave no insecticidal

residue in contrast to broadcast sprays. Furthermore, A&K avoids the up-front cost of dispensing equipment required by mating disruption (Sechser & Hoffer, 1998).

Incorporation of plant semiochemicals in A&K may increase the attraction of males and induce attraction of females (Heath *et al.*, 1992a). Host plant volatiles attracted both mated and virgin female *T. ni* but mated females were more likely to respond and contact the source (Landolt, 1989). Sexual attraction of male and female *T. ni* seemed enhanced by host plant volatiles (Landolt *et al.*, 1994). Host plant volatiles plus pheromone attracted more *C. pomonella* in walnut orchards and more corn earworms, *Heliothis zea* (Boddie), than pheromone alone (Light *et al.*, 1993, 1997). The effect of floral volatiles that were attractive to females was enhanced by a release device that resembled the flower (Morrison, 1991) of the night-blooming jessamine shrub, *Cestrum nocturnum* L., from which the volatiles were discovered (Heath *et al.*, 1992a). Many newly eclosed noctuids, including *T. ni*, nectar-feed (Raulston *et al.*, 1998), so incorporation of floral attractants in A&K formulations may attract females and males before mating. This would enhance the efficacy of an A&K tactic. As most oviposition by female *T. ni* coincided with the greatest number of open flowers per tomato plant (Zalom *et al.*, 1983), incorporation of floral attractants into an A&K tactic is further justified.

Lingren *et al.* (1998) proposed an attractive volatile blend from natural food sources for *Helicoverpa* spp. and other noctuid moths, and sought to develop an A&K formulation of feeding attractants, stimulants, and toxicants. Feeding stimulants may increase contact time with the killing agent, and thus enhance its effect.

Semiochemicals attractive to larvae may also be formulated as A&K baits.

Semiochemicals mediating host location by western corn rootworm larvae, *Diabrotica virgifera* LeConte, combined with insecticide significantly reduced *D. virgifera* damage (Hibbard *et al.*, 1995). If pathogens were utilised as the killing agent, the reduction in acute mortality achieved with conventional insecticides would be replaced by a chronic decrease in fecundity.

Practicality of A&K has been demonstrated. Brockerhoff and Suckling (1999) found ca. 50% mortality of male *C. pomonella* 48 h after a Sirene CM application and also concluded that modified Sirene A&K provided control against lightbrown apple moth, *Epiphyas postvittana* (Walker). Direct mortality and sub-lethal effects from contact with the killing agent may further prevent mating (Downham *et al.*, 1995). Sublethal doses of permethrin reduce the probability of pheromone release in female Lepidoptera (Clark & Haynes, 1992a)

Development of A&K for *T. ni* in vegetable greenhouses is justified because current tactics target egg or larval stages, and on their own do not provide the desired control. Moreover, adult moths are 10-100 times more susceptible to insecticides than larvae, and are much less likely to develop resistance. Therefore, targeting the adult female before oviposition is far more efficient than attempting to control her potential larval progeny (Lingren *et al.*, 1998). A&K for *T. ni* may be feasible, as demonstrated above for other pests, as the combination of female pheromone and plant volatiles cause *T. ni* to respond. Vegetable greenhouses in B.C. use integrated approaches and seek effective alternatives to providing control while minimizing fruit contact. Therefore, an A&K tactic may be a suitable technology for use in B.C. vegetable greenhouses.

Last Call™ CM was designed for A&K of *C. pomonella* in fruit orchards. An analogous formulation for *T. ni* control needed to be developed, and its efficacy demonstrated, in commercial vegetable greenhouses.

1.4 Research objectives

My objectives were:

1. to assess parameters for the application of Last Call™ CL (IPM Technologies Inc.), including:
 - a. determination of an effective pheromone dose
 - b. determination of an effective vertical placement
 - c. comparative attractiveness of Last Call™ CL and virgin female *T. ni*
 - d. comparative attractiveness of Last Call™ CL singly and in combination with floral semiochemicals
 - e. release rates and longevity of both Last Call™ CL and floral semiochemicals
 - f. ability of Last Call™ CL to induce contact by male and female *T. ni* ; and
2. to assess Last Call™ CL plus floral semiochemicals as a control tactic for *T. ni* populations in commercial pepper greenhouses.

2.0 Assessment of "Attract and kill" parameters

2.1 Introduction

Successful application of Last Call™ CL depends on many important parameters, including longevity, optimal dose and placement, and attractiveness relative to live virgin females. These parameters needed to be assessed prior to operational testing of the A&K tactic in commercial greenhouses for *T. ni* control. Both pheromone dose and vertical placement within a crop canopy affect attraction of males to pheromone sources (Mayer & McLaughlin, 1991; Saario *et al.*, 1970). The addition of floral semiochemicals would allow the attraction of females, in addition to males, to A&K stations. Greater attraction of males to synthetic pheromone plus floral semiochemicals than to female *T. ni* would enhance the efficacy of Last Call™ CL. Attraction of females to floral semiochemicals, also in the presence of pheromone, could allow economic, side-by-side placement of pheromonal and floral semiochemical sources. Knowledge about longevity and release rates of these compounds is necessary to determine the application schedule in greenhouses. Experimental evidence that male and female *T. ni* contact Last Call™ CL would ensure the mode of action is A&K and not mating disruption. Demonstration that honeybees do not contact droplets would ensure that the operational implementation of *T. ni* A&K does not interfere with optimal crop pollination.

2.2 Materials and Methods

2.2.1. Experimental Insects

Trichoplusia ni in Experiments 2.2 to 2.7 consisted of wild populations inside commercial greenhouses, those in Experiment 2.8 (Table 2.1) were reared at and purchased from the University of Alberta, and *T. ni* for Experiment 2.5 were wild moths supplemented by moths reared in the insectary at SFU, following Quiring's procedure (Table 2.1) (Don Quiring, Agriculture and Agri-Food Canada, PARC, Agassiz, pers. comm.). In this procedure, larvae were placed in inverted Styrofoam cups (250 ml, Unisource Canada, Inc.) provisioned with 50 mL of insect diet (Bio-serv, Frenchtown, NJ 08825). Pupae were removed, separated by sex, sterilized in a 10% bleach solution, and transferred to separate mesh cages (45 cm diam. x 45 cm high; 0.3175 cm mesh) maintained at 26°C and a photoperiod of 16:8h (L:D). Cages were set on sawdust-covered plant saucers that were sprinkled with tap water daily to increase humidity. Eclosed adults were sustained on a 10% honey water solution dispensed from three dental cotton wicks (15 cm, Sinclair Dental Company Ltd., North Vancouver, B.C., V7P 3P9) inside cups. Cages used for colony propagation contained both males and females. Paper towelling attached to the outside of wire cages served as an oviposition site for females. Egg-laden paper towels were rinsed with a sterilizing 1% bleach solution, and dried strips (1.25 x 20 cm) were placed in Tupperware boxes (25.4 x 30.5 cm) containing diet. Emerged larvae that migrated to the diet were transferred to Styrofoam cups as above.

Table 2.1. Summary of experiments (Exp.) conducted to determine release rates of pheromonal and floral volatile components from Last Call™ CL, and to optimize lure attractiveness and trap placement. n=number of replicates. AAFC=Agriculture and Agri-Food Canada.

Exp. No.	n	Description	Location	Source of experimental insects
2.1	3	Long-term release of Z7-12:OAc	Greenhouse & laboratory at SFU	n/a
2.2	3	Long-term release of floral semiochemicals	Greenhouse & laboratory at SFU	n/a
2.3	8	Optimal dose of Z7-12:OAc	Commercial greenhouse	wild
2.4	10	Optimal vertical trap placement	Commercial greenhouse	wild
2.5	10	Comparative attractiveness of <i>T. ni</i> and synthetic Z7-12:OAc	Commercial greenhouse	wild+ SFU-reared colony (♀s)
2.6	10	Optimal dose of floral semiochemicals	Commercial greenhouse	wild
2.7	10	Comparative attractiveness of synthetic Z7-12:OAc and floral semiochemicals	Commercial greenhouse	wild
2.8	1	Physical contact by ♂ + ♀ <i>T. ni</i> with Last Call™ CL	Experimental greenhouse at AAFC	University of Alberta, laboratory colony
2.9	1	Physical contact by <i>Apis mellifera</i> with Last Call™ CL	Experimental greenhouse at AAFC	Experimental bee hive in greenhouse

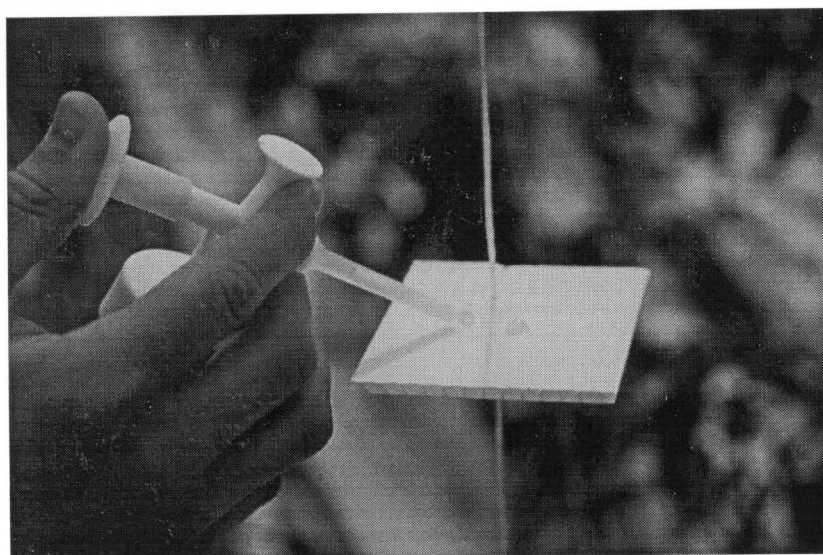
2.2.2. Last Call™ CL

Last Call™ CL paste used in Experiments 2.1 - 2.9 was supplied by IPM Technologies Inc. (4134 N. Vancouver Ave, #105, Portland, Oregon, 97217, USA). The paste was dispensed through a hand held pump calibrated to eject 50 mg droplets (Figure 2.1). Three paste formulations were created, corresponding to experimental treatments. Control paste contained the insecticidal synthetic pyrethroid permethrin (6.0 % by weight) only. A pheromone paste also contained Z7-12:OAc at concentrations from 0.2-8.0 % by weight. The third paste contained a floral volatile blend (16%): phenylacetaldehyde (PA), (+/-)-limonene, methyl salicylate, phenyl ethanol, and methyl-2-methoxybenzoate. When not in experimental use, Last Call™ CL paste was stored in the dark to avoid photodegradation of semiochemicals.

2.2.2.1. Release rates of pheromone & floral semiochemicals from Last Call™ CL

In each of three replicates in experiments 2.1 and 2.2 (Table 2.1), 10 droplets impregnated with Z7-12:OAc (2%) or the floral semiochemical blend (16% by weight) were placed on separate Templast® (Home Depot) platforms (8 x 8 cm). Once per week for 8 weeks, each platform was removed from storage in the experimental greenhouse at SFU, and placed for 24 h into a Pyrex glass aeration chamber through which a charcoal-filtered, aspirator-driven air stream passed at 1.0 L/sec, and then through glass tubing (14 x 1.3 cm OD) filled with Porapak-Q (50-80 mesh, Waters Associates, Inc. Milford, Massachusetts). Captured volatiles were desorbed with 1 mL of pentane,

Figure 2.1. Delivery of a Last Call™ CL droplet onto a Templast platform in a commercial greenhouse.



(*E*)-8-undecenyl acetate was added as an internal standard, and aliquots of concentrated extracts were analyzed by splitless injection gas chromatography, employing a Hewlett Packard (HP) 5890 Series 2 gas chromatograph equipped with a fused silica column (30 m x 0.25 mm ID) coated with DB-5 (J & W Scientific, Folsom, California 95630), with temperature of injection point and flame ionization detector at 240°C, and a temperature program of 50°C (1 min) then rising 10°C per min to 250°C.

2.2.2.2. Optimal pheromone dose and placement of pheromone-baited traps

To determine the optimal dose of Z7-12:OAc in Last Call™ CL paste droplets (Table 2.1, Experiment 2.3) 2-L milk carton sticky non-commercial delta traps (Gray *et al.*, 1984) were baited with a paste droplet impregnated with 0.0, 0.2, 2.0, or 8.0% of pheromone. In each of eight replicates, treatments were randomly assigned to one of four traps deployed 15 m apart and 2 m above ground in a commercial pepper greenhouse. Traps were moved such that each treatment occupied each position for one day. After each trap had occupied each possible position, captured male *T. ni* were counted.

To determine the optimal vertical placement of pheromone-baited traps (Table 2.1, Experiment 2.4), delta traps were baited with one paste droplet containing 8.0% of pheromone. In each of 10 randomized blocks, traps were placed 0.0, 1.0, and 2.0 m above ground at 16-m apart in a commercial pepper greenhouse. After six days, captured male *T. ni* were counted.

2.2.2.3. Relative attractiveness of synthetic pheromone and virgin female *T. ni*

A&K stations must successfully compete with calling females. The attractiveness of synthetic pheromone and virgin female *T. ni* was compared in a commercial greenhouse (Table 2.1, Experiment 2.5). Delta traps were baited with one paste droplet containing 8.0 % pheromone or one 3-day old virgin female *T. ni* from colonies at SFU in a cylindrical mesh cage (3 x 4 cm) provisioned with a cotton dental wick (1 x 3 cm, Sinclair Dental Company Ltd., North Vancouver, B.C., V7P 3P9) soaked in 10% honey-water. Control traps were unbaited. Each day, dead females and dry cotton wicks were replaced. In each of 10 randomized complete blocks, traps were suspended at 10 m spacing just above crop canopy. After three days captured wild males were counted.

2.2.2.4. Dose of floral volatiles and comparative attractiveness with synthetic pheromone

The optimal dose of the blend of floral volatiles was determined (Table 2.1, Experiment 2.6). Delta traps were baited with 0, 1, 10 or 20 paste droplets each containing 16.0 % of the floral volatile blend. In each of 10 randomized complete blocks, traps were suspended at 10 m spacing 3 cm above crop canopy. Due to low *T. ni* population density, captured moths were counted after 21 days.

To compare the relative attractiveness of synthetic pheromone *versus* that of the floral volatile blend (Table 2.1, Experiment 2.7), delta traps were baited with one droplet containing either pheromone (8.0 %), or floral volatiles (16.0 %), or with one droplet of each. Control traps were unbaited. In each of 10 randomized complete blocks, traps

were suspended at 15 m spacing 3 cm above crop canopy. After seven days, captured moths were counted.

2.2.2.5. Contact with paste droplets by *T. ni* and by *Apis mellifera*

To test for contact by *T. ni* with semiochemical-impregnated paste droplets (Table 2.1, Experiment 2.8), four Templast platforms were secured on vertical strings in each of two greenhouse compartments without plants. In one compartment, each platform received one paste droplet containing Z7-12:OAc (2.0 %) and one droplet containing the floral semiochemical blend (16.0 %). The platform surface surrounding the droplets was covered with Day-Glo™ UV fluorescent powder (50 mg) (Day-Glo Colour Corp., Cleveland, Ohio 44103). In the other compartment, control platforms were covered with UV fluorescent powder but lacked the two droplets. Eighty male and 80 female *T. ni* were released into each compartment. One week later, moths were recaptured and viewed under UV-light to determine the presence of fluorescent powder as an indicator that moths had contacted the droplets.

To test for contact by honeybees, *A. mellifera*, with semiochemical-impregnated droplets (Table 2.1, Experiment 2.9), 10 Templast platforms were secured on vertical strings just above the pepper crop canopy inside a 25 m² experimental greenhouse. Through random assignment, five treatment platforms received a paste droplet with the 16.0 % floral volatile blend and five control platforms received no droplet. All droplets were covered with a small wire cage to prevent physical contact by bees foraging from a hive inside the greenhouse at a level sufficient to pollinate the crop (H. Sabara, M.P.M. student, pers. comm.). At 2-min intervals, platforms were examined for bee visits.

2.2.3. Statistical Analyses

Mean release rates were calculated but not statistically analyzed for compounds in Experiments 2.1 and 2.2. For Experiments 2.3-2.5 and -2.7, data were subjected to completely randomized design ANOVA, followed by Tukey-Kramer HSD test ($\alpha=0.05$) (JMP IN, 1989-95 SAS Institute Inc.). An orthogonal contrast ANOVA was performed on the data from Experiment 2.4 ($\alpha=0.05$) (JMP IN, 1989-95 SAS Institute Inc.). Data from Experiment 2.6 were square root transformed to ensure conditions of normality prior to ANOVA followed by the Dunnett's means comparison test ($\alpha=0.05$) (JMP IN, 1989-95 SAS Institute Inc.). Experiments 2.8 and 2.9 were unreplicated and data were not statistically analyzed.

2.4. Results

Release rates of synthetic pheromone from paste droplets decreased from 2.2 to 0.7 ng/h over the eight week period (Figure 2.2). Release rates of floral semiochemicals also decreased over time (Figure 2.3), but release rates of phenyl ethanol, (+/-)-limonene, and methyl salicylate sharply declined by week 2, and for the latter two components remained very low thereafter, while release rates of methyl-2-methoxybenzoate and, to a lesser degree, phenyl acetaldehyde declined gradually during the 8-week period. Due to technical complications in volatile acquisitions, there was only one replicate for analysis in week 0 for methyl-2-methoxybenzoate and phenyl ethanol as well as for (+/-)-limonene in week 2.

Figure 2.2. Mean amount (ng/h) of (*Z*)-7-dodecenyl acetate (*Z*7-12:OAc) Porapak-Q-captured from Last Call™ CL paste droplets during each of nine discrete 24 h periods at weeks 0-8. Each of 3 replicates consisted of 10 droplets (50 mg each), each containing 2.0 % by weight of *Z*7-12:OAc, placed on a Templast platform, and stored in the experimental greenhouse at SFU in between periods of volatile capture.

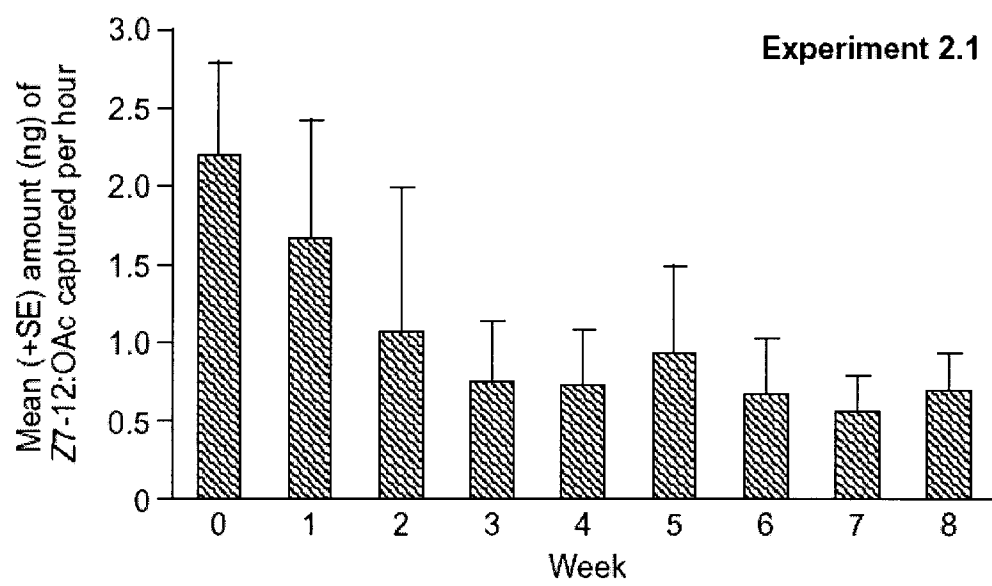
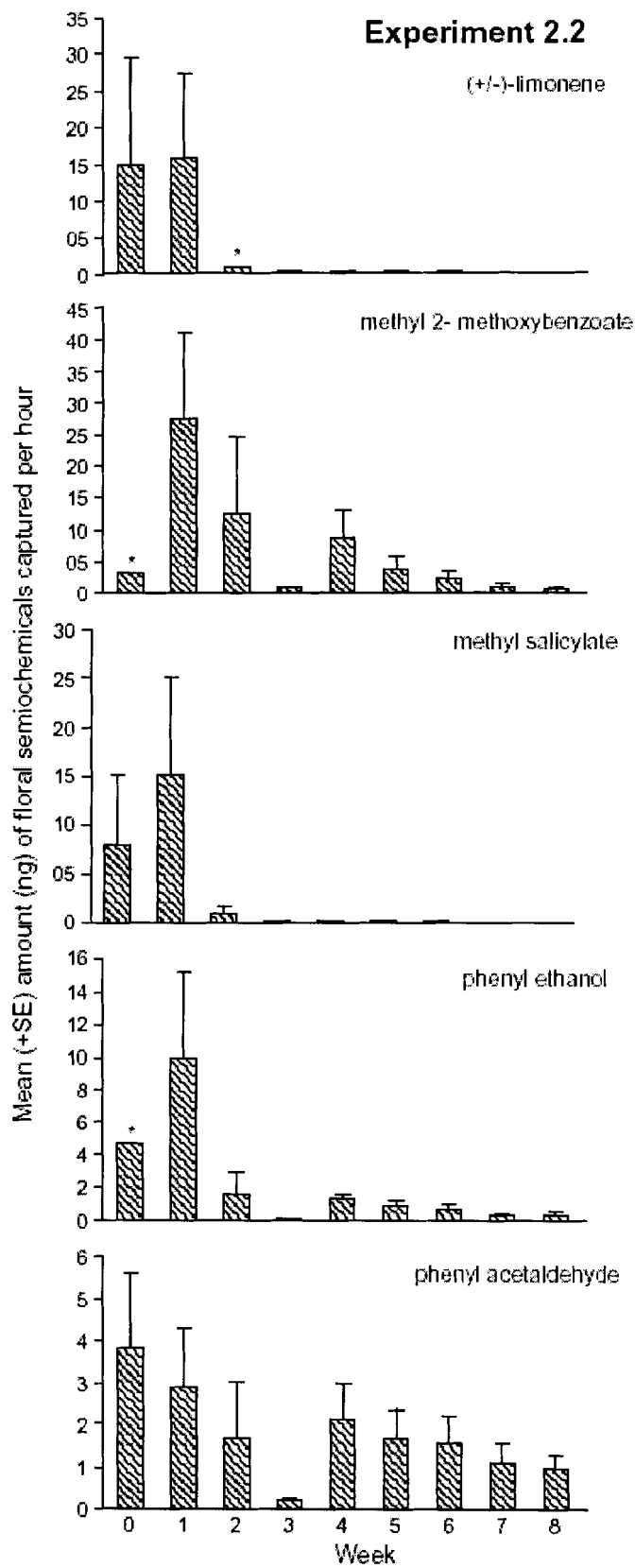


Figure 2.3. Mean amount (ng/h) of five floral volatile components Porapak Q-captured from Last Call™ CL paste droplets during each of nine discrete 24 h periods at weeks 0-8. Each of 3 replicates consisted of 10 droplets (50 mg each), each containing 16.0 % (by weight) of the floral volatile blend, placed on a Templast platform and stored in the experimental greenhouse at SFU in between periods of volatile capture. Asterisks indicate that data from only one replicate were available.



Paste droplets containing 2.0% and 8.0% of synthetic Z7-12:OAc were equally effective, and more effective than droplets with 0.2% of pheromone, in attracting male *T. ni* to traps ($F=10.6129$, $df=3, 28$, $P=<0.0001$) (Figure 2.4). Catches of *T. ni* in pheromone-baited traps at 0, 1, and 2 m above ground were not significantly different ($F=2.2833$, $df=2, 24$, $P=0.1237$), but orthogonal contrast ANOVA indicated that significantly more males were captured in traps above ground than at ground level ($F=6.3877$, $df=1, 25$, $P=0.0182$) (Figure 2.5). Traps baited with a live virgin female *T. ni* or with synthetic pheromone captured similar number of males, both significantly more than unbaited control traps ($F=15.9075$, $df=2, 34$, $P=<0.0001$) (Figure 2.6).

Captures of *T. ni* in traps baited with 10 and 20 droplets releasing floral volatiles were significantly higher than those baited with 1 or 0 droplets ($F=3.1910$, $df=3, 36$, $P=0.0351$) (Figure 2.7). Floral volatiles plus synthetic pheromone were more effective as trap baits than floral volatiles alone for males ($F=32.3071$, $df=3, 36$, $P=<0.0001$) but not females ($F=24.8400$, $df=3, 36$, $P=<0.0001$) (Figure 2.8). Floral volatiles attracted more females but not more males than pheromone baits, and more males and females than unbaited control traps (Figure 2.8).

In Experiment 2.8 (Table 2.1), 11 males (26.1%) and 13 females (33%) contacted at least once platforms baited with paste droplets releasing pheromone and floral volatiles. Control platforms not carrying droplets were not contacted. In Experiment 2.9, *A. mellifera* were not attracted to and did not contact platforms carrying paste droplets with or without floral volatiles.

Figure 2.4. Mean number of male *Trichoplusia ni* captured per day in delta traps baited with a Last Call™ CL paste droplet containing 0.0, 0.2, 2.0, or 8.0% of (Z)-7-dodecenyl acetate. Commercial greenhouse with pepper crop, May 1999, Abbotsford, B.C. Bars with the same letter are not significantly different, Tukey-Kramer HSD test, $F = 10.6129$, $df = 3, 28$, $P < 0.0001$, $n = 8$.

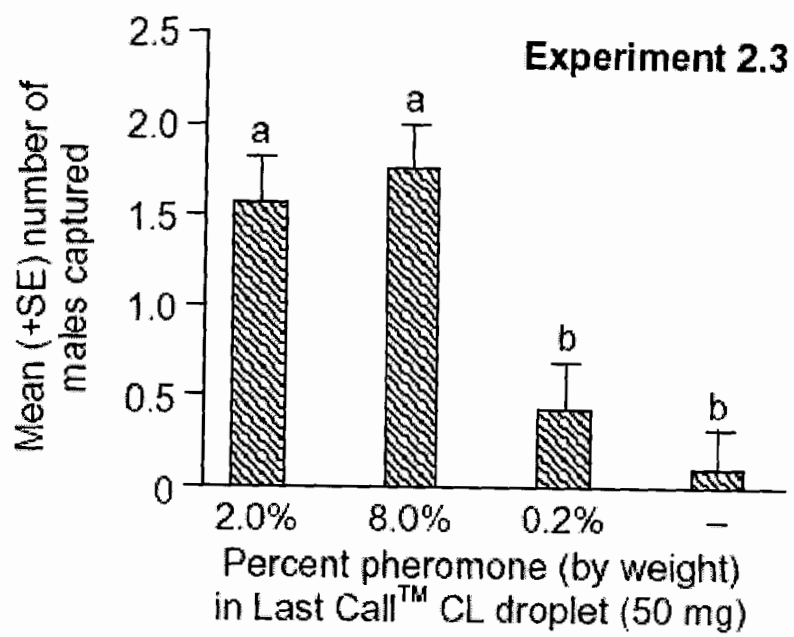


Figure 2.5. Mean number of male *Trichoplusia ni* captured per day in delta traps baited with a Last Call™ CL paste droplet and suspended at 0, 1, or 2 m above ground in a commercial greenhouse with pepper crop, May 1999, Abbotsford, B.C. Catches of traps above ground (both heights combined) (A) are significantly greater than at ground level (B), orthogonal contrast ANOVA ($F=6.3877$, $df=1, 25$, $P=0.0182$).

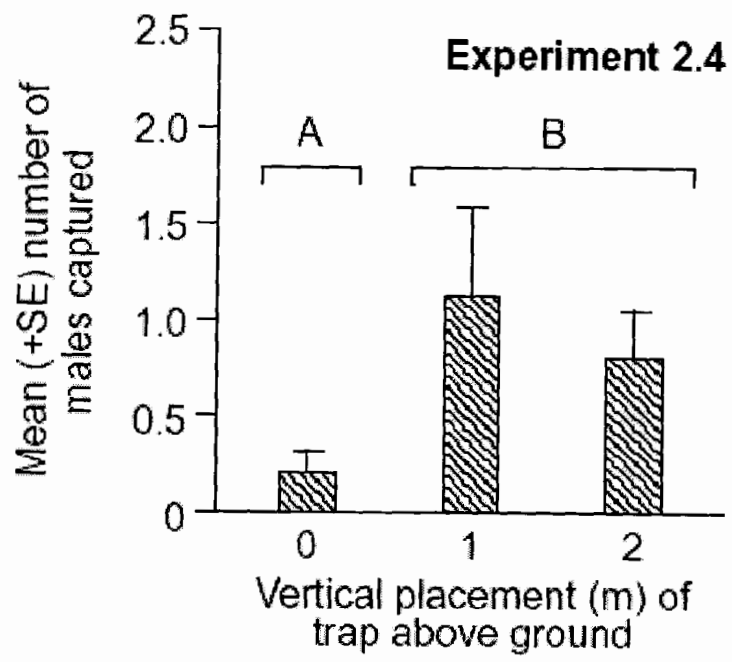


Figure 2.6. Mean number of male *Trichoplusia ni* captured per day in delta traps baited with a Last Call™ CL paste droplet (~50 mg) containing 8.0 % (by weight) of (Z)-7-12:OAc, or a live caged virgin female *T. ni* in a commercial greenhouse with pepper crop, June 1999, Abbotsford, B.C. Bars with the same letter are not significantly different, Tukey-Kramer HSD test (F=15.9075, df=2, 34, P=<0.0001).

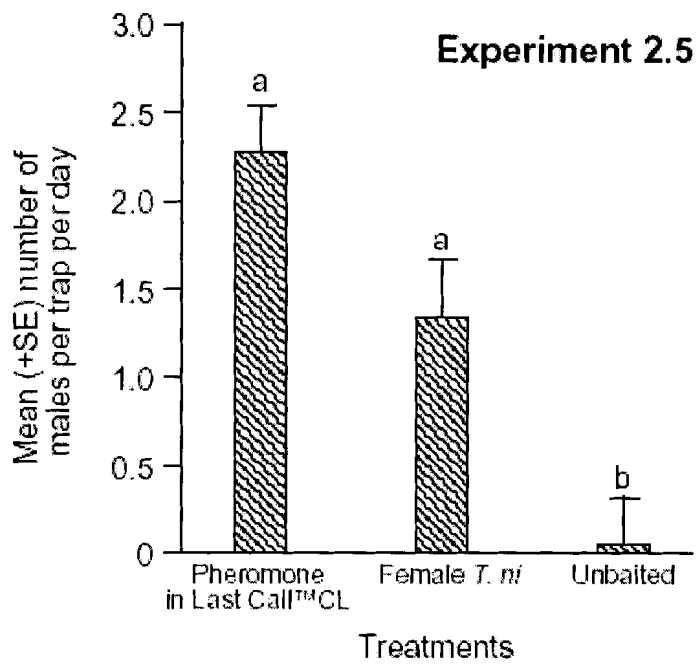


Figure 2.7. Mean (+SE) number of male *Trichoplusia ni* captured in delta traps baited with 1, 10 or 20 Last Call™ paste droplets (50 mg) containing 16.0% (by weight) of a floral volatile blend (phenylacetaldehyde, (+/-)-limonene, methyl salicylate, phenyl ethanol, and methyl-2-methoxybenzoate) in a commercial greenhouse with pepper crop, November 1999, Abbotsford, B.C., Dunnet's test, $F=3.1910$, $df=3, 36$, $P=0.0351$, $n = 10$. Data were square root transformed to ensure conditions of normality. Bars with the same letter superscript are not significantly different.

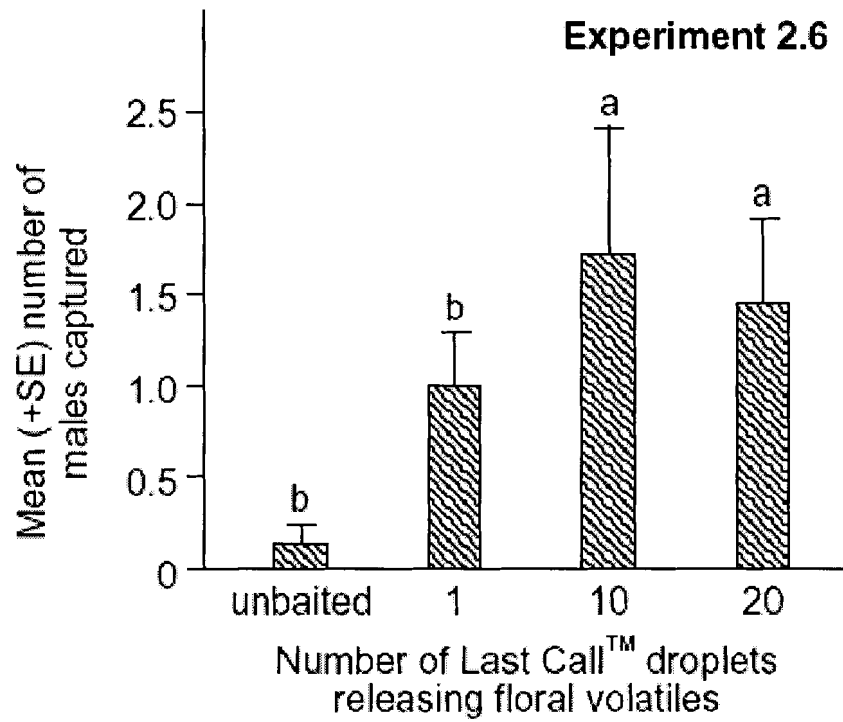
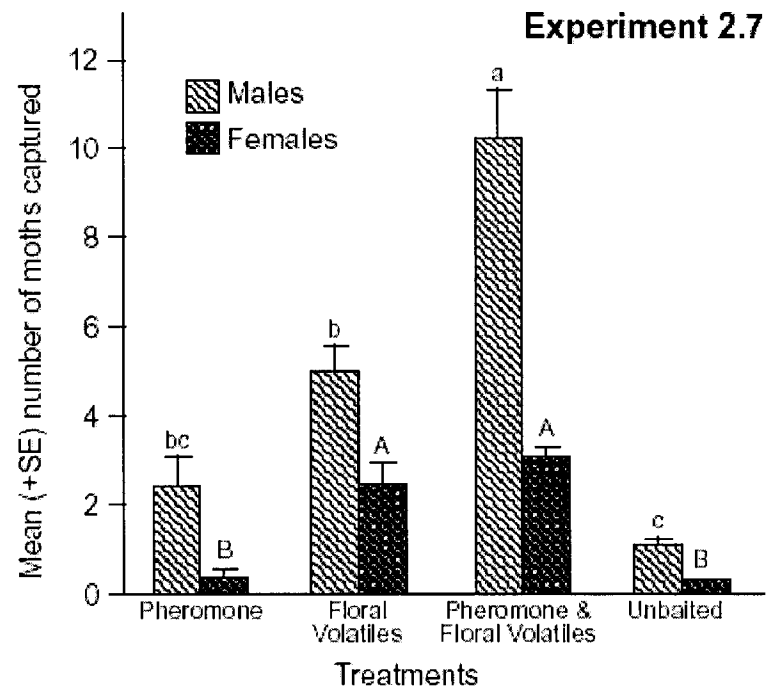


Figure 2.8. Mean (+SE) number of male and female *Trichoplusia ni* captured in delta traps baited with either one droplet (50 mg) of Last Call™ CL containing 8.0% (*Z*)-7-12:OAc, one droplet containing 16.0% of a floral volatile blend (phenylacetaldehyde, (+/-)-limonene, methyl salicylate, phenyl ethanol, and methyl-2-methoxybenzoate) or both in a commercial greenhouse with pepper crop, November 1999, Langley, B.C. Bars with the same letter superscript (lower case males, uppercase females) are not significantly different. Tukey-Kramer HSD test, $P < 0.0001$, $n = 10$. Note: data for males and females were analyzed separately.



2.5. Discussion

Similar captures of male *T. ni* in traps baited with Last Call™ CL paste droplets containing 2.0% or 8.0% of pheromone (Figure 2.4), and in traps baited with virgin female *T. ni* or 2.0 %-pheromone paste droplets (Figure 2.6), indicated that the latter dose ought to be appropriate for operational use. This conclusion was confirmed by pheromone release-rate studies (Figure 2.2) demonstrating a declining release of Z7-12:OAc for two weeks and a constant release rate for the following six. I decided that the paste droplets containing pheromone should be replaced every eight weeks in the ensuing operational trial.

Experiment 2.4 (Figure 2.5) suggested that any placement of A&K droplets above ground should be operationally acceptable. Because A&K droplets would be placed in operational greenhouses on Templast® platforms secured to the vertical strings that support plants and continually raised so as to not interfere with fruit harvest, it seemed appropriate to secure them above crop canopy for the operational trial.

Floral volatiles were as effective as Z7-12:OAc in attracting male *T. ni*, significantly enhanced attractiveness of the pheromone for males, and also attracted females (Figure 2.8) suggesting that a combination of pheromone and floral volatiles would enhance the efficacy of the A&K tactic. The attraction of females to floral volatiles was not significantly affected by the addition of pheromone. Because females as well as males contacted droplets containing semiochemicals and permethrin (Experiment 2.8), both males and females should be affected during operational A&K of *T. ni* populations.

The decline in release rates of limonene and methyl salicylate to near zero in week 2 (Figure 2.3) would have significantly altered the floral blend composition. Should these two components be critically important for blend attractiveness, their release from different dispensers should be considered. Alternatively, paste droplets with floral semiochemicals may need re-application every 2-4 weeks during operational *T. ni* control.

Experiment 2.8 confirmed that both male and female *T. ni* contact the droplets. This eliminated concern that the A&K tactic would induce long-range orientation without contact. Linn and Gaston (1981) found that dodecyl acetate alone increases the amount of time spent by males in close-range orientation as well as increases the number of attempts to copulate. The addition of this compound should be investigated to see if contact could be increased significantly.

The fact that floral semiochemicals did not attract honeybees in Experiment 2.9 was expected, because the volatiles were identified from night blooming Jessamine (*Gaura* spp.) which rely on attraction of nocturnal pollinators like *T. ni* (Lingren *et al.*, 1998). Nonetheless, demonstration of lack of attraction to honeybees by these semiochemicals instills confidence that operational A&K control of *T. ni* would not interfere with crop pollination. Considering that bumblebees are responsible for much of the pollination in greenhouses, the same type of investigation should be conducted with them.

3.0 Operational Trial

3.1 Materials and Methods

3.1.1 Justification of Methodology

Even though Z7-12:OAc was continuously released from paste droplets over 8 weeks (Figure 2.2), release rates of floral semiochemicals markedly decreased in just 4 weeks (Figure 2.3). Thus, the replacement intervals for droplets with floral semiochemicals and Z7-12:OAc were set at 4 and 8 weeks, respectively. Because above-ground traps captured more male *T. ni* than those on the ground (Figure 2.5), and because of concerns about potential fruit contamination and worker safety, droplets were placed on small platforms above the crop's canopy. Each platform received one droplet with pheromone and one with floral volatiles to exploit the enhanced attraction of male *T. ni* compared to either attractant alone and to also attract females (Figure 2.8).

Manufacturers recommend Last Call CM to be applied at 1400-3000 droplets per ha in an orchard setting. The decision to deploy significantly fewer droplets (800 of each type per ha) than recommended or deployed in field trials (Mitchel *et al.*, 1997) was made so as not to risk permeation of the enclosed greenhouse environment with semiochemicals, which could have disrupted orientation to A&K droplets.

Deployment of A&K alone for *T. ni* control in the operational trial would have been best to assess its efficacy. However, growers could not be expected to jeopardize their potential earnings, and therefore were free to use other control measures, including insecticidal sprays and biocontrol programs, when deemed necessary. In order to determine the impact of the A&K treatment, growers were asked to keep careful records of all crop protection measures and costs, as well as crop productivity

3.1.2 Experimental Methodology

Sixteen compartments (0.1-2 ha in size) in eight greenhouses were selected (Table 3.1). Greenhouses with two compartments were treated as an experimental block, with A&K and control treatments randomly assigned to each compartment. Greenhouses with one or more than two compartments were treated as an incomplete block. The A&K treatment stimulus was randomly assigned to the odd compartment in these cases.

In each compartment at the beginning of May 2000, 800 Templast platforms (8 x 8 cm) per ha were secured to separate vertical strings (supporting pepper plants). Each platform was secured within 30 cm above the top of plants, and (under Research Permit 99-RP-99, Pest Management Regulatory Agency, Health Canada) received one paste droplet of Last Call™ CL containing 2.0% of Z7-12:OAc and one droplet containing floral volatiles (16.0%). Platforms in control compartments received droplets containing only permethrin. This way, growers did not know whether a treatment or control stimulus was applied which might have affected their management program. Pheromone and floral volatile droplets were replaced every eight and four weeks, respectively.

Table 3.1. Location and size of greenhouse compartments with pepper crops in which replicates of the operational Last Call™ CL experiment were conducted.

Compartment no.	Size (ha)	Test Stimulus^a	Name of Greenhouse	Address in B.C.
1	1	Treatment	Century Pacific	266 Ross Rd, Abbotsford
2	0.1	Control	Century Pacific	266 Ross Rd, Abbotsford
3	0.1	Control	Hazelmere	18752 16 th Ave, Surrey
4	2	Control	Mt. Lehman	8592 Mt.Lehman Rd, Abbotsford
5	2	Treatment	Mt. Lehman	8592 Mt.Lehman Rd, Abbotsford
6	0.4	Treatment	Drooghendyk	50357 Chilliwack Central Rd, Chilliwack
7	1	Treatment	Cheam View	1501 Johnson Rd, Agassiz
8	0.2	Control	BMW 1	1264 McCallum Rd, Abbotsford
9	0.4	Control	BMW 1	1264 McCallum Rd, Abbotsford
10	0.4	Treatment	BMW 1	1264 McCallum Rd, Abbotsford
11	1	Control	BMW 2	1264 McCallum Rd, Abbotsford
12	1	Control	Victoria	21422 4 th Ave, Langley
13	0.1	Treatment	Victoria	21422 4 th Ave, Langley
14	1	Control	Peppertree Farms	270 Gladwin Rd, Abbotsford
15	0.5	Treatment	Peppertree Farms	270 Gladwin Rd, Abbotsford
16	0.5	Treatment	Peppertree Farms	270 Gladwin Rd, Abbotsford

^a Treatment: Two Last Call™ CL droplets (50 mg) containing permethrin and Z7-12:OAc (2%) or a floral semiochemical blend (16%) consisting of phenylacetaldehyde, (+/-)-limonene, methyl salicylate, phenyl ethanol, and methyl-2-methoxybenzoate on each of 400 platforms per ha secured to vertical strings.

Control: As treatment except that droplets did not contain Z7-12:OAc or floral volatiles.

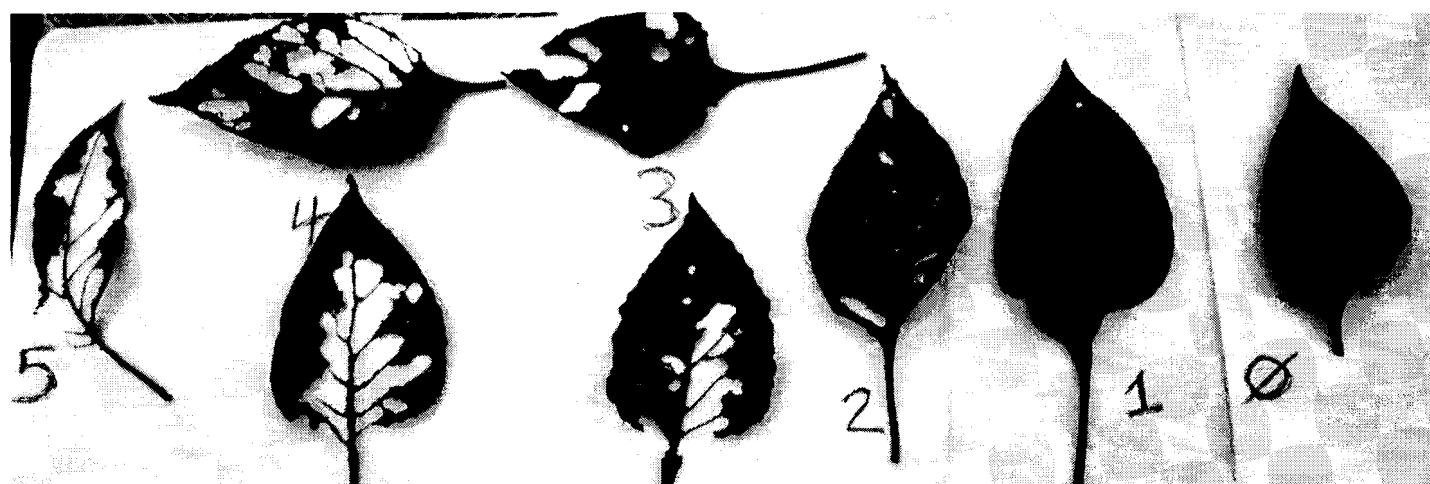
Six criteria were measured to assess efficacy of the treatment: 1) number of *T. ni* eggs per plant; 2) number of *T. ni* larvae per plant; 3) weight of larval fecal pellets accumulating beneath plants; 4) number of *T. ni* pupae per plant; 5) number of *T. ni* adults per row; and 6) degree of plant damage. These criteria with respect to *T. ni* pest impact were recorded every 4 weeks for 20 weeks in treatment and control compartments, providing substantial data to help evaluate efficacy of the A&K control tactic.

For each assessment every four weeks, 100 plants per ha were randomly selected in each compartment. For plants >1 m tall, only the uppermost 30 cm (4 weeks of growth) were inspected. This decision was based, in part, on findings that female *T. ni* preferentially oviposited and larvae fed on upper portions of cotton plants (Wilson *et al.*, 1982). For each pepper plant, the number of *T. ni* eggs, larvae and pupae was recorded, and larval fecal pellets which had accumulated through four weeks in a tinfoil tray (23 x 28 cm) beneath the plant were weighed. Numbers of adult *T. ni* per row were assessed by walking through 100 randomly selected rows per ha, slightly brushing plants and recording each moth taking flight. Leaf damage inflicted by *T. ni* larvae was graded on a 5-point scale (Leskivar & Boales, 1996; Luther *et al.*, 1996), with 0 and 5, respectively, representing no damage and complete skeletonization (Figure 3.1).

3.3 Statistical analyses

To ensure normality of data, numbers of eggs, larvae, and pupae per plant, moths per row, and fecal weights were square root transformed, and outliers were removed (I. Bercowitz, Director Statistical Consulting, SFU, pers. comm.) prior to analysis by

Figure 3.1. Graphical illustration of the five-point scale employed to assess the degree of damage to pepper plant leaves inflicted by *Trichoplusia ni* larvae.



incomplete block design repeated measure ANOVA using SAS version 8.1, with the Proc Mixed procedure for the repeated measurements program designed by the Statistical Consulting Department, Simon Fraser University.

Damage assessment data were ranked and analyzed by the likelihood-ratio χ^2 test. Data collected in month 6 were excluded from analysis because replicates were not completed in several greenhouse compartments, which had already undergone year-end clean-ups. Pearson correlation coefficients were determined for all factors using SAS version 8.1 with the Proc Corr option (program created by the Statistical Consulting Department, SFU).

3.4 Results

Mean numbers of *T. ni* eggs per plants (Figure 3.2), larvae per plant (Figure 3.3), and pupae per plant (Figure 3.4) were significantly lower in treatment than in control compartments. There were also fewer moths per row in treatment than in control compartments, but the difference only approached significance (Figure 3.5). Both the degree of plant damage (Figure 3.6) and the mean weight of accumulated larval fecal pellets (Figure 3.7) were lower in treatment than in control compartments, but these differences were not significant. Repeated measures ANOVA results for all assessment factors are summarized in Table 3.2.

Positive and significant correlations (Table 3.3) were found for: 1) number of *T. ni* eggs per plant and numbers of larvae and pupae per plant, moths per row and the degree of plant damage, but not the weight of accumulated larval fecal pellets; 2) number

Figure 3.2. Mean number of *Trichoplusia ni* eggs per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 6.28$, $df= 1, 10.7$, $P= 0.0298$, $n=8$. Treatment compartments received 400 pairs of paste droplets per ha, with one droplet (50 mg) Last Call™ CL containing (Z)7-12:OAc (2.0% by weight) and one containing a floral volatile blend (16.0% by weight). Control compartments received 400 pairs of paste droplets containing only permethrin; growers were not told which compartments had received treatment or control stimuli, and were allowed to apply other means of control as deemed necessary.

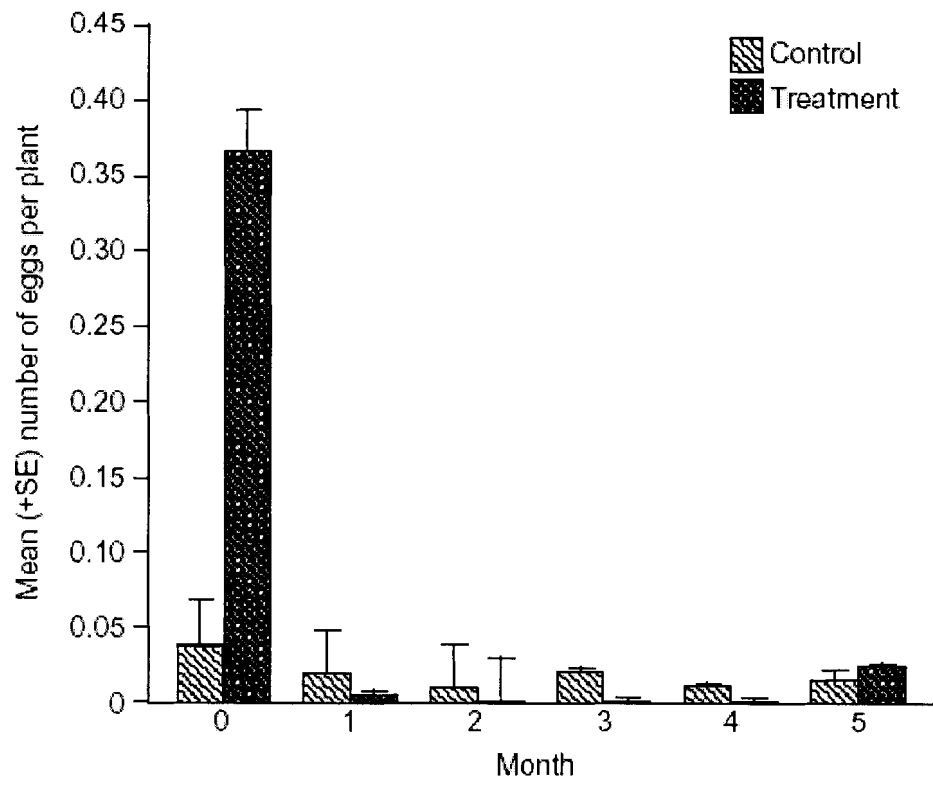


Figure 3.3. Mean number of *Trichoplusia ni* larvae per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 8.06$, $df= 1, 13.3$, $P=0.0137$, $n=8$. Additional information in caption of Figure 3.2.

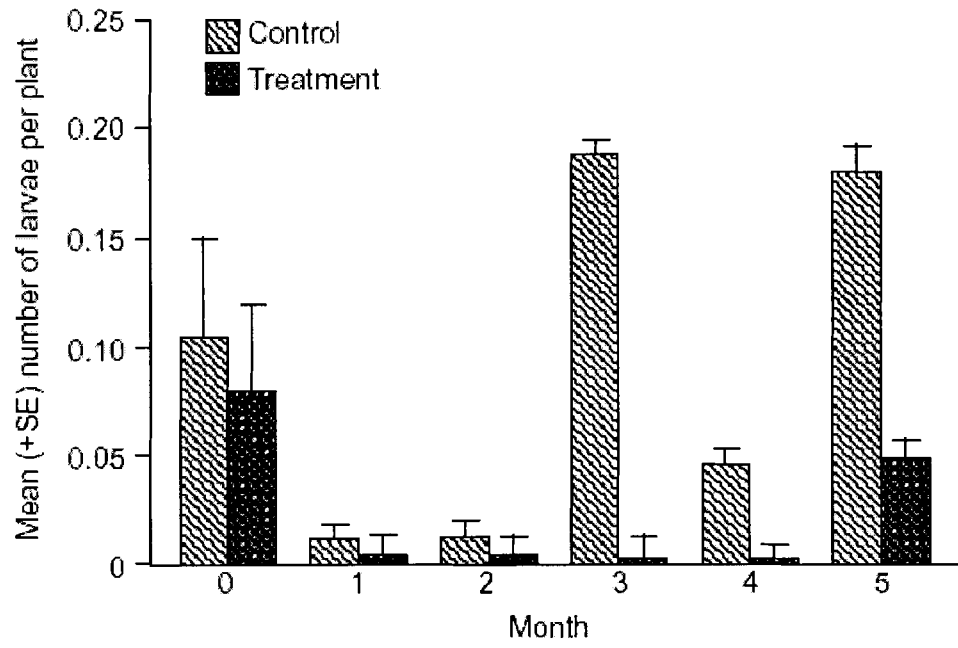


Figure 3.4. Mean number of *Trichoplusia ni* pupae per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 10.19$, $df= 1,14.4$, $P=0.0063$, $n=8$. Additional information in caption of Figure 3.2.

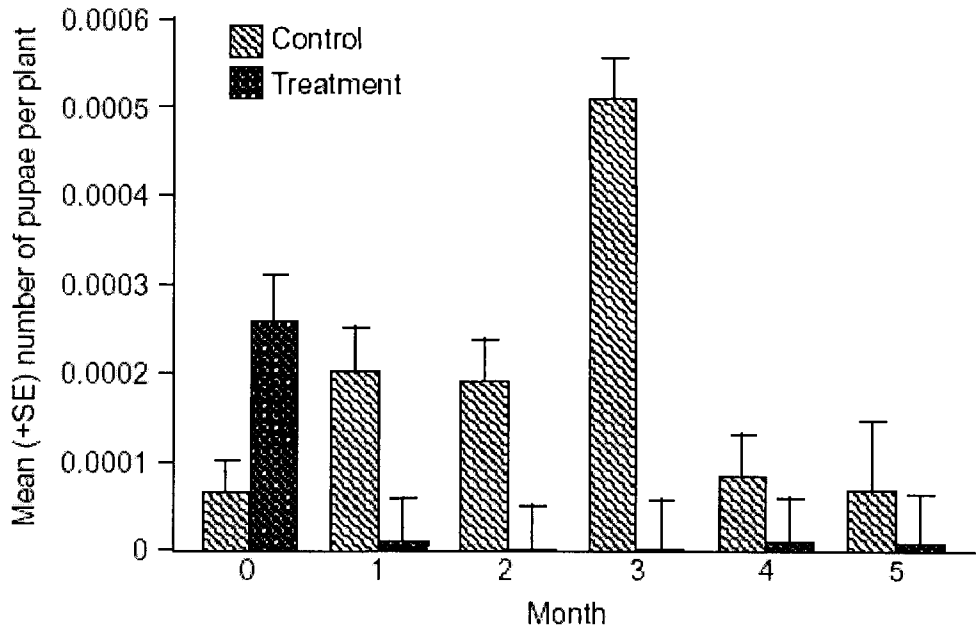


Figure 3.5. Mean number of *Trichoplusia ni* moths per randomly selected row of peppers (100 per ha) in treatment and control compartments of commercial green houses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 3.55$, $df=1, 14.8$, $P=0.0794$, $n=8$. Additional information in caption of Figure 3.2.

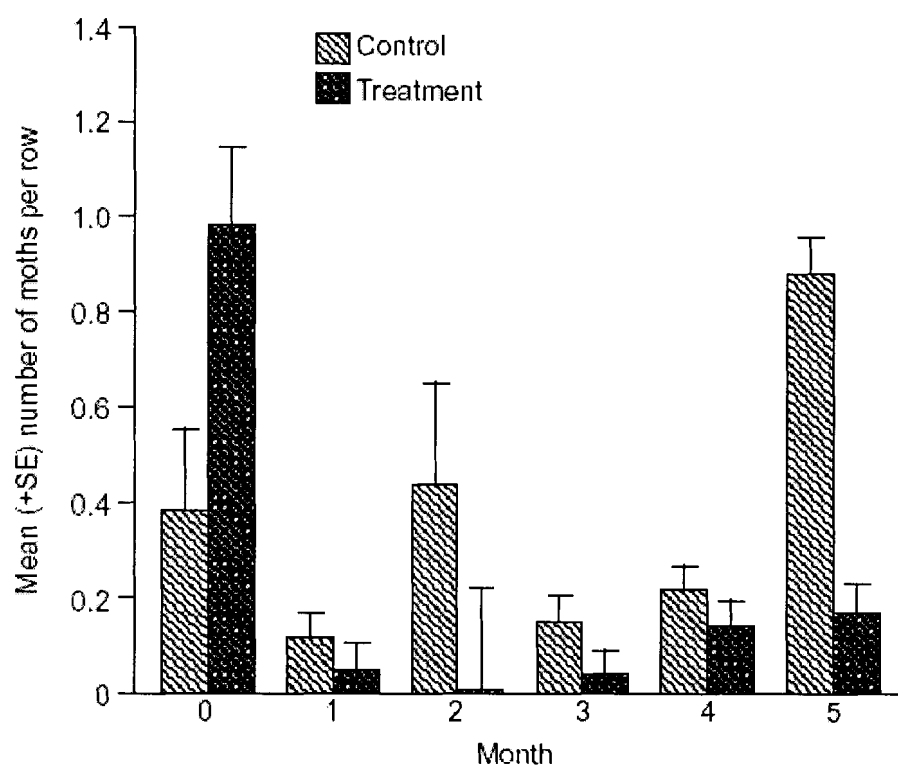


Figure 3.6. Mean degree of damage on a five-point scale (Figure 3.1) per randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Likelihood-ratio Chi-square test, $\chi^2=16.636$, $df= 11$, $P=0.1191$, $n=8$. Additional information in caption of Figure 3.2.

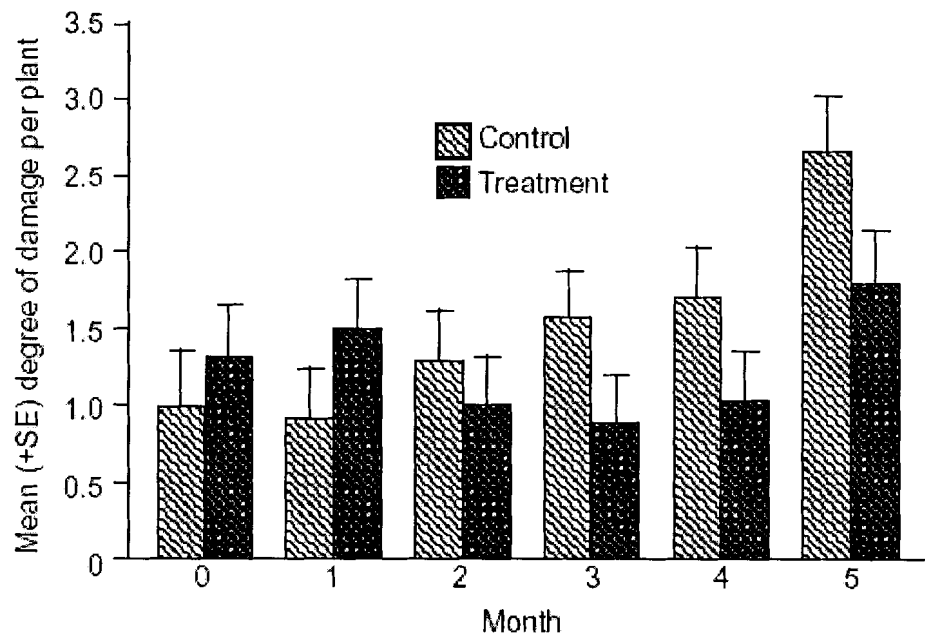


Figure 3.7. Mean weight of *Trichoplusia ni* larval feces beneath 100 randomly selected pepper plants in treatment and control compartments of commercial greenhouses (Table 3.1), May-October 2000 during operational testing of an “attract and kill” tactic. Repeated Measures ANOVA, $F= 0.89$, $df= 1, 22.7$, $P=0.3567$, $n=8$. Additional information in caption of Figure 3.2.

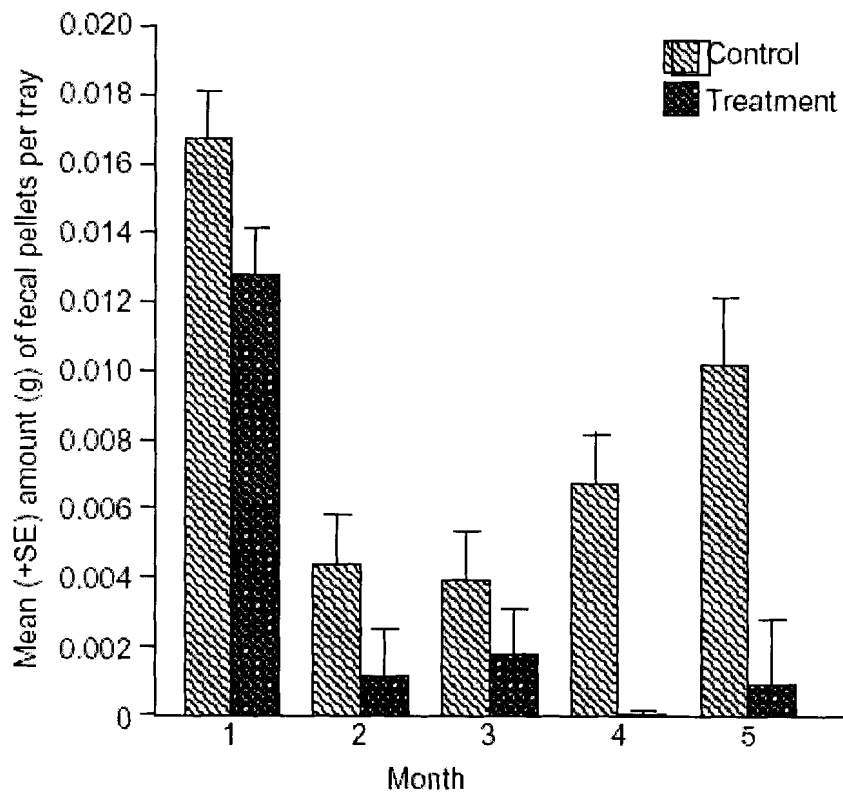


Table 3.2. Repeated measures ANOVA results for all criteria used to assess the “Attract and kill” tactic for reducing incidence and damage of *Trichoplusia ni* populations in commercial greenhouses.

Assessment	Effect	Num DF	Den DF	F	Pr > F
Eggs per plant	Treatment	1	10.7	6.28	0.0298
	Month	4	48.8	0.60	0.6657
	Treatment*Month	4	48.8	0.38	0.8219
Larvae per plant	Treatment	1	13.3	8.06	0.0137
	Month	4	51.7	2.65	0.0436
	Treatment*Month	4	51.7	1.38	0.2537
Pupae per plant	Treatment	1	14	10.19	0.0063
	Month	4	54.1	0.19	0.9448
	Treatment*Month	4	54.1	0.39	0.8161
Moths per row	Treatment	1	14.8	3.55	0.0794
	Month	4	55.3	1.21	0.3158
	Treatment*Month	4	55.3	0.23	0.9210
Plant Damage	χ^2	11	-	16.636	0.1191
Fecal pellet weight	Treatment	1	22.7	1.10	0.3230
	Month	4	51.3	1.28	0.2917
	Treatment*Month	4	51.4	0.22	0.9285

Table 3.3. Grid of Pearson correlation coefficients (r) and p-values for all criteria used to assess the “Attract and kill” tactic (Table 3.2) for reducing incidence and damage of *Trichoplusia ni* populations in commercial greenhouses. n=number of observations.

Statistical parameters		Eggs	Larvae	Pupae	Moths	Damage	Fecal Weight
r	Eggs		0.36062	0.21795	0.56544	0.22510	0.03817
p			0.003	0.0329	<0.001	0.0266	0.7417
n			97	96	97	97	77
r	Larvae			0.26654	0.31329	0.35839	0.12319
p				0.0087	0.0018	0.0003	0.2858
n				96	97	97	77
r	Pupae				-0.01652	0.00012	0.00654
p					0.8731	0.9991	0.9550
n					96	96	77
r	Moths					0.15093	0.03289
p						0.1379	0.7750
n						98	78
r	Damage						0.34444
p							0.0020
n							78
r	Fecal Weight						
p							
n							

of larvae per plant and number of pupae per plant, moths per row, and the degree of plant damage, but not the weight of accumulated larval fecal pellets; and 3) number of moths per row and the degree of plant damage and weight of accumulated larval fecal pellets. There was no significant correlation between the number of pupae per plant, and moths per row, degree of plant damage or weight of larval fecal pellets.

3.5 Discussion

The data provide evidence that Last Call™ CL, in combination with floral semiochemicals, has potential for *T. ni* control in commercial greenhouses (Table 3.2). Numbers of *T. ni* eggs, larvae and pupae were significantly lower in treatment than in control compartments (Figures 3.2, 3.3, 3.4). There were also fewer adult moths (Figure 3.5) and less plant damage (Figure 3.6) in treatment compartments, but the difference was not statistically significant.

Plant damage in control unlike treatment compartments continuously rose during the 5-month experimental period (Figure 3.6). The apparently greater damage in control compartments might have been confirmed as significant: a) had damage been assessed for the entire plant rather than the uppermost 30 cm, b) had it been possible to run the experiment as complete randomized blocks (with treatment and control compartment in the same greenhouse) which would have reduced data variation and would have increased the power of statistical analysis¹; c) had the PMRA research permit been issued and the experiment been initiated at the start of the growing season (January); and d) had there not been more initial damage in randomly selected treatment houses than in control

¹ Ian Bercovitz, Director Statistical Consulting Service, Simon Fraser University, pers. comm.

houses. Initiation of the A&K treatment early in the growing season would be advantageous because there are no crop flowers yet competing with A&K sources.

The finding that fecal weights were not correlated with the number of feeding larvae (Table 3.2), and did not differ between treatment and control houses is likely to be attributed to the sampling method. As plants grew taller and carried more foliage, larval fecal pellets were more likely to bounce off leaves and to be directed toward the plant's perimeter than to be collected in the tray beneath plants.

The impact of the A&K treatment is even more remarkable in light of the fact that control greenhouses were not true controls. Growers were allowed to apply every tactic against *T. ni* eggs and larvae they deemed necessary, including the release of biological control agents and sprays of Btk and/or chemical insecticides. Comparable control measures for *T. ni* in treatment and control compartments (Table 3.2; Appendix A) support the conclusion that the A&K tactic had a significant impact on the incidence of *T. ni* populations. The economic benefit of the A&K treatment may become obvious through comparative cost-benefit and fruit production analyses in treatment and control houses.

The A&K tactic targets the adult stage of *T. ni* populations, and is compatible and complementary with tactics aimed at *T. ni* eggs or larvae. If proven cost-effective, it should become part of operational, integrated *T. ni* control in commercial greenhouses.

Table 3.4. Summary of control measures employed by growers to reduce *Trichoplusia ni* populations in greenhouses.

Control Measures in Control Compartments									
<i>Bacillus thuringiensis</i>									
Compartment No. ²	Biological Control Agents ¹ (# of adults)			<i>Bacillus thuringiensis</i> (# applications)			Chemical Insecticides		
	<i>Trichogramma</i> (10 ² per m ²)	<i>Podisus</i> (per ha)	<i>Cotesia</i> Ladybirds (per m ²)	Dipel	Foray	Name	(# applications)		
2	44.1	170	0	0	2	1	0		
3	4.80	0	0	0	1	0	0		
4	4.50	6800	500	0	0	1	0		
8	0.78	0	0	0	0	2	0		
9	0.78	0	0	0	0	2	0		
11	0.50	0	0	0	0	0	Nicotine (1), Thiodan (1), Avid (1)		
12	2.55	0	0	0	0	1	0		
14	1.26	0	0	3.6	0	0	Nicotine (1)		
Total	59.27	6970	500	3.6	3	7	4		

Control Measures in Treatment Compartments									
<i>Bacillus thuringiensis</i>									
Compartment No. ²	Biological Control Agents (# of adults)			<i>Bacillus thuringiensis</i> (# applications)			Chemical Insecticides		
	<i>Trichogramma</i> (10 ² per m ²)	<i>Podisus</i> (per ha)	<i>Cotesia</i> Ladybirds (per m ²)	Dipel	Foray	Name	(# applications)		
1	45.7	167	0	0	0	1	0		
5	1.10	2000	500	0	0	0	0		
6	0	0	0	0	1	0	0		
7	1.08	100	0	7.5	2	0	Nicotine (2)		
10	0.625	0	0	0	0	2	0		
13	15.0	0	0	0	0	1	0		
15	1.02	0	0	1.8	0	0	Nicotine (1)		
16	1.02	0	0	1.8	0	0	0		
Total	65.55	2267	500	11.1	3	4	3		

¹ *Trichogramma brassicae* Bezdenko, *Podisus maculiventris* (Say), *Cotesia marginiventris* (Cresson), Ladybirds = *Coleomegilla maculata* (DeGeer)

² Numbers as reported in Table 3.1

4.0 Concluding Discussion

Integrated pest management (IPM) is founded on the principle that integration of multiple tactics provides a robust strategy that results in reduced use of conventional chemical pesticides. Modelling efforts have shown that combining pest management tactics that partition mortality across different life stages are often complementary, and provide a more powerful strategy than combining tactics that target the same lifestage (Barclay, 1992). For example, tree trunk banding to reduce overwintering populations of *C. pomonella* larvae (Newcomer *et al.*, 1933) coupled with pheromone-based mating disruption of *C. pomonella* adults allowed effective *C. pomonella* control in organic apple orchards with initially high *C. pomonella* populations (Judd *et al.*, 1997). My results provide evidence that A&K of *T. ni* adults is a tactic that can be integrated into IPM programs for *T. ni* control in commercial greenhouses. By targeting the adults, A&K may be complementary with parasitoids that target *T. ni* eggs and with pathogens, such as Btk and NPV, that target larval stages. Integration of A&K in an IPM program will certainly help partition mortality across several *T. ni* lifestages. It may also retard the development of resistance which occurs when a single tactic is used repetitively, and may help reduce crop protection costs. Trumble (1997) concluded that IPM programs utilizing pheromones in the vegetable production industry are financially more viable than conventional chemical controls.

If integration of the A&K tactic into IPM programs for *T. ni* proceeds, there are several ways in which it could be improved. Physical contact of moths with the insecticide-laced paste droplet is needed to kill moths. (*Z*)-7-Dodecenyl acetate induces anemotactic response of males (Mayer *et al.*, 1995), and as trap bait in A&K droplets

appeared equally effective as virgin females in attracting males (Figure 2.6). However, dodecyl acetate in combination with Z7-12:OAc enhanced the accuracy of males orientation to, and the number of contacts with, a point source (Shorey & Gaston, 1970; Linn & Gaston, 1981), and should be considered for incorporation in commercial A&K formulations. Prolonged or repeated contacts with a paste droplet should enhance male mortality and further impair mating ability.

Templast platforms as a carrier for A&K droplets added to the expense of the A&K program through both their cost and handling. These added costs may be justified if platforms were to be rented and/or shaped to enhance the visual stimulus for foraging moths. For example, moth-shaped silhouettes combined with pheromone-impregnated and insecticide-laced dispensers increased lethal and sub-lethal effects on the Mediterranean flour moth, *Ephiestia kuehniella* Zeller (Trematerra, 1995). Similarly, the presence of a moth image near a pheromone source increased the number of male *T. ni* that oriented toward, and attempted copulation with, the source (Birch & Haynes, 1982; Shorey & Gaston, 1970). Finally, flower model (Morrison, 1991) “platforms” may enhance the males’ and females’ response to and frequency of their contacts with A&K droplets disseminating floral semiochemicals. Platform colour should also be investigated as Lopez (1998) found that multicoloured traps were more attractive to beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera:Noctuidae), than single coloured traps alone. Costs associated with the incorporation of visual stimuli in A&K tactics may be offset by enhanced attractiveness and lethal potency of A&K, or by fewer droplets required for *T. ni* control.

Deployment of 800 A&K droplets pairs per ha, half impregnated with pheromone and the other half with floral semiochemicals, significantly reduced the pest impact of *T. ni* (Chapter 3). Employing >800 droplets per ha may have provided even better control, if this increased the probability of males approaching a droplet rather than a female. However, too many droplets might have disrupted the males' ability to perceive point sources of pheromone, reducing the efficacy of the A&K tactic (Westbrook & Lingren, 1998, Downham *et al.*, 1995). Further research is needed to determine the number of A&K droplets in commercial greenhouses required for optimal control of *T. ni* adults.

To be effective, the A&K tactic should be applied when *T. ni* population densities are low, which is typical early in the greenhouse growing season. At that time, A&K droplets would not be competing as much with calling female *T. ni*, nor would floral semiochemicals be competing with floral scent from crop plants that would not be in bloom. Similarly, Hofer & Angst (1995) concluded that low to moderate rather than high population densities of pink bollworm, *Pectinophora gossypiella* (Saunders), are most effectively controlled by A&K.

In concluding, I have presented experimental data suggesting that the A&K tactic could be incorporated into IPM programs for *T. ni* control in commercial greenhouses. If operational implementation proceeds, research should continue to optimise the efficacy of this tactic.

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Appendix A: Control Measures Applied to Suppress *T. ni* populations in commercial pepper greenhouses during the operational trial. T=Treatment and C=control

BMW

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (C) (0.16ha)	Trichogramma: 5000/week		•July 26 •September 29	
2 (C) (0.17ha)	Trichogramma: 5000/week		•July 26 •September 29	
3 (T)(0.33ha)	Trichogramma: 5000/week		•July 26 •September 29	
4 (C) (1.0ha)	Trichogramma: 20,000/week			Nicotine: •August 1
				Thiodan: •August 28
				Avid: •September 9

Set up May 22-25 2000

Century Pacific

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (T) (1.0ha)	Trichogramma: •May 11, 18, 25, June 1: each w/ 10,000,000 @ \$0.12/1000 •June 8, 15, 22, 29, July 6, 13, 20, 28: each w/ 300,000 •Aug 2, 8, 15, 22, 29, Sep 5, 12, 19, 26, Oct 4: @ 33/m ² (\$0.13/1000)		•Nov 1: 3mL/m ²	
	Podopsis: •July 25: 167 adults			
2 (C) (0.1ha)	Trichogramma: •May 11, 18, 25, June 1: each w/ 1,000,000 @ \$0.12/1000 •June 8, 15, 22, 29, July 6, 13, 20, 28: each w/ 30,000 •Aug 2, 8, 15: @ 33/m ² (\$0.13/1000) •Aug 22, 31	•September 3: 6 cans @ 500g •September 18: 6 cans @ 500g (3kg total = \$103.50)	•Oct 27: 3mL/m ²	
	Podopsis: •July 25: 17 adults			

Set up May 08 & 09 2000

Cheamview

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (T) (1.0ha)	<p>Trichogramma:</p> <ul style="list-style-type: none"> •May 23, 31, June 7: each w/ 120,000 @ \$0.74/1000 •June 16, 21: each w/ 120,000 @ \$0.55/1000 •June 29, July 5, 12: each w/ 120,000 @ \$0.74/1000 •July 19, 25: each w/ 120,000 @ \$0.44/1000 <p>Noctupar:</p> <ul style="list-style-type: none"> •May 23: 250 @ \$191.98 <p>Ladybird:</p> <ul style="list-style-type: none"> •February 23: 75,000 @ \$303.96 •August: 75,000 @ \$151.98 <p>Podesis:</p> <ul style="list-style-type: none"> •May 23: 100 @ \$104.80 	<ul style="list-style-type: none"> •June 6, June 13: each w/ 12 X 0.5kg @ \$18.25 per kg 		<p>Nicotine:</p> <ul style="list-style-type: none"> •August 17: 3 cases •September 27: 4 cases

Set up May 18 2000

Droogendyke

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (T) (0.4ha)		<ul style="list-style-type: none"> •June/July (spot treatment) 1 X 0.5kg @ \$18.25 per kg •August 19 (outside bays) 2 X 0.5kg @ \$18.25 per kg 		

Set up May 09 2000

Hazelmere

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (C) (0.1ha)	<p>Trichogramma:</p> <ul style="list-style-type: none"> •Weekly: 20 Females/m² 	<ul style="list-style-type: none"> •August 3 		

Set up June 07 2000

Mt. Lehman

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (C) (2.0ha)	Trichogramma: ●May 18: each w/ 400,000 ●May 24: 1,000,000 ●May 31, June 8: each w/ 500,000 ●June 15: 600,000 ●June 21, 28, July 5, 12, 19, 26: each w/ 1,000,000			
	Podesis: ●May 18: 2500 ●May 24: 1000 ●May 31: 1100 ●June 8: 2000 ●June 15: 5000 ●June 21: 2000			
	Cotesia: ●May 24: 500			
2 (T) (2.0ha)	Trichogramma: ●May 13, 19, 27: each w/ 400,000 ●June 6: 600,000 ●June 11, 18: each w/ 400,000			
	Podesis: ●June 4: 2500 ●June 11: 1000 ●June 18: 500			
	Cotesia: ●June 18: 500			

Set up May 11 & 15 2000

Peppertree

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (C) (1.0ha)	Trichogramma: ●60,000 /week till September 20 ●40,000 /week October 11 to end date			Nicotine: ●Oct 27: 1/8 can per 1200m ²
	Ladybirds: ●October 13, 20 150,000 ●October 27 75,000			
2 (T) (0.4ha)	Trichogramma: ●30,000 /week till September 20 ●20,000 /week October 11 to end date			Nicotine: ●Oct 27: ¼ can per 1200m ²
	Ladybirds: ●October 13, 20 150,000 ●October 27 75,000			
3 (T) (0.4ha)	Trichogramma: ●30,000 /week till September 20 ●20,000 /week October 11 to end date			
	Ladybirds: ●October 13, 20 150,000 ●October 27 75,000			

Set up May 26, 30 and 31 2000

Victoria

Compartment	Biocontrol	Dipel	Foray	Chemical
1 (C) (1.0ha)	Trichogramma: ●May 20: 850,000 ●July 15, August 2, 16: 850,000		●July 20	
	Podesis: ●May 20: 175 insects			
2 (T) (0.01ha)	Trichogramma: ●May 9, 10, 20: 150,000 ●July 15: 150,000		●July 20	

Set up May 23 2000