

University of Alberta

Development of a Semiochemical-based Monitoring System for
Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae),
in Canola in Alberta

by

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In memory of my Mom, Marge Miluch...

ABSTRACT

Studies focused on developing a semiochemical-based monitoring system for *Plutella xylostella* (L.) using sex pheromone and Z3-hexenyl acetate. A commercially available pheromone trapping system was used to capture male moths at sites in Alberta in 2007 and 2008. Larval sampling occurred every two weeks after the first males were captured. Male moth capture was predictive of larval density on individual sample dates during the growing season. The predictive capability of pheromone-baited trap capture was not in direct proportion to population density and was inconsistent. Modifications to the trapping system were tested to improve attractiveness. Adding Z3-hexenyl acetate at various doses to pheromone did not improve the attractiveness to males over pheromone alone and did not attract significant numbers of females when tested at various times during the flight season. Trap height and colour did not influence male capture. Pheromone dose and lure type did influence male moth capture in traps.

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List of Abbreviations

DD	Degree Days
EAG	Electroantennogram
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
IPM	Integrated Pest Management
PPMN	Prairie Pest Monitoring Network
Z11-16: Ac	(Z)11-hexadecenyl acetate
Z11-16: Ald	(Z)11-hexadecanal
Z11-16: OH	(Z)11-hexadecenol
Z9-14:Ac	(Z)9-tetradecenyl acetate
Z9-14: OH	(Z)9-tetradecenyl alcohol

Chapter 1. Literature Review and Research Objectives

1.1 Pheromone-based Monitoring

The reliance of individuals of many insect species on pheromone communication for mate finding, aggregation and dispersion makes the use of synthetic pheromones a valuable tool in integrated pest management (IPM) (Foster and Harris, 1997). Currently, the primary use for synthetic pheromones in IPM is to monitor populations to assist in timing and decision-making for insecticide applications (Baker and Heath, 2005) in order to minimize spray applications and optimize control. For example, pheromone-based monitoring of the tortricid moth complex that affects apple production in New York State, Michigan and the Pacific Northwest resulted in the reduction of pesticide applications by more than 50% during the mid-1970's to late 1980's (Madsen, 1981). Further reduction in pesticide application continues with the refinement of these monitoring programs (Agnello et al., 1994).

Many of the pheromone-based monitoring programs developed to date target lepidopteran and coleopteran species that are pests on valued resources. In stored-product facilities, the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), and cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), are monitored with pheromone-baited traps to provide information on pest presence. Detection leads to effective pest management decisions as there is zero-tolerance for insect infestations in these settings (Arbogast et al., 2000). Capture of male pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), moths in pheromone-baited

traps in cotton was related to population densities later in the season (Carrière et al., 2003). A decline in overall populations of pink bollworm was demonstrated by pheromone trap captures at sites where cotton is commonly grown and modified transgenically to carry genes for expression of the *Bacillus thuringiensis* (*Bt*) endotoxin (Carrière et al., 2003). Recent research on chemical communication in other insect orders (Baker and Heath, 2005) will undoubtedly lead to the development of monitoring systems in these species. Male-produced pheromones of two species of stinkbugs, *Chlorochroa sayi* (Stål) (Hemiptera: Pentatomidae) (Ho and Millar, 2001) and *Piezodorus hybneri* (Gmelin) (Hemiptera: Pentatomidae) (Leal et al., 1998), have been identified and research to develop effective monitoring systems continues. A system of trapping male and female peach aphids, *Tuberocephalus momonis* (Matsumura) (Hemiptera: Aphidae), utilizing a female-produced semiochemical has the potential to become an effective monitoring system (Boo et al., 2000). Interestingly, trap capture with this blend included numerous pre-sexual females and 20 other aphid species (Boo et al., 2000).

To date, there are several cases in which pheromone-based monitoring systems have been developed to predict population densities of pest species. In forest systems, pheromone monitoring is used as a predictive tool for potential outbreaks of several lepidopteran defoliators. Pheromone-based monitoring of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), has led to the development of an operational predictive-monitoring system in eastern North America (Ramaswamy et al., 1983; Allen et al., 1986; Sanders,

1988). Capture of male pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae), a defoliator of pines in the Mediterranean Basin, in pheromone-baited traps is related to densities of larval winter nests the following year and predicts incipient outbreaks (Jactel et al., 2006). Pheromone-based monitoring of the western hemlock looper, *Lambdina fiscelaria lugubrosa* (Hulst) (Lepidoptera: Geometridae), is a good predictor of subsequent egg populations, especially at the beginning of the flight period (Evenden et al., 1995a, b). Pupal counts for forest tent caterpillar, *Malacosoma disstria* (Hübner) (Lepidoptera: Lasiocampidae), and larval counts of large aspen tortrix, *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae), provided the best relationship with males caught in traps baited with lures of their combined sex pheromones in aspen (*Populus tremuloides* Michx.) forests in Alberta (Jones et al., 2009). There is a relationship between male pheromone trap capture of European pine sawfly, *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae), a defoliator of Scots pine (*Pinus sylvestris* L.), and egg densities in subsequent generations at peak population densities (Lyytikäinen-Saarenmaa et al., 2006). Pheromone monitoring programs for Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae), use the population trends indicated by trap capture in combination with egg-mass surveys to predict population location and potential defoliation in Douglas fir stands (Shepherd et al., 1985). Male gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), numbers in pheromone-baited traps predict egg mass densities (Thorpe et al., 1993). Predictions of outbreaks of the Nantucket pine tip moth,

Rhyacionia frustrana (Comstock) (Lepidoptera: Tortricidae), with pheromone-baited traps show a strong relationship in the first generation with subsequent larval damage to pine seedlings and saplings in Georgia, USA (Asaro and Berisford, 2001).

In crop systems there are also examples of the use of pheromones to predict incipient outbreaks of lepidopteran pests. Adult trap capture with pheromone-baited traps is an early predictor of outbreaks for bertha armyworm, *Mamestra configurata* (Wlk.) (Lepidoptera: Noctuidae), in Argentine canola (*Brassica napus* L.) (Turnock, 1987). Pheromone-baited trap captures of potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), adults is used to predict larval foliar damage (Shelton and Wyman, 1979). The use of low dose pheromone lures to attract male moths of *Argyrotaenia pulchellana* (Haw.) (Lepidoptera: Tortricidae) is predictive of subsequent larval populations in Italian orchards (Faccioli et al., 1993). Pheromone-based monitoring of the European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae), acts as a predictor of early oviposition of females and initiates monitoring for other life stages in corn crops (Ngollo et al., 2000). There is a relationship between pheromone-baited adult trap capture of corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), predicting larval infestations in corn crops (Latheef et al., 1991). Pheromone trap captures of the lingonberry fruitworm, *Grapholita libertina* (Heinrich) (Lepidoptera: Tortricidae), can predict larval damage to the fruit during seasons of fruit abundance (Hillier et al., 2004).

The predictive capability of pheromone-baited traps that target the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), has been tested in cabbage (Baker et al., 1982) and other cole crops (Walker et al., 2003). There is a relationship between mean male adult trap catch in a 24-hour period with mean larvae per plant with a lag of 7 to 24 days in cabbage (Baker et al., 1982). There is a significant correlation between moth capture in traps located on the edge and in the centre of fields indicating similar moth flight activity in both areas of the fields (Baker et al., 1982). Walker et al. (2003) determined that adult trap catches in cabbage, broccoli and cauliflower crops predict *P. xylostella* larval infestations in the spring and summer in New Zealand. However, large catches in pheromone-baited traps late in the summer do not predict larval populations. External abiotic (climate) and biotic (natural enemies) factors influence pheromone-baited trap capture of *P. xylostella* and limit the usefulness of pheromone-based monitoring as a predictive tool of this species (Walker et al., 2003). The current project aims to develop a semiochemical-based monitoring system for *P. xylostella* in canola in western Canada that can detect and predict population densities. Strategies to enhance the attractiveness of commercially available lures to male moths are also explored in these studies.

1.2 *Plutella xylostella* (L.)

Plutella xylostella (L.) (Lepidoptera: Plutellidae) is considered one of the most destructive insect pests worldwide on crops in the family Brassicaceae (Talekar and Shelton, 1993; He et al., 2003; Sarfraz et al., 2006). The estimated annual cost to manage this insect is approximately one billion dollars (U.S.)

(Talekar and Shelton, 1993). During outbreaks in western Canada the cost of controlling *P. xylostella* have been significant for example, 1995 \$42 million, 2001 \$86 million, 2003 \$4 million and 2005 \$3.5 million (Dosdall et al., 2008). *Plutella xylostella* occurs wherever brassicaceous crops are grown and is the most widely distributed of all Lepidoptera (Talekar and Shelton, 1993). *Plutella xylostella* is primarily oligophagous on members of the Brassicaceae, but in rare instances *P. xylostella* exploits alternate host plants. For example, a population in Kenya recently shifted hosts from cabbage to feed on sugar-snap and snow peas (*Pisum sativum* L.) (Fabaceae) (Rossbach et al., 2006). In western Canada, *P. xylostella* is a significant pest on canola, *Brassica rapa* L. and *B. napus* (Palaniswamy et al., 1986; Philip and Mengersen, 1989; Dosdall et al., 2004). *Plutella xylostella* can also be pestiferous in other areas of canola production including additional regions of Canada, the U.S.A., Australia and Europe (Shelton, 2001).

Plutella xylostella is multivoltine with up to four generations annually in the Canadian prairies and six in southern regions of Ontario (Harcourt, 1957). All life stages can be present in the field simultaneously. The life cycle of *P. xylostella* averages 32 days (from egg to adult) and ranges from 21 to 51 days under field conditions (Harcourt, 1957). Time to maturity is highly dependent upon climatic conditions especially temperature. *Plutella xylostella* reared at constant temperatures between 8-32°C were able to complete development, however insects reared at constant temperatures outside this range did not complete development from egg to adult (Liu et al., 2002). Food quality (Sarfrac

et al., 2009) and genotype (Sarfranz et al., 2007) can also influence *P. xylostella* development. Varying fertilizer regimes affect the quality of host plant nutrients available to developing larvae, fastest larval development was observed on *B. napus* with moderate fertility regimes (Sarfranz et al., 2009). Host plant species and cultivar significantly affected the development time of both larvae and pupae (Sarfranz et al., 2007).

Plutella xylostella eggs are flat, yellowish in colour and appear glued to the leaves on host plants, particularly the adaxial surface (Harcourt, 1957; Justus et al., 2000). Eggs hatch in four to eight days depending on temperature. There are four larval instars. Newly hatched first-instar larvae bore into the epidermis and mine the leaf tissue. At the end of the first instar, larvae emerge from the leaf tissue and become surface feeders (Harcourt, 1957). Eventually all leaf material can be consumed excluding the veins and upper epidermal layer which gives damaged leaves a windowpane effect. Late-instar larvae may move to other parts of the plant to forage, such as the siliques, flowers and buds, as the leaf surface area is depleted. *Plutella xylostella* larvae feed efficiently on brassicaceous hosts because larvae deactivate defensive glucosinolate compounds (Sarfranz et al., 2006) with glucosinolate-sulfatase found in the larval gut (Ratzka et al., 2002). The sulfatase prevents formation of highly toxic hydrolysis products of glucosinolates (Sarfranz et al., 2006). Elevated levels of glucosinolate-sulfatase in the larval gut of *P. xylostella* enhance the ability of larvae to feed on a wide range of brassicaceous species with diverse glucosinolate structures (Sarfranz et al., 2006; Sarfranz et al., 2010).

Final-instar larvae spin a delicate mesh cocoon and remain in quiescence for one to two days pre-pupation. Pupae are usually found on the host plant and pupation lasts between five and 15 days depending upon climatic conditions (Harcourt, 1957). Adult activity is crepuscular and moths often sit motionless on plants during the day unless disturbed when they fly upwards in a narrow spiralling pattern or crawl and fly rapidly in search of shelter (Harcourt, 1957).

Mating and oviposition occur after dusk and peak about two hours into the scotophase (Harcourt, 1957; Pivnick et al., 1994). Females normally mate only once whereas males can mate up to five times during their life span (Wang et al., 2005). Mate finding behaviour is mediated by a female-produced sex pheromone (Tamaki et al., 1977; Chow et al., 1977; Ando et al., 1979). The sex pheromone consists of three identified components: (Z)11-hexadecenyl acetate (Z11-16:Ac), (Z)11-hexadecenal (Z11-16:Ald) and (Z)11-hexadecen-1-ol (Z11-16:OH) (Table 1.1). Ratios of Z11-16:Ac, Z11-16:Ald and Z11-16:OH analyzed in a Korean population from female gland extracts are 100:7.5:18.3 (Suwon, Korea) and 100:7.9:17.7 (Jeju Island, Korea) (Yang et al., 2007), whereas the blend extracted from female glands in a New Zealand population has a ratio of 60:10:30 (Suckling et al., 2002). There is also evidence for a fourth, as of yet unidentified component, that may be a long chain hydrocarbon found in female gland extracts in New Zealand (Suckling et al., 2002). Gland extracts from *P. xylostella* females in a Texas population were analyzed and have a ratio of 1:3:2, Z11-16:Ac, Z11-16:Ald and Z11-16:OH (He et al., 2003). Response to sex pheromone by *P. xylostella* males also shows geographic variation across its broad range (Maa et

al., 1984; Zihali-Balogh et al., 1995; Môtus et al., 1997; Yang et al., 2007) (Table 1.1).

Host plants stimulate the onset of reproductive activities of *P. xylostella*. In the presence of host plants, females call at a younger age, earlier in the scotophase, and spend more time calling (Pittendrigh and Pivnick, 1993). Host volatiles also enhance male orientation to sex pheromone sources (Reddy and Guerrero, 2000a; Reddy and Guerro, 2004).

Females search out plants for oviposition using host volatile cues (Palaniswamy et al., 1986; Reed et al., 1989; Reddy et al., 2003; Renwick et al., 2006). Female *P. xylostella* respond to a variety of compounds released by crucifer plants including terpenes or green leaf volatiles from intact plants, isothiocyanates from damaged plants and combinations of these compounds in Y-Tube choice and electroantennogram (EAG) experiments (Pivnick et al., 1994). Females normally lay eggs singly and oviposit approximately 160 eggs within an oviposition period lasting about 10 days (Harcourt, 1957; Sarfraz et al., 2010). The volatile metabolites of glucosinolates (allyl isothiocyanates) act as oviposition stimulants for females (Talekar and Shelton, 1993; Sarfraz et al., 2006). In addition to olfactory stimulants for oviposition, female *P. xylostella* also use tactile (Justus and Mitchell, 1996) and visual cues. Females oviposit more frequently on plants with less phylloplane wax than on surfaces high in wax content (Justus et al., 2000). The waxiness acts as a visual cue because there is a different light spectrum reflected from waxy versus glossy leaves (Justus et al., 2000).

Plutella xylostella adults are small, weak fliers and easily are carried by wind currents, which facilitate long distance migration on trajectory winds up to 1000 km per day (Talekar and Shelton, 1993). In the Canadian prairies, *P. xylostella* populations originate primarily from the southern United States or Mexico and are transported northward via trajectory winds (Dosdall et al., 2004; Hopkinson and Soroka, 2010). Small overwintering populations of *P. xylostella* in the United Kingdom are augmented by immigrants from continental Europe that are carried to the U.K. by similar air-flow mechanisms (Chapman et al., 2002).

Plutella xylostella are capable of overwintering in the Canadian prairies (Dosdall 1994), but this occurs rarely (Dosdall et al., 2004). No living specimens were found at sites in Vegreville, Alberta (1994, 1995, 1996 and 1997) or Saskatoon, Saskatchewan (1996 and 1997) after attempts to overwinter different life stages (eggs, larvae, pupae and adults) under a wide range of conditions (Dosdall et al., 2004). With predictions of global warming, there is potential for a dramatic increase in the pest status of this species in western Canada (Dosdall et al., 2008).

1.3 Management of *Plutella xylostella* (L.)

Plutella xylostella populations are difficult to manage because of their reproductive potential and the rapidity with which they develop insecticide resistance. For example, *P. xylostella* was the first species to become resistant to DDT (dichloro-diphenyl-trichloroethane) (Johnson, 1953) and to *Bt* (*Bacillus thuringiensis* Berliner var. *kurstaki*) (Kirsch and Schmutterer, 1988; Tabashnik et

al., 1990). *Plutella xylostella* can also demonstrate rapid behavioural resistance to foliar pesticides as field-collected females often lay eggs in a band near the soil-stem interface as a potential adaptation to avoid exposure to foliar-applied insecticides (Sarfraz et al., 2005). In some tropical countries, particularly portions of Southeast Asia, Central America, and the Caribbean, cultivation of brassicaceous crops is nearly impossible because the cost of *P. xylostella* control is not economical (Talekar and Shelton, 1993). The severe worldwide pest status of *P. xylostella* is due to the wide range of brassicaceous crops it attacks, the low density or disruption of natural enemies that often accompany infestations, its high reproductive potential, and its genetic plasticity that facilitates the rapid development of pesticide resistance (Sarfraz et al., 2006).

Plutella xylostella populations in some regions, especially areas of milder climates, are resistant to almost all commercial products used against it (Tabashnik et al., 1987; Perng et al., 1988; Kao et al., 1989; Kobayashi et al., 1992; Yu and Nguyen, 1992; Tabashnik et al., 1993; Baker and Kovaliski, 1999; Pérez et al., 2000; Shelton et al., 2000; Zhao et al., 2000; Zhao et al., 2002; Mohan and Gujar, 2003; Ninsin, 2004; Sayyed et al., 2004; Mau and Gusukuma-Minuto, 2004; see Table 1 *In*: Sarfraz and Keddie, 2005). There are reported cases of success with tank-mixing products with different modes of action once resistance has occurred (Jansson and Lecrone, 1988; Leibe and Savage, 1992); however, the long-term sustainability of this practice is questionable (Talekar and Shelton, 1993).

Bacillus thuringiensis is a gram-positive soil bacterium which is a complex of subspecies that are highly pathogenic to insects (Bauer, 1995). *Bacillus thuringiensis* (Berl.) var. *kurstaki* (*Btk*) is a promising biopesticide for many lepidopteran insect pests including *P. xylostella*, as it has little impact on non-target organisms (Sarfraz et al., 2005). The first incidence of *Btk* resistance in the field occurred in a population of *P. xylostella* in Hawaii in 1990 (Tabashnik et al., 1990). In many crucifer-producing areas in temperate climates, *Btk* is still effective against *P. xylostella* if used at registered application rates (Braun et al., 2004; Sarfraz et al., 2005). The use of *Btk* transgenic crops to manage *P. xylostella* looks promising for the future, but good agronomic practices such as refugia of non-transgenic crops and integrated pest management with additional tactics like bio-control agents and pheromone-based management need to be used in conjunction with *Bt* transgenic crops to maintain its efficacy in the long run (Talekar and Shelton, 1993).

Other microbes (including bacteria, fungi, viruses, protozoans, and nematodes) infect *P. xylostella*, but relatively few studies demonstrate control with these agents (Sarfraz et al., 2005). Two fungal pathogens isolated from *P. xylostella*, *Zoophthora radicans* (Brefeld) Batko and *Beauveria bassiana* (Balsamo), can cause epizootics in *P. xylostella* populations (Furlong and Pell, 2001) and reduce localized populations under favourable environmental conditions (Reithmacher et al., 1992). There is potential for the utilization of both of these fungi as tools in an IPM strategy for *P. xylostella* (Vickers et al., 2004).

Selection for resistant morphological and chemical plant traits in *Brassica* species breeding programs may enhance crop plant resistance to *P. xylostella* (Andrahennadi and Gillott, 1998). Biochemical resistance breeding programs manipulate the structure and function of glucosinolates, which normally act as oviposition and feeding stimulants for *P. xylostella* (Thorsteinson, 1953; Gupta and Thorsteinson, 1960; Justus and Mitchell, 1996). Identification of glucosinolates that elicit egg-laying or feeding behaviour could lead to the reduction of those compounds in selected varieties (Reed et al., 1989). Plant volatile compounds like allyl isothiocyanate that attract adult *P. xylostella* also function to arrest larval development (Reed et al., 1989) and in high concentrations can be toxic to diamondback moth adults and neonates (Sarfranz et al., 2005).

Morphological traits that might be targeted for crop resistance to *P. xylostella* include leaf colour, size, and position. Glossy leaf surfaces are more attractive than leaves with a waxy surface (Justus et al., 2000), and leaf colour is one of the most important host selection criteria for *P. xylostella* (Sarfranz et al., 2005). Host plants high in cuticular waxes and trichomes delay oviposition and development of *P. xylostella* (Eigenbrode and Shelton, 1990; Talekar et al., 1994; Eigenbrode et al., 1991a; Eigenbrode et al., 1991b; Sarfranz et al., 2005).

Trap cropping is another tactic that can be employed in the integrated management of *P. xylostella*. Planting strips of preferred, non-commercial host plants (e.g., *Sinapis alba* L. [white mustard] or *Brassica juncea* (L.) Czern. [brown mustard]) with the commercial brassicaceous crops directs *P. xylostella*

away from and minimizes damage on the crop plant species. Additional control can occur as larvae feed on the preferred host and natural enemies aggregate at the feeding site (Talekar and Shelton, 1993). For example, *Barbarea vulgaris* R. Br., a biennial brassicaceous weed, is five times more attractive to ovipositing female *P. xylostella* than *B. napus*. The effectiveness of a trap crop comprised of *B. vulgaris* plants is maximized as larvae do not survive on *B. vulgaris* due to the presence of a feeding deterrent, monodesmosidic triterpenoid saponin (Shelton and Nault, 2004). Further investigations have demonstrated that *Brassica oleracea* L. var. *acephala* planted early at high seeding rates is an effective trap crop in cabbage systems. Females are more attracted to older plants for oviposition and larval survival is decreased on older plants (Badenes-Perez et al., 2005).

Intercropping of non-host crops with brassicaceous host crops can act as a barrier to normal behaviour in crucifer specialists (Finch and Collier, 2000; Dossdall et al., 2003; Dixon et al., 2004; Hummel et al., 2009). Natural enemy populations are often higher in more biodiverse environments and heterogeneous systems can interrupt host-finding behaviour of specialist herbivores (Talekar and Shelton, 1993; Åsman et al., 2001). Cole crops intercropped with tomato (*Lycopersicon esculentum* L.) (Perrin and Phillips, 1978) and red clover (*Trifolium pratense* L.) (Åsman et al., 2001) contribute to effective management of *P. xylostella* but success in other systems has been limited (Talekar and Shelton, 1993).

Biological control of *P. xylostella* can significantly influence populations during outbreaks. Parasitoid populations can maintain *P. xylostella* at levels that avoid economic loss (Sarfraz et al., 2005). There are over 135 species of parasitoids that attack *P. xylostella* at various life stages (Delvare, 2004). In addition, generalist predators including ants, flies, lacewings, beetles, hemipterans, spiders and birds can prey on the larval stages though their impact is not significant in population control of *P. xylostella* (Sarfraz et al., 2005). In Canada, there are three main species of hymenopteran parasitoids that attack *P. xylostella*: the ichneumonid *Diadegma insulare* (Cresson) (Putnam, 1978; Harcourt, 1986; Braun et al., 2004; Dossdall et al., 2004; Sarfraz et al., 2005) and the braconid *Microplitis plutellae* (Muesbeck) (Putnam, 1978; Harcourt, 1986; Braun et al., 2004; Dossdall et al., 2004; Sarfraz et al., 2005) which are both parasitic on the larvae, and the ichneumonid *Diadromus subtilicornis* (Gravenhorst) that attacks the pre-pupal and pupal stages (Harcourt, 1986; Braun et al., 2004; Dossdall et al., 2004; Sarfraz et al., 2005). In Alberta and Saskatchewan, the principal parasitoid of *P. xylostella* is *D. insulare* that accounts for 45% and 30% of total parasitism in each province, respectively. Parasitism by *M. plutellae* and *D. subtilicornis* has significantly less impact on *P. xylostella* populations in western Canada (Braun et al., 2004; Sarfraz et al., 2005).

Pest managers have attempted to exploit sex pheromone-mediated behaviours of *P. xylostella* to control populations with mixed results. The potential for mating disruption with synthetic sex pheromone was tested with little success in cabbage in New York State (Schroeder et al., 2000), but these

experiments were conducted in field cages that would enhance the mate finding behaviour of adult moths. Mating disruption with a supplemental insecticide application protects cabbage from *P. xylostella* in Florida (McLaughlin et al., 1994). Application of a synthetic pheromone blend of Z11-16: aldehyde and Z11-16: acetate effectively disrupted chemical communication of *P. xylostella* in field plots of canola near Saskatoon, Saskatchewan as measured by reduced trap capture in pheromone-baited traps in treated plots (Chisholm et al., 1984).

Mitchell (2002) tested an attracticide formulation in cabbage that consisted of the sex pheromone of *P. xylostella* and permethrin; the potential to use this formulation to manage *P. xylostella* looks promising. Similar formulations have suppressed sexual communication, killed the attracted moths and decreased mating in other insect pests, for example, codling moth [*Cydia pomonella* L. (Lepidoptera: Tortricidae)] in apple orchards (Charmillot et al., 2000) and pink bollworm [*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)] in cotton (Hofer and Brassel, 1992). A similar attracticide formulation tested against *P. xylostella* in cabbage and collards in Alabama was more effective at low to moderate populations in the fall in comparison to high populations in the spring (Maxwell et al., 2006). Attracticide formulations are not likely to be effective as stand-alone tactics but work best in combination with other IPM strategies (Maxwell et al., 2006).

A mass trapping study in Vietnam investigated the effectiveness of pheromone-baited traps to reduce populations of *P. xylostella* (Wang et al., 2004). Pheromone-baited plastic basin traps in combination with *Bt* applications

decreased population densities in kohlrabi and cabbage fields (Wang et al., 2004). An IPM program that combined pheromone-based mass trapping for *P. xylostella* with application of *Bt* and the release of predatory green lacewings, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and the larval parasitoid, *Cotesia plutellae* Kurdj (Hymenoptera: Braconidae), yielded profits ranging from \$777 to \$810 per ha compared to \$456 to \$462 in the traditional insecticide-treated cabbage fields in India (Reddy and Guerrero, 2000b). These studies provide evidence that pheromone-based management is suited for inclusion in an IPM system rather than as an independent management strategy for *P. xylostella*.

1.4 The Role of Pheromones in Population Monitoring for *Plutella xylostella* (L.)

Plutella xylostella use female-produced sex pheromones to mediate mate finding and initiate reproduction; exploiting male attraction to pheromones makes the use of pheromone trap monitoring a component of IPM programs as discussed in Section 1.2. *Plutella xylostella* female sex pheromone compounds have been isolated (Table 1.1) by several lab groups. There are three components found in the majority of the attractive blends published to date, which comprise (Z)11-hexadecenyl acetate (Z11-16: Ac), (Z)11-hexadecanal (Z11-16: Ald) and (Z)11-hexadecen-1-ol (Z11-16: OH). In western Canada, (Z)9-tetradecenyl acetate (Z9-14:Ac) or (Z)9-tetradecen-1-ol was added as a fourth component, which increased attractiveness of the blend to males (Chisholm et al. 1983). Several different pheromone blends attract *P. xylostella* males in different regions of the world (Table 1.1).

The majority of sex pheromone-based monitoring has been in cabbage and other cole crops and to a lesser extent in canola (Dai et al., 2008; Yang et al., 2007; He et al., 2003; Suckling et al., 2002; Reddy and Guerrero, 2000b; Môtus et al., 1997; Zilahi-Balogh et al., 1995; Reddy and Urs, 1996; Maa et al., 1984; Chisholm et al., 1983; Lin et al., 1982; Chisholm et al., 1979; Ando et al., 1979; Koshihara et al., 1978; Chow et al., 1977; Tamaki et al., 1977) (Table 1.1). Synthetic pheromone of *P. xylostella* is used to monitor and detect pest populations (Baker et al., 1982; Chisholm et al., 1983; Reddy and Guerrero, 2000b; Suckling et al., 2002). A pheromone-trapping network is in place across the Canadian Prairie Provinces called the Prairie Pest Monitoring Network (PPMN) (Hopkinson and Soroka, 2010). The attractiveness of synthetic pheromones used to bait monitoring traps is variable (He et al. 2003; Evenden and Gries, 2010) and female-baited traps routinely capture more males than traps baited with synthetic blends (Chow et al., 1977; Koshihara et al., 1978; Zilahi-Balogh et al., 1995; Suckling et al., 2002; Evenden and Gries, 2010). The significance of male trap capture to predict field population densities has not been examined for *P. xylostella* in canola systems. The use of host volatiles in combination with synthetic sex pheromone blends in lures to improve moth attraction to traps has been successful in cabbage systems. Reddy and Guerrero (2000a) tested (*Z*)-3-hexenyl acetate, a common green leaf volatile, in combination with an attractive pheromone blend and increased adult moth trap capture. Significantly more male moths were captured in traps baited with pheromone + (*Z*)-3-hexenyl acetate + (*Z*)-3-hexen-1-ol + allyl isothiocyanate

compared to the blend of pheromone + (Z)-3-hexenyl acetate; the former blend also increased the number of female moths captured in traps (Dai et al., 2008). The Dai et al. (2008) study determined that the combination of pheromone + (Z)-3-hexenyl acetate + (Z)-3-hexen-1-ol + allyl isothiocyanate would be the most effective bisexual attractant for *P. xylostella*.

1.5 Research Objectives

The purposes of this thesis are: 1) to determine the predictive capability of commercially available pheromone lures for populations of *P. xylostella* in canola systems in western Canada; and 2) to identify factors that increase attractiveness of pheromone-baited traps to male *P. xylostella*. To address the first objective, season-long experiments were conducted over two field seasons in which trap capture in pheromone-baited traps was related to immature populations of *P. xylostella* in the same locations throughout the season. To address the second objective factors investigated to evaluate the attractiveness of pheromone-baited traps included: 1) trap height throughout the season; 2) lure type (grey versus red rubber septa); 3) pheromone dose; and 4) the incorporation of visual (trap colour) and olfactory (green leaf volatile) host cues.

Pheromone monitoring of *P. xylostella* in canola currently is not a consistent indicator of outbreaks. Through the development of a system to relate adult trap capture to immatures in the field and improve the attractiveness of the lures, this project aims to enhance the ability of canola producers and pest management professionals to manage this pest in western Canada.

Table 1.1. *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) sex pheromone blends shown to be attractive in various locations.

Year	Author	Testing Location	Pheromone Components (μg)				Crop(s)
			Z11-16: Ac	Z11-16: Ald	Z11-16:OH	Z9-14:Ac	
2008	Dai et al.	China	3	7	1	-	Cabbage
			90	10	1	-	
2007	Yang et al.	Korea	90	10	10	-	Chinese
			100	8	18	-*	Cabbage
2003	He et al.	USA	1	3	2	-*	Cabbage
			60	40	-	-	
2002	Suckling et al.	New Zealand	30	60	10	-	Brassica spp.
			60	10	30	-*	
2000	Reddy & Guerrero	India	1	1	0.01	-	Cabbage
1997	Mõttus et al.	Estonia	1	1	0.01	-	Cabbage
1995	Zilahi-Balogh et al.	Indonesia	6	4	-	-	Cabbage
			4	6	-	-	
1996	Reddy and Urs	India	50	50	1	-	Cabbage and Cauliflower
1984	Maa et al.	Northern Taiwan	7	3	0.1	-	Cabbage
1983	Chisholm et al.	Canada	30	70	1	0.1	Canola
1982	Lin et al.	Taiwan	5	5	0.1	-	Cabbage
1979	Chisholm et al.	Canada	3	7	-	-	Canola
1979	Ando et al.	Japan	1	1	0.1	-	Cabbage
1978	Koshihara et al.	Japan	4	6	-	-	Cabbage
1977	Chow et al.	Taiwan	1	1	-	-	Cabbage
1977	Tamaki et al.	Japan	1	1	-	-	Cabbage
			6	4	-	-	

*-Indicates a ratio determined from female gland extracts

1.6 Literature Cited

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Chapter 2. Development of a Pheromone-based Monitoring System to Predict Populations of *Plutella xylostella* (L.) in Canola

2.1 Introduction

Models that can predict insect population density based on adult capture in pheromone-baited traps are used for a number of pest species in various managed systems. For instance, adult trap capture with pheromone-baited traps is an early predictor of outbreaks for bertha armyworm, *Mamestra configurata* (Wlk.) (Lepidoptera: Noctuidae), in canola (*Brassica napus* L.) (Turnock, 1987). Pheromone-baited trap capture of potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is used to predict larval foliar damage in potato (Shelton and Wyman, 1979). Pheromone-based monitoring of the European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae), predicts early oviposition of females and initiates monitoring for other life stages in corn crops (Ngollo et al., 2000). Pheromone-baited adult trap capture of corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), predicts larval infestations in corn (Latheef et al., 1991).

The predictive capability of pheromone-baited traps that target the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), has been tested in cabbage (Baker et al. 1982) and other cole crops (Walker et al. 2003). A relationship exists between mean male adult trap catch in a 24-hour period and mean larvae per plant with a lag of 7 to 24 days in cabbage (Baker et al. 1982). Trap catch in pheromone-baited traps predicts population density of *P. xylostella* in *Brassica* vegetables in New Zealand (Walker et al., 2003). Male moth capture

in pheromone-baited traps predicts larval infestations in crops established in spring and early summer, but not late summer (Walker et al., 2003). A predictive model based on moth capture in pheromone-baited traps has not been developed for *P. xylostella* in canola systems. *Plutella xylostella* demonstrate wide geographic variation in response to pheromone, which may necessitate the development of different predictive models in different growing regions (Zilahi-Balogh et al., 1995; Yang et al., 2007).

Plutella xylostella is a cosmopolitan pest of cruciferous crops that costs producers upwards of \$1 billion (US) annually on pest management (Talekar and Shelton, 1993) and can render growing regions unsuitable for cruciferous crop production. *Plutella xylostella* is multivoltine with up to four generations annually in the Canadian Prairies (Harcourt, 1957). Overlapping generations occur once the adults arrive on trajectory winds from the southern US or Mexico in early spring (Doddall et al., 2004; Hopkinson and Soroka, 2010). Detection of the arrival of moths is best achieved with a sensitive monitoring tool such as pheromone-baited traps. Sex pheromone-baited traps are used to monitor the adult activity of *P. xylostella* across the Canadian Prairie provinces in the government-funded Prairie Pest Monitoring Network (<http://nlwis-snite2.agr.gc.ca/ppmn/loginFormEn.jsp>) but it is not known if the number of male moths captured is related to population density of the damaging larval stage.

The purpose of these experiments is to determine whether *P. xylostella* male moth captures in synthetic sex pheromone-baited traps is a reliable indicator of larval densities in canola, and therefore can be used to predict the temporal and

spatial distributions of *P. xylostella* populations. Experiments were conducted in 2007 and 2008 when overall diamondback densities in Alberta were moderate and low, respectively. Pheromone-baited trap capture of male *P. xylostella* was compared to estimates of abundance for larvae sampled at the same sites over a similar and delayed sample period to account for larval development. An additional experiment conducted in 2008 compared the attractiveness of sex pheromone-baited traps positioned at different heights in the crop canopy throughout the field season.

2.2 Materials and Methods

2.2.1 Capability of Pheromone-Baited Trap Capture to Predict Larval Densities

Site Selection

In early May of 2007 and 2008, 15 sites were established on canola field margins in south-central Alberta, Canada (Appendix 1). The study sites encompassed an area of approximately 40,000 km² and extended from near Red Deer (52°16'N; 113°48'W) to south of Lethbridge (49°42'N; 112°49'W), near the U.S.A. border. Experiments were established on different fields, but within the same general proximity in the two trapping seasons (Appendix 1) because of the agronomic practice of crop rotation. The distance between individual sites within each experimental season was at least 1 km. Crop type on adjacent fields was recorded in both years (Appendix 2). Where access to fields permitted, the southwest corner of the field was chosen for trap placement as *P. xylostella* passively migrate on trajectory winds from the southwest.

Male Moth Sampling with Pheromone-Baited Traps

In early May 2007, three plastic delta traps (Contech Enterprises Inc., Delta, British Columbia, Canada) fitted with removable sticky inserts were baited with commercially available *P. xylostella* pheromone lures (Contech Enterprises Inc., Delta, British Columbia, Canada) at each site. Lures were transported to sites in refrigerated containers. Traps were erected 50 m apart along a linear transect at the field margin ~1.5 m above the ground on L-shaped metal hangers fashioned from rebar (Totem Welding Co. Ltd., Edmonton, Alberta, Canada). Six traps were erected at each site in a similar manner in early May 2008. Traps were checked at two-week intervals throughout the growing season from early May to mid-August in both years. At each two-week check, sticky inserts were changed and moths were counted. In 2007, lures were changed at six-week intervals. In 2008, lures in three of the six traps at each site were changed on a six-week schedule and lures in the other three traps were changed at three-week intervals as recommended by the Prairie Pest Monitoring Network.

Immature Sampling

In both 2007 and 2008, sampling for immature stages of *P. xylostella* commenced in the sampling period after the first male moths were captured in pheromone-baited traps. There were five larval collection dates in 2007 and six in 2008 due to the variable arrival time of moths on trajectory winds between the two years. At each site and collection date, 50 canola plants were sampled systematically along a zigzag pattern approximately 15 m into the field along the length of the linear trap transect. Plants were collected, bagged individually, kept

in refrigerated containers and transported to the laboratory for processing. To remove insects from the plants, each plant was washed in 95% ethanol. Insects were counted, and the larval instar was determined based on body length and head capsule width (Harcourt, 1986). Larval collections were stored in 70% ethanol. Voucher larval specimens from several collection sites have been deposited in the entomology collection of Olds College, Olds, Alberta.

Predictive Model

The trap capture of male moths was regressed against the number of immatures sampled at the same site on each sample date and the subsequent sample date using Negative Binomial Regression Models with the PROC GENMOD procedure (SAS Institute, 2005) as the collected data (adults and immatures) were highly overdispersed. In 2007, the total number of males captured in all three traps at each site and the number of males captured in an individual trap located in the middle of the transect and the respective quadratic terms were used as the independent variables in separate models. Models were generated separately for each sample date to examine relationships between adults and immatures sampled on the same day and when adults were collected in the sampling period prior to the immature sampling. In 2008, a similar approach was used with Negative Binomial Regression Models (PROC GENMOD) (SAS Institute, 2005) except that additional models were developed based on moth captures in traps with lures changed at three-week intervals.

Degree-days

Degree-days were calculated to compare the developmental stages of *P. xylostella* sampled between the two study years. Cumulative degree-days throughout the trapping period in 2007 and 2008 were calculated using the single sine method (Zalom et al., 1983) with the 'ddsine' program (Snyder, 2001). The developmental threshold used in the calculation was 7.3°C for *P. xylostella* (Harcourt, 1954; Butts and McEwen, 1981; Liu et al., 2002). Degree-days were accumulated from 3 May to 20 August of each year. Maximum and minimum daily temperature data for Lethbridge, Alberta, Canada were obtained from Environment Canada (http://www.climate.weatheroffice.ec.gc.ca/climateData/Canada_e.html).

Sweep Net versus Pheromone Trap Sampling (2008)

In 2008, samples with a standard insect sweep net (40 cm diameter) were taken throughout the season at each of the pheromone-trapping sites. The number of *P. xylostella* immatures and adults in five 180° sweeps at each site was recorded and compared to the number of male moths captured in pheromone-baited traps with lures changed at 3- and 6-week intervals. Data were normally distributed and insect capture using the two sample techniques over the entire season was compared by a One-Way ANOVA using PROC GLM (SAS Institute, 2005) followed by a Tukey's test to compare individual treatments.

2.2.2 The Effect of Trap Height on Adult Male Moth Trap Capture (2008)

In 2008, a season-long experiment tested the hypothesis that trap height would affect male moth capture in traps baited with commercial pheromone lures (Contech Enterprises Inc., Delta, British Columbia, Canada). The experiment was replicated at eight sites in central and southern Alberta, Canada (Appendix 3). In early May 2008, three plastic delta traps (Contech Enterprises Inc., Delta, British Columbia, Canada) fitted with removable sticky inserts were baited with commercially available *P. xylostella* pheromone lures (Contech Enterprises Inc., Delta, British Columbia, Canada) at each site. Lures were transported to sites in refrigerated containers. Traps were erected 50 m apart on L-shaped metal hangers fashioned from rebar (Totem Welding Co. Ltd., Edmonton, Alberta, Canada) along a linear transect at the field margin. Traps were attached to hangers using metal clamps at each of three heights: 1) the industry standard of approximately 1.5 m above the ground; 2) 50 cm above the ground; and 3) at canopy height in which the height of the trap changed throughout the season to match the height of the canola canopy on sampling days. The canopy height treatment was initially placed on the soil surface prior to crop emergence. Traps were checked at two-week intervals throughout the growing season from early May to mid-August, 2008. At each two-week check, sticky inserts were changed, moths were counted, and the canopy height treatment was adjusted to match the height of the canola canopy (Appendix 2). Lures were changed at three-week intervals.

The number of males captured in traps positioned at different heights in the canola canopy was compared with a Repeated Measures ANOVA (PROC

MIXED) (SAS Institute, 2005). Data were $\log(x+0.5)$ transformed prior to analysis. Factors in the model were site, trap height and sample date, with trap height specified as the repeated measure.

2.3 Results

2.3.1 Capability of Pheromone-Baited Trap Capture to Predict Larval Densities

In 2007, *P. xylostella* population densities in Alberta were moderate. Male moth capture and larval density were significantly related on several sample dates throughout the flight season (Tables 2.1, 2.2). Interestingly, the three-trap capture was a better predictor of larval density than individual trap capture in models when the independent variable was larvae sampled on the subsequent sample date from when moths were sampled (Table 2.2). In models that used the number of moths and larvae collected over the same sample period, moth capture in individual traps resulted in significant relationships more often than moth capture in three traps per site (Tables 2.1).

Although the number of males captured in individual pheromone-baited traps at each site in 2007 significantly predicted the number of larvae collected on the same day early in the season (18-22 June) this relationship was not strong and appears to be negative (Figure 2.1a). Later in 2007, the quadratic of trap capture in individual traps significantly predicted larval numbers on samples collected 1-3 August (Figure 2.1b) and 15-17 August (Figure 2.1c). During the last sampling period in 2007 (15-17 August), there was also a significant relationship between the quadratic of moth trap capture in three traps per site and larvae sampled

during the same period (Table 2.1, Figure 2.2). These non-linear relationships demonstrated that intermediate levels of trap catch were associated with high larval densities (Figures 2.1b, c, 2.2)

In 2007 when the male moths captured in pheromone-baited traps were used to predict the number of larvae sampled during the subsequent sample period, models were again significant early (Figures 2.3a,b) and late (Figure 2.3c) in the season (Table 2.2). Early-season models appeared to be driven by a single point (Figures 2.2a, b). There was a negative exponential relationship late in the season in which low levels of male moth trap capture were associated with high densities of larvae in the subsequent sample period (Figure 2.3c).

In 2008, *P. xylostella* densities were low throughout the province. Analyses separated by sample date revealed that trap catch in an individual trap per site was related to larvae sampled during the same sample period early (23-26 June) and late in the season (6-8 August) (Table 2.1). As in 2007, the curvilinear relationships showed that moderate trap catch was most closely associated with high larval densities (Figure 2.4). Models designed to test the predictive capability of male moth trap capture on larvae sampled during the subsequent sample period in 2008 were significant only when trap capture in individual traps was used as the dependent variable (Figure 2.5, Table 2.2). The predictive capability of pheromone-baited trap capture in 2008 between moth capture and larvae sampled on the subsequent sample date occurred both early (Figure 2.5a) and late in the season (Figure 2.5b) and showed a similar curvilinear relationship (Figure 2.5).

Degree-days

Plutella xylostella is a multivoltine species and all life stages were present near or on host plants after the adults arrived on trajectory winds in 2007 and 2008 and were first detected in pheromone traps (Figure 2.6). A total of 1057 and 875.9 degree days ($DD_{7.3}$) accumulated throughout the sample season from 3 May to 20 August in 2007 and 2008, respectively. The peak sample of immatures occurred at 885.4 DD in 2007 and was dominated by second-instar larvae and pupae (Figure 2.6). Although far fewer immatures were collected in 2008, the peak sample occurred earlier in the season at 458 DD and was dominated by second- and third-instar larvae (Figure 2.6).

Sweep Net versus Pheromone Trap Sampling (2008)

In 2008, pheromone-baited traps with lures changed at three- or six-week intervals captured significantly more moths than sweep net samples ($F=47.26$; $P<0.0001$) (Figure 2.7). There was a significant difference between the number of male moths captured over the entire season in traps baited with lures changed at 6- than at 3-week intervals in 2008 numerically more males were captured in traps baited with lures changed at 6 week intervals ($F=4.52$; $P\leq 0.05$) (Figure 2.7).

2.3.2 The Effect of Trap Height on Adult Male Moth Trap Capture (2008)

A statistically similar number of adults were caught in pheromone-baited traps positioned at the three heights tested in 2008 (Figure 2.8). Numerically more adults were captured during the season in traps positioned at the lower trap heights compared to the standard height of ~1.5 m above the soil surface. The 50

cm trap height throughout the season was equally as effective and less labour intensive than moving the trap as the crop grows.

2.4 Discussion

This study assessed the capacity of capture of male *P. xylostella* in commercial pheromone-baited traps to predict densities of immature stages of the insect in canola fields across southern Alberta, Canada. Capture of male moths was predictive of larval densities within the same sample period and in the subsequent sample period following a curvilinear relationship (Figures 2.1, 2.2, 2.4, 2.5) mainly at the end of the flight period in both years (Tables 2.1, 2.2). Predictive models have been developed for *P. xylostella* in cabbage (Baker et al., 1982) and other high value cole crops (Walker et al., 2003). In contrast to my findings, Walker et al. (2003) found that male moth catch in pheromone-baited traps was predictive of larval populations early but not late in the growing season. Differences between the two studies may be driven by geographic variation in pheromone response (Zilahi-Balogh et al., 1995; Yang et al., 2007), environmental conditions (Mayer and Mitchell, 1999) or differences in the physiology of the insect. For example, insecticide-resistant *P. xylostella* males showed increased EAG response to sex pheromone compared to susceptible males (Xu et al., 2010). Similarly, pheromone-baited traps were effective in some regions and not others as tools for predicting larval populations of the Nantucket pine tip moth (*Rhyacionia frustrana* Comstock (Lepidoptera: Tortricidae) (Asaro and Berisford, 2001).

In my studies, a better correlation between moth trap catch and larval densities late in the season may reflect the establishment of local populations as compared to migratory populations early in the season. Male pine processionary moths, *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Lepidoptera: Thaumetopoeidae), captured in pheromone-baited traps are genetically representative of larvae sampled at the same sites in core populations throughout their range in Italy (Salvato et al., 2005). Male pine processionary moths recovered from pheromone traps in an expanding population were genetically different from larvae sampled from the same location, suggesting that males from different populations were recruited into the area (Salvato et al., 2005). The distribution of *P. xylostella* populations on canola in the Prairie Provinces is variable because habitats are colonized by wind-driven migrations (Dosdall et al., 2004; Hopkinson and Soroka, 2010). Although multiple migrations can occur on wind currents that originate from different areas of infestation (Hopkinson and Soroka, 2010), migrations that permit the establishment of populations in canola crops are concentrated early in the season. Therefore, relationships between male moth capture and immature samples would be expected to be weakest early in the season in the Prairie Provinces.

The relationships between male *P. xylostella* captured in pheromone-baited traps and immature samples in this study were mainly curvilinear. Non-linear relationships between male moth capture and immature samples were also discovered in the development of a sex pheromone-based monitoring system for the forest tent caterpillar (Jones et al., 2009). In my study, intermediate levels of

male *P. xylostella* capture in pheromone-baited traps were most indicative of high immature densities (Figures 2.1, 2.2, 2.4, 2.5). High larval samples may indicate that many female *P. xylostella* are present at the sample location and male *P. xylostella* are known to be more attracted to female-produced pheromone plumes than to synthetic pheromone lures (Chow et al., 1977; Koshihara, et al., 1978; Zilahi-Balogh et al., 1995; Suckling et al., 2002; Evenden and Gries, 2010). Higher male moth trap capture may be associated with low larval densities if there are multiple migration events to a site over the season (Hopkinson and Soroka, 2010).

This study did not evaluate the presence of natural enemies at the sampled sites. Mortality from predation and parasitism can reduce the strength of relationships between the different life stages in herbivorous insects. Assessment of levels of parasitism and incorporation of a weighting factor to account for this mortality may improve the predictive capability of the model (Evenden et al., 1995).

The number of male *P. xylostella* captured in traps baited with commercially available pheromone lures was weakly or not related to larval samples in the early and mid-season in both years of this study. Although pheromone-baited traps did not always predict larval population densities, they were more sensitive than sweep net samples when the two techniques were compared directly in 2008 (Figure 2.7). Pheromone-baited traps are an indicator of male moths in the area and do not indicate the number of egg-laying females in the vicinity (Sweeney et al., 1990). There may be a disconnect between the

number of ovipositing females and male moths present in the population that are responsive to pheromone. Although the sex ratio of reared *P. xylostella* is close to 1:1, (52.9:47.1 males: females) (Harcourt, 1957), it is not known if this ratio is similar in migrating populations. More female moths may be transported on trajectory wind currents than males. Males that do migrate may be less responsive to sex pheromone than resident males, as has been demonstrated in other migratory moth species, including *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Gemeno and Haynes, 2000) and *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) (Turgeon et al., 1983). Further research needs to address the sex ratio and mating status of migratory populations of *P. xylostella*.

In 2008 when little *P. xylostella* migration occurred, there were significant relationships between moth capture in pheromone-baited traps and the immatures sampled on specific collection dates when capture from one trap was used in the analyses. This suggests that one pheromone trap in a canola field may be the most suitable technique to monitor moth flight. These findings are in contrast to those of Baker et al. (1982), in which more than one trap provided a better assessment of field populations of *P. xylostella*. The better predictive efficacy of male moth trap capture from a single trap versus three traps per site in my study may be a function of the low population densities in 2008. Conducting this experiment when populations are high (Evenden et al., 1995) would contribute further to the establishment of a pheromone-based predictive model of *P. xylostella*.

Overall, there were stronger relationships between moths captured in traps baited with lures changed at 6-week than 3-week intervals. More male *P. xylostella* are attracted to traps baited with commercially-available lures when field-aged for eight weeks prior to deployment compared to traps baited with fresh lures in canola fields in Alberta (Evenden and Gries, 2010). Other studies have shown decreased male *P. xylostella* capture with pheromone lure age (Môttus et al., 1997; Mayer and Mitchell, 1999), a result that underlines the influence of variable environmental conditions on pheromone trap capture of this insect.

The relationship between moth capture in pheromone-baited traps and larval samples may be greater if the number of male moths captured is more indicative of the number of male moths flying in the vicinity of the traps. Traps positioned at the two lower heights (50 cm and canopy height) in the season-long study conducted in 2008 captured numerically but not statistically more male moths than traps positioned at the standard height (1.5 m) used in the Prairie Pest Monitoring Network. Traps placed closer to host plants increased male moth trap capture of *P. xylostella* in trials with *Brassica* vegetables in India (Reddy and Urs, 1996) and cole crops in New Zealand (Walker et al., 2003). The greatest numbers of male *P. xylostella* moths were collected in traps located 30 cm above the soil surface in Saskatchewan, Canada (Chisholm et al. 1979). Because *P. xylostella* are weak fliers, they prefer the protection of plant cover (Harcourt, 1957) and may more easily orient to pheromone positioned in a trap close to the canopy (Chisholm et al., 1979). Data from this study suggest that a lower trap height of

50 cm above the soil should be adopted for pheromone-based monitoring of *P. xylostella* in canola. Maintenance of the traps at 50 cm would be less labour-intensive than changing trap height with the crop canopy for canola and may make monitoring more appealing for pest managers in the Canadian Prairies. Further research needs to be conducted to determine if moth capture in traps positioned at 50 cm above the ground is a better indicator of larval densities than traps positioned 1.5 m above the ground.

Table 2.1. Same day sampling results of predictive model for male moth captures in traps baited with lures changed at 6-week (2007) and 3- and 6-week (2008) intervals. Values in bold indicate a significant relationship in which adults predict larval populations in the field. Samples of adults and immatures were collected on the same day.

Year	Sample Date(s)	# Traps per Sample	Lure Change Interval (weeks)	Independent Variable	Negative Binomial GLM Results	
					χ^2	P-value
2007	18-22 Jun	1	6	Adult	4.70	0.0301
				Adult*Adult	0.00	0.9961
	3	6	Adult	0.40	0.5289	
			Adult*Adult	1.88	0.1704	
2007	5-8 Jul	1	6	Adult	1.05	0.3063
				Adult*Adult	0.31	0.5774
	3	6	Adult	2.04	0.1535	
			Adult*Adult	0.85	0.3563	
2007	19-21 Jul	1	6	Adult	0.060	0.8102
				Adult*Adult	1.67	0.1961
	3	6	Adult	2.00	0.1573	
			Adult*Adult	2.41	0.1209	
2007	1-3 Aug	1	6	Adult	0.18	0.6689
				Adult*Adult	7.00	0.0081
	3	6	Adult	0.15	0.7031	
			Adult*Adult	3.34	0.0677	
2007	15-17 Aug	1	6	Adult	2.34	0.1258
				Adult*Adult	5.50	0.0190
	3	6	Adult	0.00	0.9865	
			Adult*Adult	12.21	0.0005	
2008	9-11 Jun	1	3	Adult	0.00	-
				Adult*Adult	0.00	-
		3	3	Adult	0.46	0.4962
				Adult*Adult	0.36	0.5509
	1	6	Adult	1.36	0.2436	
			Adult*Adult	0.00	-	
	3	6	Adult	1.93	0.1648	
			Adult*Adult	0.00	1.0000	
2008	23-26 Jun	1	3	Adult	1.19	0.2744
				Adult*Adult	5.51	0.0189
		3	3	Adult	0.26	0.6097
				Adult*Adult	3.41	0.0649
	1	6	Adult	0.36	0.5483	
			Adult*Adult	1.23	0.2673	
	3	6	Adult	0.20	0.6551	
			Adult*Adult	1.17	0.2791	
2008	7-10 Jul	1	3	Adult	0.16	0.6909
				Adult*Adult	0.03	0.8634
	3	3	Adult	0.03	0.8603	
			Adult*Adult	0.00	0.9679	
	1	6	Adult	0.14	0.7068	
			Adult*Adult	0.02	0.8963	

		3	6	Adult	0.28	0.5976
				Adult*Adult	0.36	0.5476
2008	21-24 Jul	1	3	Adult	0.14	0.7055
				Adult*Adult	0.35	0.5535
		3	3	Adult	0.21	0.6490
				Adult*Adult	2.54	0.1110
		1	6	Adult	0.04	0.8384
				Adult*Adult	1.57	0.2099
		3	6	Adult	0.01	0.9374
				Adult*Adult	1.04	0.3087
2008	6-8 Aug	1	3	Adult	4.85	0.0277
				Adult*Adult	4.01	0.0452
		3	3	Adult	0.12	0.7268
				Adult*Adult	1.79	0.1812
		1	6	Adult	0.86	0.3543
				Adult*Adult	0.28	0.5996
		3	6	Adult	0.70	0.4042
				Adult*Adult	1.86	0.1730
2008	18-20 Aug	1	3	Adult	1.82	0.1777
				Adult*Adult	0.59	0.4408
		3	3	Adult	0.00	0.9465
				Adult*Adult	2.54	0.1110
		1	6	Adult	2.14	0.1434
				Adult*Adult	0.12	0.7331
		3	6	Adult	0.51	0.4759
				Adult*Adult	1.53	0.2164

Table 2.2. Lag sampling results of predictive model for male moth captures in traps baited with lures changed at 6-week (2007) and 3- and 6-week (2008) intervals. Values in bold indicate a significant relationship in which adults predict larval populations in the field. Adults were collected in the sample period prior to immature samples.

Year	Sample date(s) ¹	Sample date(s) ²	# Traps per sample	Lure change interval (wks)	Independent Variable	Negative Binomial GLM Results		
						χ^2	P-value	
2007	7-8 Jun	18-22 Jun	1	6	Adult	3.74	0.0530	
					Adult*Adult	5.79	0.0161	
	3	6	Adult	9.56	0.0020			
			Adult*Adult	0.68	0.4106			
2007	18-22 Jun	5-8 Jul	1	6	Adult	0.21	0.6498	
					Adult*Adult	1.90	0.1678	
	3	6	Adult	1.68	0.1954			
			Adult*Adult	0.71	0.3989			
2007	5-8 Jul	19-21 Jul	1	6	Adult	2.34	0.1263	
					Adult*Adult	1.63	0.2022	
	3	6	Adult	1.44	0.2302			
			Adult*Adult	3.54	0.0600			
2007	19-21 Jul	1-3 Aug	1	6	Adult	0.41	0.5201	
					Adult*Adult	3.08	0.794	
	3	6	Adult	5.12	0.0236			
			Adult*Adult	5.29	0.0214			
2007	1-3 Aug	15-17 Aug	1	6	Adult	2.01	0.1566	
					Adult*Adult	0.12	0.7281	
	3	6	Adult	1.07	0.3007			
			Adult*Adult	2.17	0.1407			
	2008	29-30 May	9-11 Jun	1	3	Adult	0.00	-
						Adult*Adult	0.00	-
3		3	Adult	0.50	0.4776			
			Adult*Adult	1.18	0.2777			
2008	9-11 Jun	23-26 Jun	1	6	Adult	3.66	0.0556	
					Adult*Adult	0.00	-	
	3	6	Adult	0.87	0.3503			
			Adult*Adult	0.43	0.5110			
2008	9-11 Jun	23-26 Jun	1	3	Adult	0.00	-	
					Adult*Adult	0.00	-	
	3	3	Adult	0.86	0.3549			
			Adult*Adult	2.87	0.0902			
	1	6	Adult	0.84	0.3596			
			Adult*Adult	0.00	-			
	3	6	Adult	0.40	0.5283			
			Adult*Adult	2.221	0.1386			
2008	23-26 Jun	7-10 Jul	1	3	Adult	0.58	0.4462	
					Adult*Adult	0.00	0.9552	
	3	3	Adult	0.48	0.4884			
			Adult*Adult	0.05	0.8232			
1	6	Adult	0.65	0.4213				

			3	6	Adult*Adult	6.44	0.0112
					Adult	0.53	0.4681
					Adult*Adult	4.84	0.0278
2008	7-10 Jul	21-24 Jul	1	3	Adult	0.76	0.3832
					Adult*Adult	0.93	0.3344
			3	3	Adult	0.12	0.7253
					Adult*Adult	0.30	0.5811
			1	6	Adult	0.55	0.4570
					Adult*Adult	0.42	0.5148
			3	6	Adult	0.24	0.6250
					Adult*Adult	3.29	0.0699
2008	21-24 Jul	6-8 Aug	1	3	Adult	2.67	0.1023
					Adult*Adult	0.60	0.4384
			3	3	Adult	1.44	0.2308
					Adult*Adult	1.72	0.1899
			1	6	Adult	2.08	0.1493
					Adult*Adult	0.38	0.5393
			3	6	Adult	2.13	0.1444
					Adult*Adult	0.22	0.6387
2008	6-8 Aug	18-20 Aug	1	3	Adult	1.37	0.2410
					Adult*Adult	3.42	0.0646
			3	3	Adult	0.77	0.3794
					Adult*Adult	1.31	0.2526
			1	6	Adult	0.61	0.4346
					Adult*Adult	3.16	0.0756
			3	6	Adult	3.09	0.0787
					Adult*Adult	2.48	0.1152

¹Adult sample dates.

²Immature sample dates.

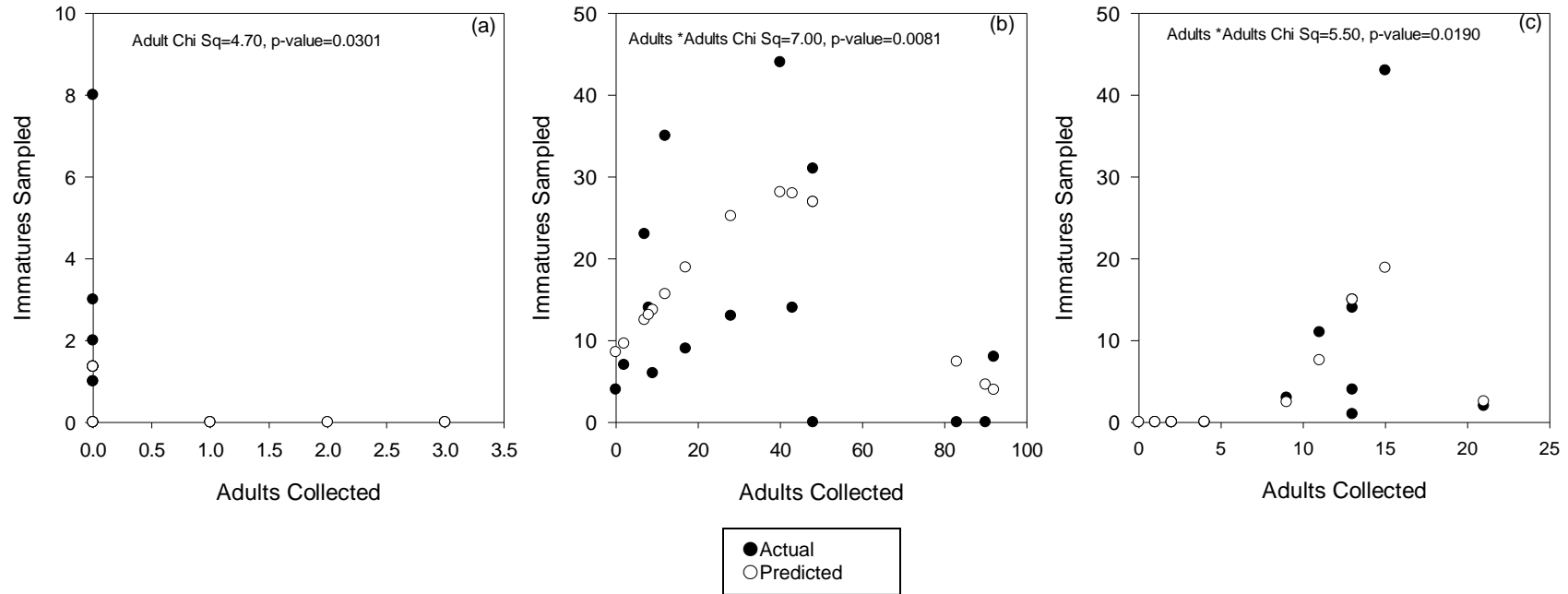


Figure 2.1. Significant actual and predicted relationships between male *Plutella xylostella* captured in a single pheromone-baited trap with a 6-week lure change schedule at each site ($n=15$) and immatures sampled at the same sites during the same sample period in 2007. Relationships were significant for three sample periods in 2007: a) 18-22 June, b) 1-3 August, c) 15-17 August.

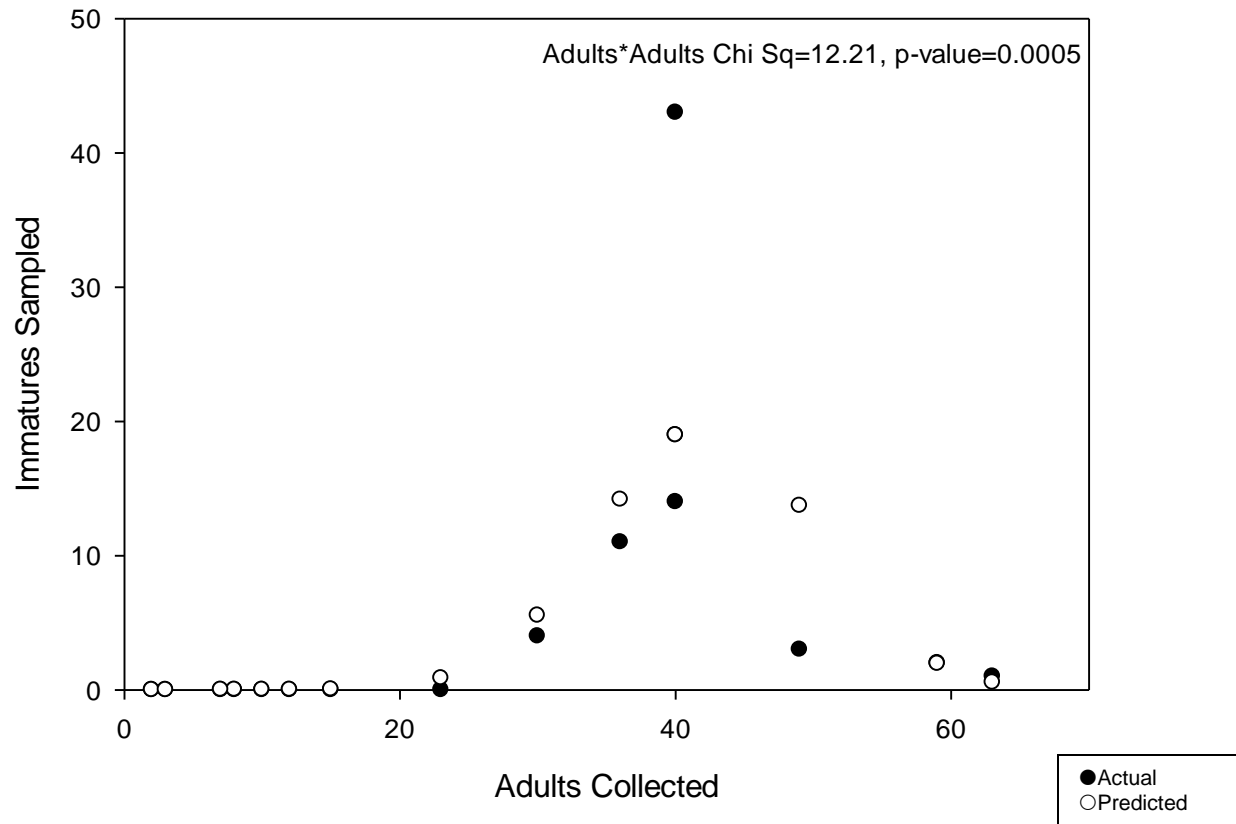


Figure 2.2. Significant actual and predicted relationships between male *Plutella xylostella* captured in three pheromone-baited traps with a 6-week lure change schedule at each site (n= 15) and immatures sampled at the same sites during the same sample period in 2007. Relationships were significant for one sample period in 2007: 15-17 August.

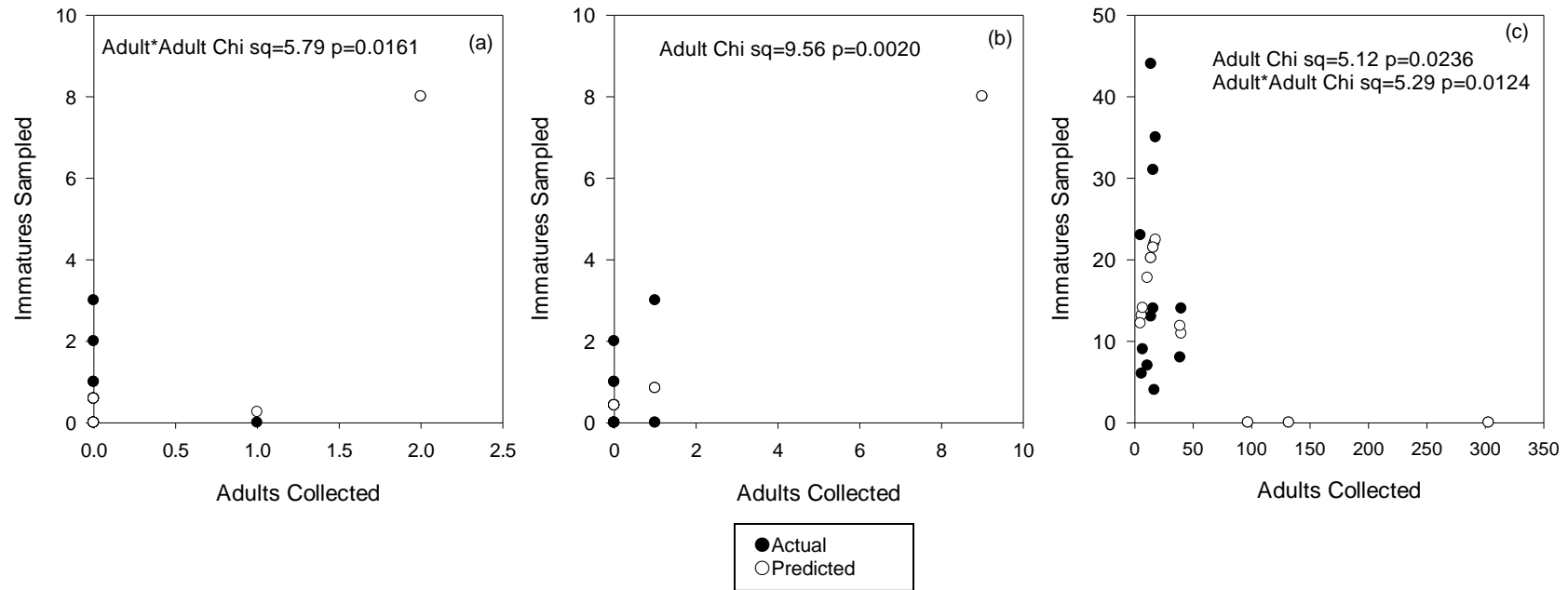


Figure 2.3. Significant actual and predicted relationships between male *Plutella xylostella* captured in a) one pheromone-baited trap, or b) and c) in three pheromone-baited traps with a 6-week lure change schedule at each site ($n=15$) and immatures sampled at the same sites during the subsequent sample period in 2007. Relationships were significant for two sample periods in 2007: a) and b) Adult sample: 7-8 June; Immature sample: 18-22 June; and c) Adult sample: 19-21 July; Immature sample: 1-3 August.

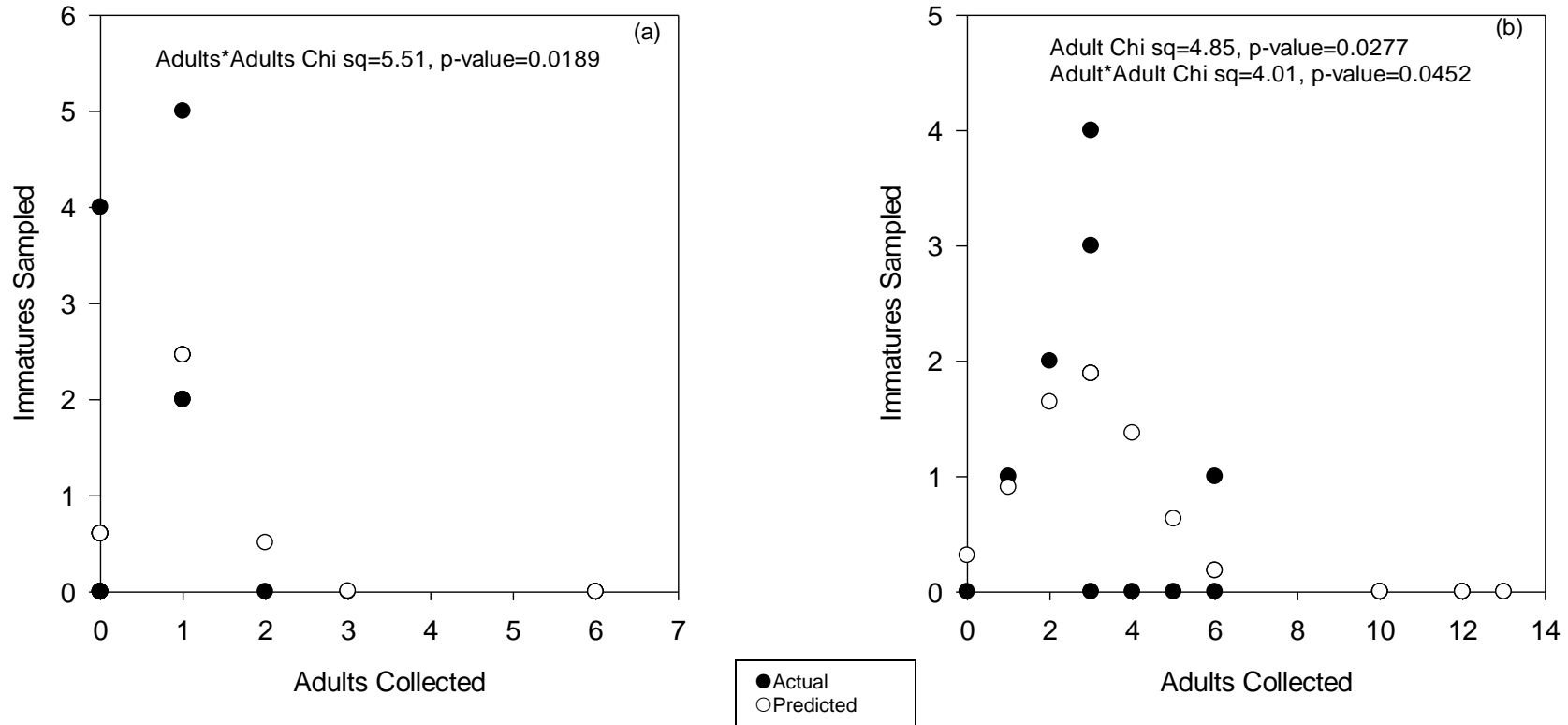


Figure 2.4. Significant actual and predicted relationships between male *Plutella xylostella* captured in a single pheromone-baited trap with a 3-week lure change schedule at each site ($n=15$) and immatures sampled at the same sites during the same sample period in 2008. Relationships were significant at two sample periods in 2008: a) 23-26 June, and b) 6-8 August.

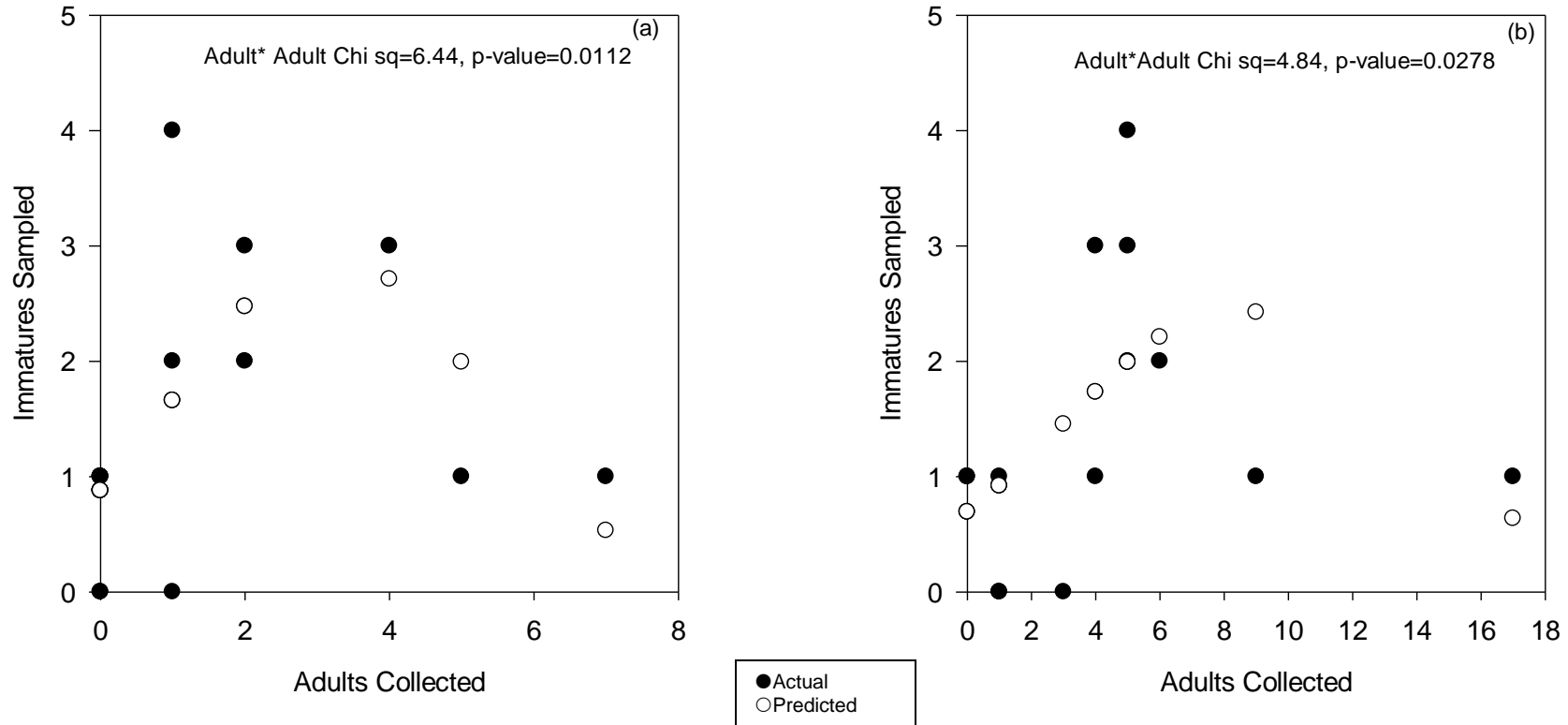


Figure 2.5. Significant actual and predicted relationships between male *Plutella xylostella* captured in a) a single pheromone-baited trap or b) three pheromone-baited traps with a 6-week lure change schedule at each site (n= 15) and immatures sampled at the same sites during the subsequent sample period in 2008. Relationships were significant at one sample period in 2008: a) and b) Adult sample: 23-26 June; Immature sample: 7-10 July.

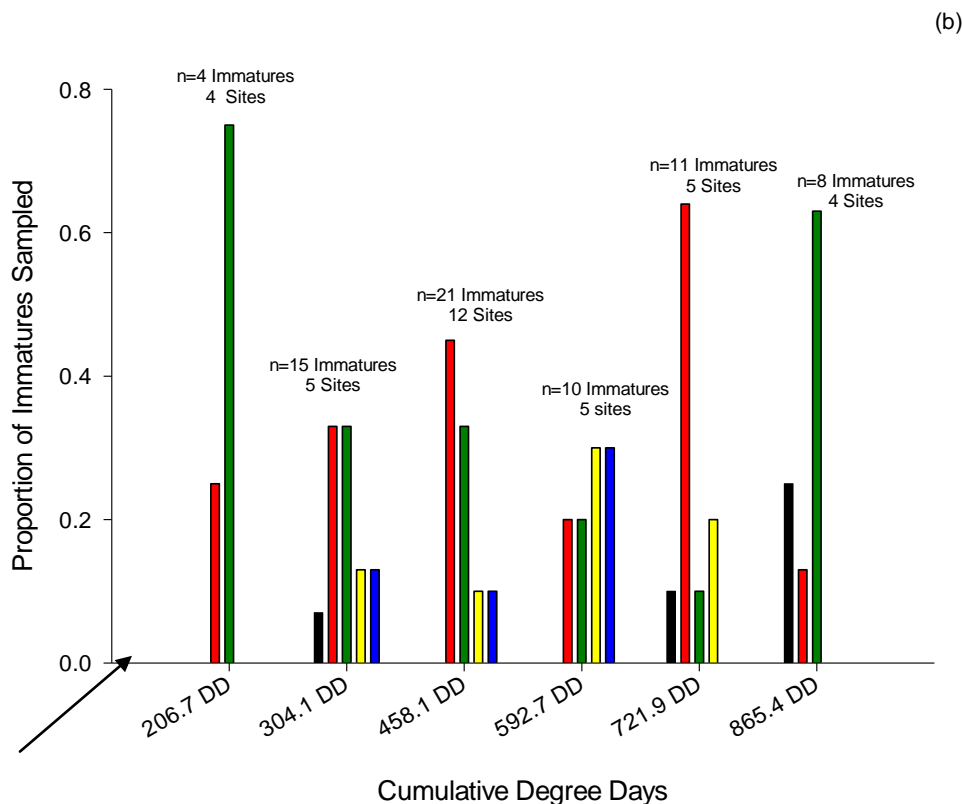
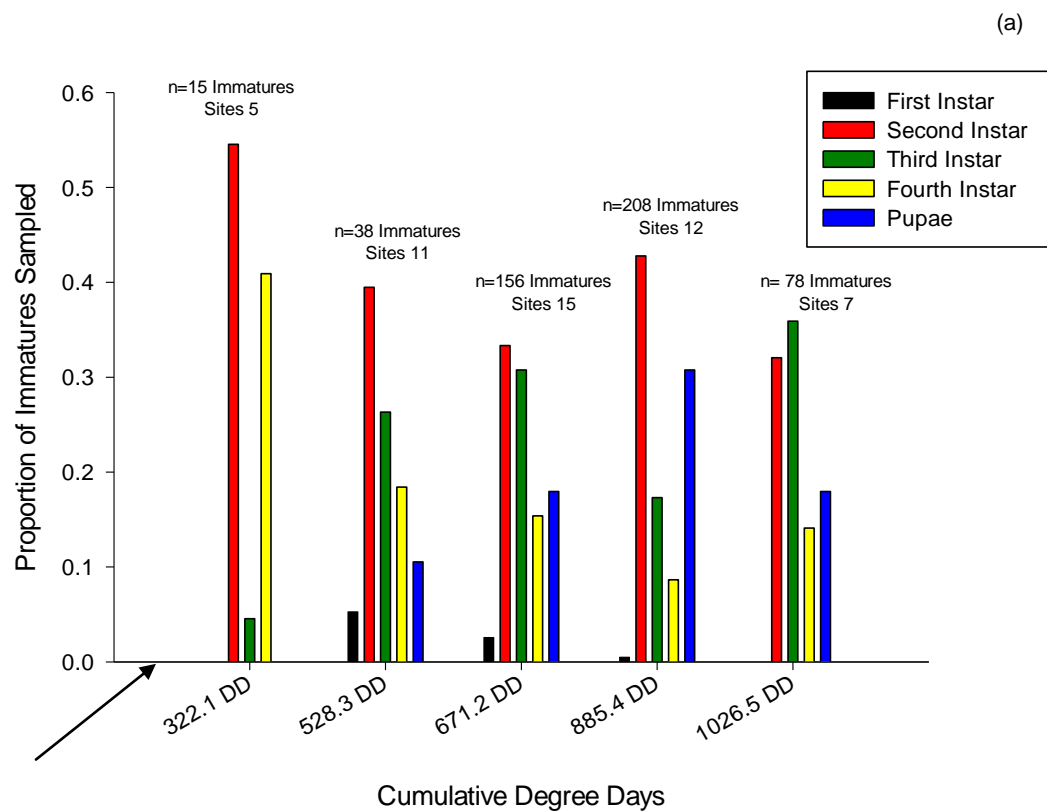


Figure 2.6. Immature stages of *Plutella xylostella* sampled from 50 canola plants per sampling period in a) 2007 and b) 2008. Numbers above bars indicate the total number of individuals collected at all positive sites from the total $n = 15$ sites sampled. Cumulative degree days (DD) calculated from 3 May each season. Arrows indicate time of moth detection in traps (2007=Week of 4-8 June, 2008=Week of 26-30 May).

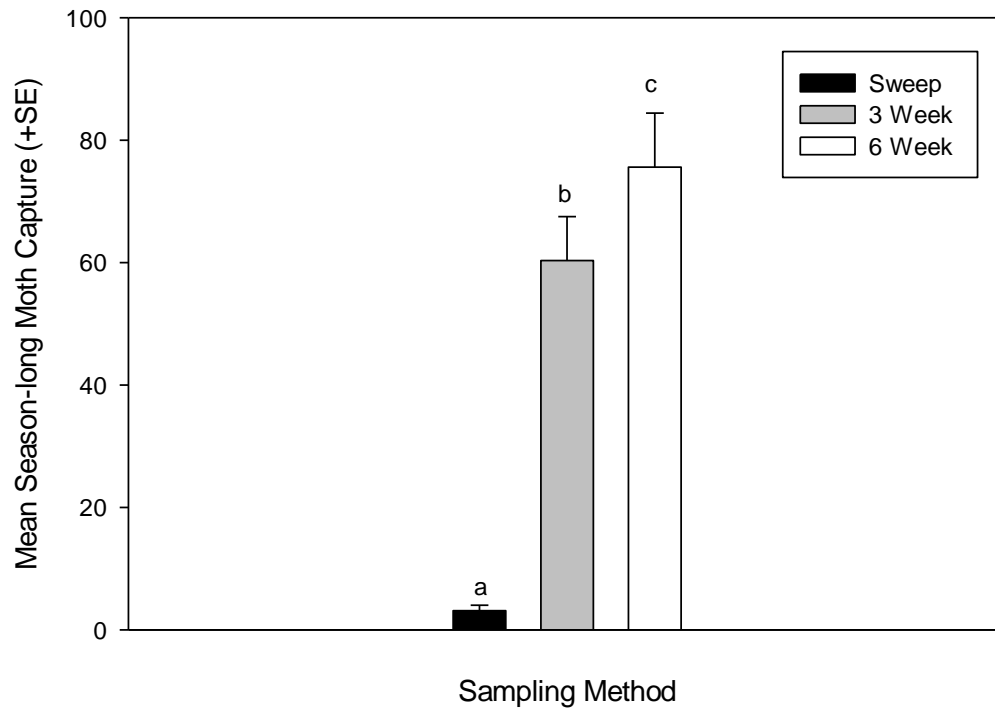


Figure 2.7. Comparison of *Plutella xylostella* captured in season-long sweep net samples and pheromone-baited traps at $n=15$ sites in 2008. Bars labelled with different letters are significantly different (Tukey's $P \leq 0.05$).

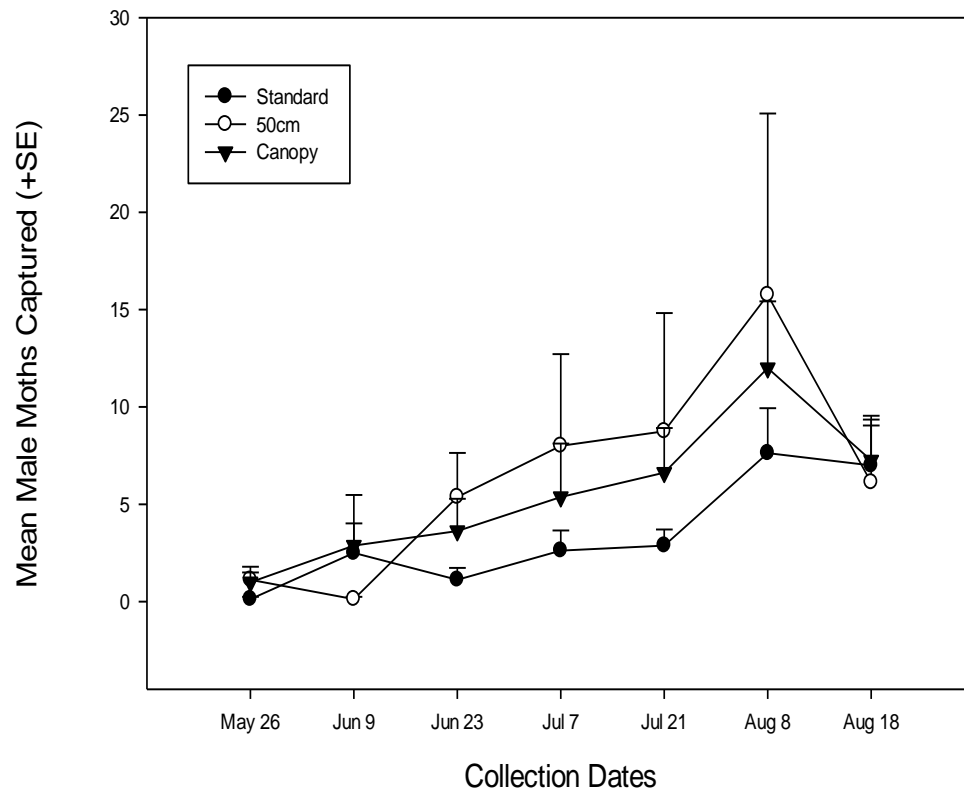


Figure 2.8. Mean (+SE) number of male *Plutella xylostella* captured in pheromone-baited traps positioned at different heights within the crop canopy at n= 8 sites in 2008. A statistically similar number of males was captured in traps positioned at each height (Repeated Measures ANOVA, $P=0.6705$).

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Chapter 3. Factors Influencing Capture of Male *Plutella xylostella* (L.) in Pheromone-baited Traps in Canola Systems

3.1 Introduction

Globally, *Plutella xylostella* (L.) is a key pest of cruciferous crops because of its ability to migrate easily on trajectory winds and find suitable brassicaceous host plants (Talekar and Shelton, 1993; Sarfraz et al., 2005). The cost to control this easily adaptable insect can exceed \$1 billion US annually (Talekar and Shelton, 1993). Male moth capture in pheromone-baited traps has been used to monitor *P. xylostella* as part of Integrated Pest Management (IPM) programs to reduce pesticide use in cabbage and other cole crops (Reddy and Guerrero, 2000a; Walker et al., 2003). Sex pheromone-baited traps are used to detect the arrival of *P. xylostella* in canola crops throughout the Canadian Prairie provinces as part of the government-funded Prairie Pest Monitoring Network (<http://nlwis-snite2.agr.gc.ca/ppmn/loginFormEn.jsp>). Difficulties in relating male *P. xylostella* capture to larval population density (Chapter 2) and inconsistencies in trap capture (Meers, pers. comm.) led to the examination of means in which attraction to pheromone-baited traps can be enhanced.

Three pheromone components are found in the majority of attractive pheromone blends reported for *P. xylostella*: (Z)11-hexadecenyl acetate (Z11-16: Ac), (Z)11-hexadecanal (Z11-16: Ald) and (Z)11-hexadecenol (Z11-16: OH); a fourth component (Z)9-tetradecenyl acetate (Z9-14:Ac) or (Z)9-tetradecenyl alcohol (Z9-14: OH) increased attractiveness to a blend of these components in western Canada (Chisholm et al., 1983). Male *P. xylostella* demonstrate a wide breadth of response to different ratios of the identified pheromone components.

There is also geographic variation in response (Table 1.1) to different ratios of the identified pheromone components. Male *P. xylostella* respond best to blends that have Z11-16:Ac as the major component in Korea (Yang et al., 2007) and New Zealand (Suckling et al., 2002) (Table 1.1). In contrast, the most attractive pheromone blend to male *P. xylostella* trapped in Texas (He et al., 2003) and Saskatchewan (Chisholm et al., 1983) has Z11-16:Ald as the main component (Table 1.1). Because synthetic pheromone-baited traps consistently perform less effectively compared to virgin female-baited traps (Chow et al., 1977; Koshihara et al., 1978; Zilahi-Balogh et al., 1995; Suckling et al., 2002; Evenden and Gries, 2010), researchers have sought to improve the attractiveness of synthetic pheromone lures to male *P. xylostella* (Reddy and Guerrero, 2000b; Dai et al., 2008).

Pheromone dose and subsequent release rate influence response to pheromone differently for different moth species and can be manipulated to influence male moth capture in IPM systems. Release rates of the components of pheromone blends are affected by a number of factors, including chemical structure, dose, lure age, lure type, environmental conditions, and UV degradation (Mayer and Mitchell, 1999; Zhu, 2001). Molecular size, weight, and polarity are the principal factors affecting evaporation rates of the chemical compounds from natural rubber septa (Butler and McDonough, 1981). In wind tunnel studies with male redbanded leaf roller [*Argyrotaenia velutinana* Walker (Lepidoptera: Tortricidae)], cabbage looper [*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)] and Oriental fruit moth [*Grapholita molesta* Busck (Lepidoptera: Tortricidae)]

males respond to their specific synthetic pheromone blends in a dose-dependent manner but peak response occurs at different doses among the three species (Linn Jr. et al., 1986). Release rates of a pheromone component *E11-14*: aldehyde from a 3-day-old virgin female spruce budworm [*Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae)], and three different doses of synthetic pheromone on rubber septa (10, 100 and 1000 µg/lure) have different release rates and are differentially attractive to male moths (Ramaswamy and Cardé, 1984). Pine processionary moths (*Thaumetopoea pityocampa* Denis & Schiffermüller) (Lepidoptera: Tortricidae) in France were captured in traps baited with lures dosed with 0.5 to 20 mg of synthetic sex pheromone, but optimal attraction occurred to the 10 mg lure (Jactel et al., 2006). In *P. xylostella* (Kawasaki, 1984; Lin et al., 1982; Môtus et al., 1997; Evenden and Gries, 2010) and other moth species (Baker et al., 1981), arrestment to high release rates of pheromone occurs and results in an upper threshold of male response. Low pheromone release rates of less than 5 ng/h are most attractive to male *P. xylostella* tested in canola in western Canada (Evenden and Gries, 2010). Different substrates can influence pheromone release and subsequent attractiveness of lures to male moths. Male *P. xylostella* response to pheromone released from grey rubber septa was greater than to red rubber septa (Mayer and Mitchell, 1999).

Chemical signals used in host location can synergize response to sex pheromones. The addition of green leaf volatiles to attractive pheromone blends increased male moth trap capture for both corn earworm [*Helicoverpa zea* Boddie (Lepidoptera: Noctuidae)] and codling moth [*Cydia pomonella* L. (Lepidoptera:

Tortricidae)] (Light et al., 1993). A combination of host volatiles and sex pheromone produced a synergistic effect on the firing rate of olfactory receptor neurons as compared to the pheromone alone in male corn earworm (Ochieng et al., 2002). Pheromone in combination with plant volatiles (linalool, β -farnesene or Z3-hexen-1-ol) enhanced the behavioural response of male codling moths in wind tunnel experiments compared to pheromone alone (Yang et al., 2004). In a wind tunnel study with male European grape berry moths [*Eupoecilia ambiguella* Hübner (Lepidoptera: Tortricidae)], plant volatiles [Z3-hexen-1-ol, (*E*)- β -caryophyllene, (+)-terpinen-4-ol and methyl salicylate] attracted significantly more males to suboptimally attractive pheromone lures (Schmidt-Büsser et al., 2009).

Plutella xylostella behaviour is affected by exposure to host volatiles. Males and females respond behaviourally and electrophysiologically to leaf extracts (Palaniswamy et al., 1986) that are postulated to be important for oviposition site and mate location. The sexual maturity of males and females is accelerated by exposure to host plants (Pivnick et al., 1990) and the presence of glucosinolate host compounds stimulates females to oviposit (Reed et al., 1989). Both male and female *P. xylostella* are more attracted to traps baited with sex pheromone and the green leaf volatile (*Z*) 3-hexenyl acetate in a 1:1 ratio as compared to traps baited with pheromone alone in cabbage systems (Reddy and Guerrero, 2000b).

This study examined a variety of factors in an attempt to enhance the attractiveness of synthetic pheromone lures to male *P. xylostella* in canola in

southern Alberta. All studies were conducted with the commercially available pheromone blend (ConTech Enterprises Inc., Delta BC) (Appendix 4) and examined the addition of olfactory (green leaf volatile) and visual (trap colour) host cues, the influence of pheromone dose and pheromone release substrate (lure type) on trap capture in pheromone-baited traps.

3.2 Materials and Methods

3.2.1 The Influence of Visual and Olfactory Host Cues on Adult *Plutella xylostella* Capture in Pheromone-baited Traps

The first three experiments tested the hypothesis that a green leaf volatile (Z3-hexenyl acetate) would enhance the attractiveness of the pheromone blend currently used by the Prairie Pest Monitoring Network (ConTech Enterprises Inc., Delta, British Columbia, Canada) (Appendix 4) when lures are positioned in variously coloured traps at different times during the growing season (Table 3.1). Trapping sites (n=8 per experiment) were located in southern Alberta (Appendix 5).

Experiments 1 and 2 tested the hypothesis that semiochemical lures containing pheromone and Z3-hexenyl acetate in differently coloured traps (Table 3.1) would be differentially attractive to *P. xylostella* at different times during the canola-growing season. Experiment 1 was conducted at full bloom when the crop was primarily yellow (2007) and Experiment 2 was conducted pre-bloom (2008) when the crop was green. In both experiments, traps consisted of plastic Delta traps (ConTech Enterprises Inc., Delta, British Columbia, Canada) made of corrugated white plastic. There were three trap colour treatments: 1) unpainted

white; 2) painted white; and 3) painted yellow (Table 3.1). The painted white treatment was included to control for the non-visual effects of paint treatment. Coloured traps were painted with Behr Exterior Flat Acrylic Latex White and Citrus Splash Yellow (Home Depot, Edmonton, Alberta, Canada) in the laboratory and allowed to dry before transport to field sites. The reflective properties of one trap from each colour treatment were assessed using a dual-beam spectrophotometer operating between 250 and 750 nm (Cary 5G UV-Vis-NIR, Vairan, Inc., Mississauga, ON). The reflectance was assessed at 1 nm increments and corrected for proportion reflected by a 99% Spectralon reflectance standard (Labsphere, North Sutton, New Hampshire, USA).

Pre-extracted grey rubber septa lures were loaded with hexane solutions containing the commercial pheromone blend with and without Z3-hexenyl acetate (green leaf volatile) (Table 3.1) and held in sealed glass containers at -20°C until transport to field sites in refrigerated containers. Each lure treatment was assigned to one trap of each of the three trap colour treatments at each of eight field sites, for 12 treatments per site (Table 3.1). Traps were positioned in random order at 30 m intervals, 1.5 m above the ground on L-shaped metal hangers (Totem Welding Co. Ltd., Edmonton, Alberta, Canada) along a linear transect at the edge of each canola field. Traps remained in the field for one month, moths were counted, and sticky inserts replaced at two-week intervals. The moths captured were separated by sex. Moth catch was pooled and $\log(x+1)$ transformed prior to analysis using a Three-Way ANOVA with trap colour,

pheromone and green leaf volatile specified as the main factors with the PROC GLM procedure in SAS version 9.1 (SAS Institute, 2005).

Experiment 3 tested the hypothesis that different doses of the green leaf volatile, Z3-hexenyl acetate (Table 3.1) would vary the attractiveness of pheromone-baited traps to *P. xylostella*. Pre-extracted grey rubber septa lures were loaded with hexane solutions containing the commercial pheromone blend and various doses of Z3-hexenyl acetate (Table 3.1) and held in sealed glass containers at -20°C until transport to field sites in refrigerated containers. Each lure treatment was assigned to one unpainted white trap at each site (n=8) at full bloom in 2008, for six treatments per site (Table 3.1). Traps were positioned in random order at 30 m intervals, 1.5 m above the ground on L-shaped metal hangers (Totem Welding Co. Ltd., Edmonton, Alberta, Canada) along a linear transect at the edge of each canola field. Traps remained in the field for one month, moths were counted, and sticky inserts replaced at two-week intervals. Sticky inserts were returned to the lab, and captured moths were separated by sex. Moth catch was pooled and $\log(x+1)$ transformed prior to analysis using a Two-Way ANOVA with site and green leaf volatile dose as the main factors in the model, followed by a Tukey's multiple comparison test to compare individual treatments with the PROC GLM procedure in SAS (SAS Institute, 2005).

ISCA Technologies (Riverside, California, USA) is another supplier of commercial *P. xylostella* pheromone lures. In addition to the *P. xylostella* pheromone lure, ISCA Technologies is developing a pheromone plus greenleaf volatile lure in an effort to enhance the attractiveness of their commercial

pheromone product. The lure under development contains a *P. xylostella* pheromone blend and the greenleaf volatile Z3-hexenyl acetate. However, the composition and quantities of pheromone components and Z3-hexenyl acetate applied to each lure is proprietary information. In Experiment 4, the two types of pheromone lures (with and without Z3-hexenyl acetate) were tested for attractiveness to *P. xylostella* at the same sites as Experiment 1 in July 2007 (Appendix 5). Two plastic Delta traps (ConTech Enterprises, Inc., Delta, British Columbia, Canada) were baited with one lure of each treatment: 1) untreated control, 2) ISCA Technologies pheromone lure; and 3) ISCA Technologies pheromone + greenleaf volatile lure, at each of eight field sites. Traps were positioned ~1.5 m above the ground on L-shaped metal hangers (Totem Welding Co. Ltd., Edmonton, Alberta, Canada) and separated by 30 m. Traps remained in the field for one month, moths were counted, and sticky inserts replaced at two-week intervals; captured moths were separated by sex. Moth catch was pooled and square root transformed prior to analysis using a Randomized Block ANOVA with site as the block and pheromone treatments as the factor in the model followed by a Tukey's multiple comparison test to compare individual treatments with the PROC GLM procedure in SAS (SAS Institute, 2005).

3.2.2 The Effect of Pheromone Dose and Lure Type on Male *Plutella xylostella* Attraction

To test the hypothesis that release substrate and pheromone dose influence capture of male *P. xylostella* in pheromone-baited traps, pre-extracted red and grey rubber septa lures (ConTech Enterprises Inc., Delta, British Columbia, Canada) were loaded with 100 µl of HPLC-grade hexane containing four different

doses of the commercially available pheromone blend (Appendix 4) and compared to solvent controls for a total of 10 treatments per site (n=8) (Table 3.2).

Pheromone was dispensed into pre-extracted rubber septa lures in the laboratory; lures were stored in sealed containers at -20 °C until deployment in the field. Lures were transported in refrigerated containers to each of eight field sites in southern Alberta (Appendix 5) at the beginning of July 2007. At each field site, one plastic Delta trap (Contech Enterprises Inc., Delta, British Columbia, Canada) was baited with one lure from each treatment (Table 3.2) and traps were assigned randomly to a position along a linear transect at the edge of the canola field. Traps were separated by 30 m and were attached to L-shaped metal hangers (Totem Welding Co. Ltd., Edmonton, Alberta, Canada) at ~1.5 m above ground. Traps remained in the field for one month, moths were counted, and sticky inserts were replaced at two-week intervals. Moth catch was pooled for both sampling dates and $\log(x+1)$ transformed prior to analysis using a Three-Way ANOVA with the PROC GLM procedure in SAS (SAS Institute, 2005). Site, pheromone dose and lure type were the three main factors in the model.

Lures were aerated by collaborators at Simon Fraser University (Gries Lab, Department of Biological Sciences) to determine pheromone release rate and ratio of pheromone components. Three 100 µg lures of each type were aerated for six days at 24-28 °C. Volatile emissions were captured on an adsorbent material (Porapak-Q) and extracted in pentane. Extracts were analyzed for the presence of

Z11-16:Ald and Z11-16:Ac by Gas Chromatography-Mass Spectrometry (GC-MS).

3.3 Results

3.3.1 The Influence of Visual and Olfactory Host Cues on Adult *Plutella xylostella* L. Capture in Pheromone-baited Traps

Significantly more adults were captured in traps baited with pheromone as compared to traps baited with the hexane/solvent control, but the addition of Z3-hexenyl acetate in a 1:1 ratio did not increase the attractiveness of pheromone alone in 2007 (Figure 3.1). Z3-hexenyl acetate alone captured a similar number of moths to the solvent-baited control. There was no effect of trap colour on moth attraction and there may be a slight repellent effect of paint treatments on male moth response (Figure 3.1).

This experiment was repeated in 2008 earlier in the season prior to crop flowering. Once again, the addition of the green leaf volatile Z3-hexenyl acetate to the pheromone blend did not enhance moth capture over the pheromone alone (Figure 3.1). There was no effect of trap colour on male moth capture despite the fact that this experiment was conducted pre-bloom when yellow traps should be apparent against the green background of the crop (Figure 3.1). There was higher reflectance for the painted and unpainted white traps compared to the painted yellow traps (Appendix 7). The number of female moths captured in semiochemical-baited traps in Experiments 1 and 2 was extremely low (n=37 caught at all sites in both experiments) and not analyzed statistically in either experimental year.

There was no effect of adding the green leaf volatile Z3-hexenyl acetate at doses ranging from 10-10000 µg on the attractiveness of lures baited with 100 µg of *P. xylostella* pheromone (Figure 3.2). All semiochemical traps were significantly more attractive to males than the solvent control, but there was no difference in attractiveness with dose of Z3-hexenyl acetate (GLV) added to pheromone (Figure 3.2). The number of female moths was extremely low (n=20 caught at all sites) and not analyzed statistically.

Traps baited with the ISCA pheromone blend and the green leaf volatile Z3-hexenyl acetate were significantly more attractive to *P. xylostella* adult males compared to the ISCA pheromone blend alone (F=13.14, P=0.0027) (Figure 3.3). The number of female moths captured was extremely low (n=3 caught at all sites) and not analyzed statistically.

3.3.2 The Effect of Pheromone Dose and Lure Type on Male *Plutella xylostella* Attraction

There were significant effects of pheromone dose (F=71.94; P=<0.0001) and lure type (F=7.82; P=0.0068) on male moth trap capture (Figure 3.4). The interaction of lure type by pheromone dose was also significant (F=3.27; P=0.0169). Peak moth trap capture occurred with the 100 µg dose for each lure type (Figure 3.4). Traps baited with grey rubber septa lures were more attractive than those baited with red rubber septa lures (Figure 3.4). The 100 µg dose on red rubber septa is currently used by Contech Inc., Delta British Columbia, Canada for their commercial *P. xylostella* monitoring product. Although the results of aeration analyses cannot be statistically analyzed, they illustrate that more

pheromone was released from grey septa and the ratio of Z11-16:Ald to Z11-16:Ac was lower in the more attractive grey lures (Appendix 6).

3.4 Discussion

Behaviours of phytophagous insects are influenced by olfactory and visual cues produced by their host plants for a range of activities including host and mate location (Landolt and Philips, 1997; Ochieng et al., 2002). Exploitation of these behavioural responses can be useful in the management of pest species. For mate finding, host plant stimuli can also enhance responsiveness to species-specific insect-produced signals. For example, the addition of green leaf volatiles to sex pheromone enhances male attraction by *C. pomonella* (Light et al., 1993; Yang et al., 2004), *H. zea* (Light et al., 1993) and *P. xylostella* (Reddy and Guerrero 2000b; Dai et al., 2008). In my studies, the addition of Z3-hexenyl acetate, a common green leaf volatile, to the ConTech Inc. pheromone blend did not increase the attractiveness of the lure to moths at the concentrations tested compared to traps baited with the ConTech Inc. pheromone lure alone. In contrast, the combined lure produced by ISCA was more attractive than the ISCA pheromone alone. The addition of Z3-hexenyl acetate to *P. xylostella* pheromone increases male and female moth attraction to traps in cabbage systems in India (Reddy and Guerrero, 2000b). In a Chinese study, traps baited with pheromone and the host plant volatiles (Z3-hexenyl acetate + Z3-hexen-1-ol + allyl isothiocyanate) were more attractive to both male and female *P. xylostella* than pheromone + Z3-hexenyl acetate alone in cabbage (Dai et al., 2008). The lack of response (by male and female moths) to Z3-hexenyl acetate in combination with

the pheromone in my experiments may be a function of geographic variation of *P. xylostella* response to green leaf volatiles (Reddy and Guerrero, 2000b; Dai et al., 2008). The host volatile complex emitted from different *Brassica* species vary and subsequently affect moth response (Reddy and Guerrero, 2000b; Han et al., 2001). Z3-hexenyl acetate may be behaviourally less important to moths in western Canada.

The ISCA (Riverside, California, USA) lures with the addition of the green leaf volatile Z3-hexenyl acetate were more attractive to *P. xylostella* adults compared to the ISCA pheromone blend alone. The commercially available ISCA pheromone lure is not as attractive as the ConTech lure to male *P. xylostella* in Alberta (Evenden and Gries, 2010). The increased attractiveness to the ISCA lures with the addition of Z3-hexenyl acetate may be the result of stimulation of the pheromone receptors with a non-pheromone mimic (Priesner, 1986). The acetate functional group of the green leaf volatile may stimulate receptors for the pheromone component Z11-16:Ac. Pheromone blends containing Z11-16:Ac as the main component are preferentially attractive to *P. xylostella* in Korea (Yang et al., 2007) and New Zealand (Suckling et al., 2002), and preliminary studies show that *P. xylostella* in Alberta also prefer pheromone blends with Z11-16:Ac as the major component (Evenden, unpublished data). Non-pheromone compounds can enhance male moth response to suboptimal pheromone blends in other moth species. The combination of a green leaf volatile and pheromone to male European grape berry moth, *Eupeocilia ambiguella* (Hubner) (Lepidoptera: Tortricidae), was most effective when suboptimal doses

of pheromone were used in wind tunnel studies (Schmidt-Büsser et al., 2009). Inclusion of a heterospecific pheromone component to a suboptimal pheromone blend targeting male Oriental fruit moths increased attractiveness to male moths (Evenden and McLaughlin, 2005). It remains unresolved as to why female *P. xylostella* were not captured in traps baited with Z3-hexenyl acetate, as has been demonstrated in other brassicaceous cropping systems (Reddy and Guerrero, 2000b; Dai et al., 2008). It is possible that cabbage and cole crop systems provide more “clean air” between widely spaced plants than canola crops and may increase the signal-to-noise ratio of a host plant volatile trap. Alternatively, the physiological state of female moths that migrate long distances may influence their host finding behaviour as compared to resident moths studied in different systems. Further research needs to determine if electrophysiological and behavioural responsiveness of *P. xylostella* to Z3-hexenyl acetate is influenced by geographic or genetic variation in addition to endogenous factors such as nutrition and egg load and/or exogenous factors such as temperature, wind and host crop.

Trap colour did not influence moth response to semiochemical-baited traps. The painted traps appear to be slightly, but not statistically, repellent to *P. xylostella*; paint volatiles may negatively influence response to pheromone-baited traps. *Plutella xylostella* adults are captured on yellow sticky traps (Sivapragasam and Saito, 1986), but the yellow delta traps in my study did not increase moth trap capture at different periods of the growing season. Yellow is an effective attractant for other crucifer specialists such as the cabbage seedpod weevil [*Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae)] (Smart et al.,

1997; Tansey et al., 2010) and cabbage root fly [*Delia radicum* (L.) (Diptera: Anthomyiidae)] (Roessingh and Städler, 1990). However, diurnally-active insects are more likely to use visual cues for host location than crepuscular insects such as *P. xylostella*.

In this study, the use of different types of rubber septa lures baited with an attractive commercially-available pheromone showed that significantly more *P. xylostella* males were captured in traps baited with grey as compared to red rubber septa. Similarly, more male *P. xylostella* were captured in pheromone-baited traps with grey as compared to red rubber septa in a study conducted in cabbage (Mayer and Mitchell, 1999). It is possible that either the total pheromone release rate or the differential release of individual pheromone components varies between the two septa types. In contrast, Mayer and Mitchell (1999) were unable to demonstrate a difference in the release rate of pheromone from the two lure types tested. Although I could not statistically analyze the emission rate from lures aerated in this study, grey lures appear to release more pheromone and a lower Z11-16:Ald to Z11-16:Ac ratio than red lures (Appendix 6). In field studies conducted in Korea (Yang et al., 2007) and New Zealand (Suckling et al., 2002) male *P. xylostella* are most attracted to blends containing low Z11-16:Ald: Z11-16:Ac ratios. Red rubber septa are currently used in the commercially available lures (supplied by ConTech Inc., Delta, British Columbia, Canada) to monitor *P. xylostella* by the Prairie Pest Monitoring Network (PPMN). Grey lures should be adopted to monitor *P. xylostella* in the future so that traps are optimally sensitive as a detection tool for this pest. Further analysis of the most attractive pheromone

blend to *P. xylostella* in the Prairie provinces is warranted. Increased attractiveness may be obtained using pheromone blends with Z11-16:Ac as the major pheromone component.

The currently used commercial rate of 100 µg per lure was the most attractive for both lure types (red or grey) in this study. Moths were less attracted to traps baited with lower or higher doses of the pheromone blend. Similar to these findings, the 100 µg dose of the attractive blend has been most attractive to *P. xylostella* in western Canada (Chisholm et al., 1979; Chisholm et al., 1983), in Korea (Yang et al., 2007) and in New Zealand (Suckling et al., 2002). However, in other regions the most attractive dose varies from 0.1 to 10 µg for populations in Japan (Koshihara et al., 1978), 10 µg for Indonesian populations (Zilahi-Balogh et al., 1995) and 50-100 µg for Chinese (Lin et al., 1982; Dai et al., 2008) populations, once again demonstrating the wide geographic variation of *P. xylostella* response to pheromone. Environmental influence on release rate of pheromone also contributes to the wide variation of attractive pheromone lures in different pheromone monitoring systems targeting *P. xylostella* around the world (Mayer and Mitchell, 1999) and illustrates the importance of the development of pheromone-based monitoring systems in individual cropping regions.

Table 3.1 Trap colour and lure treatment used in Chapter 3, Experiments 1-3.

<i>Experiment (Date)</i>	<i>Treatment</i>	<i>Trap Colour</i>	<i>Pheromone Dose (μg)</i>	<i>Z3-hexenyl acetate Dose (μg)</i>
1 (July, 2007)	1	Unpainted white	0	0
1 (July, 2007)	2	Painted white	0	0
1 (July, 2007)	3	Painted yellow	0	0
1 (July, 2007)	4	Unpainted white	100	0
1 (July, 2007)	5	Painted white	100	0
1 (July, 2007)	6	Painted yellow	100	0
1 (July, 2007)	7	Unpainted white	0	100
1 (July, 2007)	8	Painted white	0	100
1 (July, 2007)	9	Painted yellow	0	100
1 (July, 2007)	10	Unpainted white	100	100
1 (July, 2007)	11	Painted white	100	100
1 (July, 2007)	12	Painted yellow	100	100
2 (June, 2008)	1	Unpainted white	0	0
2 (June, 2008)	2	Painted white	0	0
2 (June, 2008)	3	Painted yellow	0	0
2 (June, 2008)	4	Unpainted white	100	0
2 (June, 2008)	5	Painted white	100	0
2 (June, 2008)	6	Painted yellow	100	0
2 (June, 2008)	7	Unpainted white	0	100
2 (June, 2008)	8	Painted white	0	100
2 (June, 2008)	9	Painted yellow	0	100

2 (June, 2008)	10	Unpainted white	100	100
2 (June, 2008)	11	Painted white	100	100
2 (June, 2008)	12	Painted yellow	100	100
3 (July, 2008)	1	Unpainted white	0	0
3 (July, 2008)	2	Unpainted white	100	0
3 (July, 2008)	3	Unpainted white	100	10
3 (July, 2008)	4	Unpainted white	100	100
3 (July, 2008)	5	Unpainted white	100	1000
3 (July, 2008)	6	Unpainted white	100	10000

Table 3.2. Treatment list for pheromone dose and lure type experiment.

<i>Treatment</i>	<i>Lure Type</i>	<i>Pheromone Dose (μg)</i>
1	Red	0
2	Red	1
3	Red	10
4	Red	100
5	Red	1000
6	Grey	0
7	Grey	1
8	Grey	10
9	Grey	100
10	Grey	1000

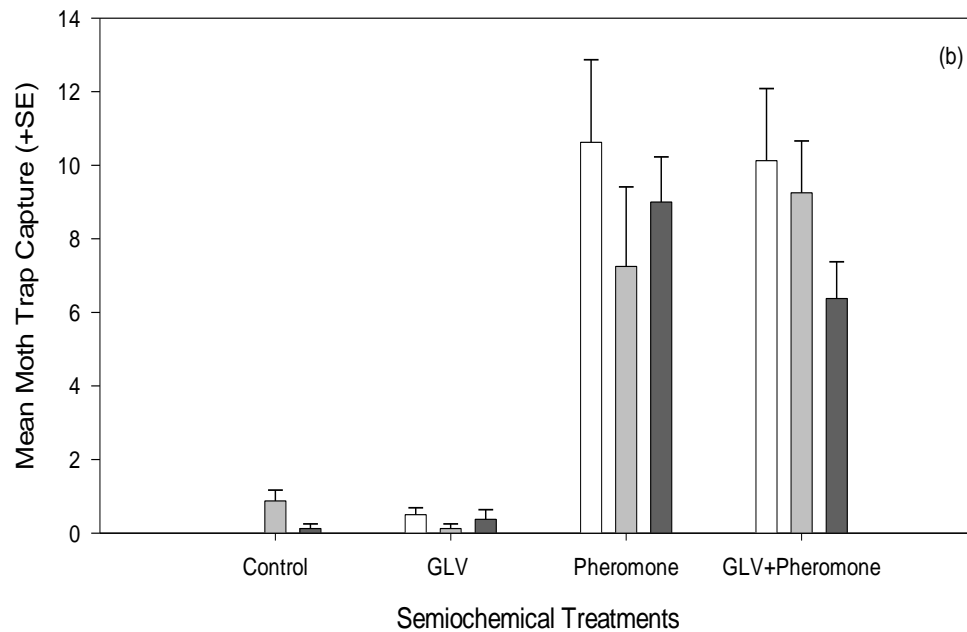
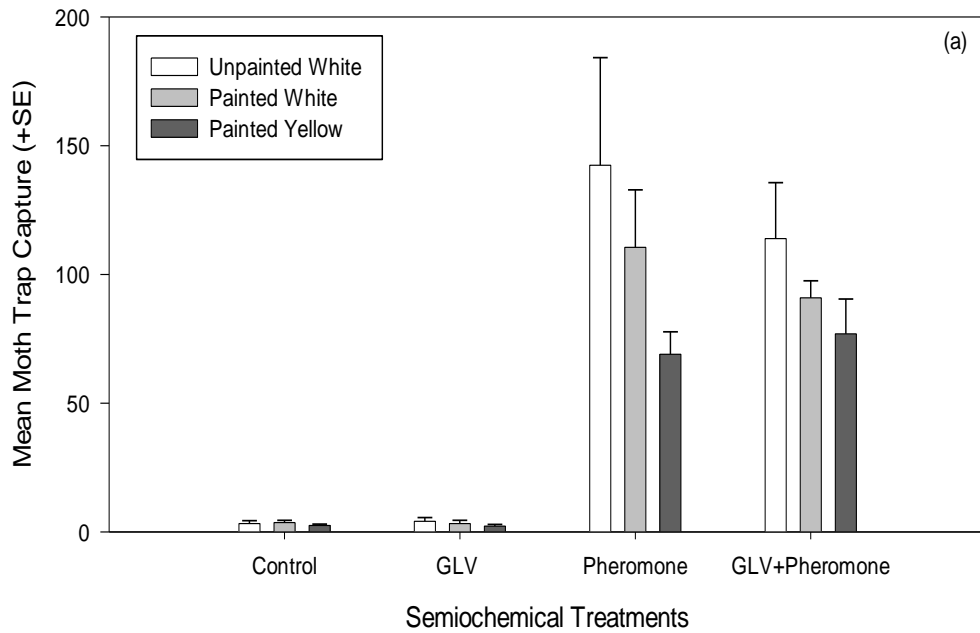


Figure 3.1. Mean (+SE) male moths captured a) during full-bloom, 2007 and b) pre-bloom 2008, in coloured traps baited with semiochemical treatments consisting of the *Plutella xylostella* pheromone blend (Contech Inc., Delta, British Columbia, Canada) and green leaf volatile (GLV) (Z3-hexenyl acetate) in a 1:1 ratio. n= 8 sites.

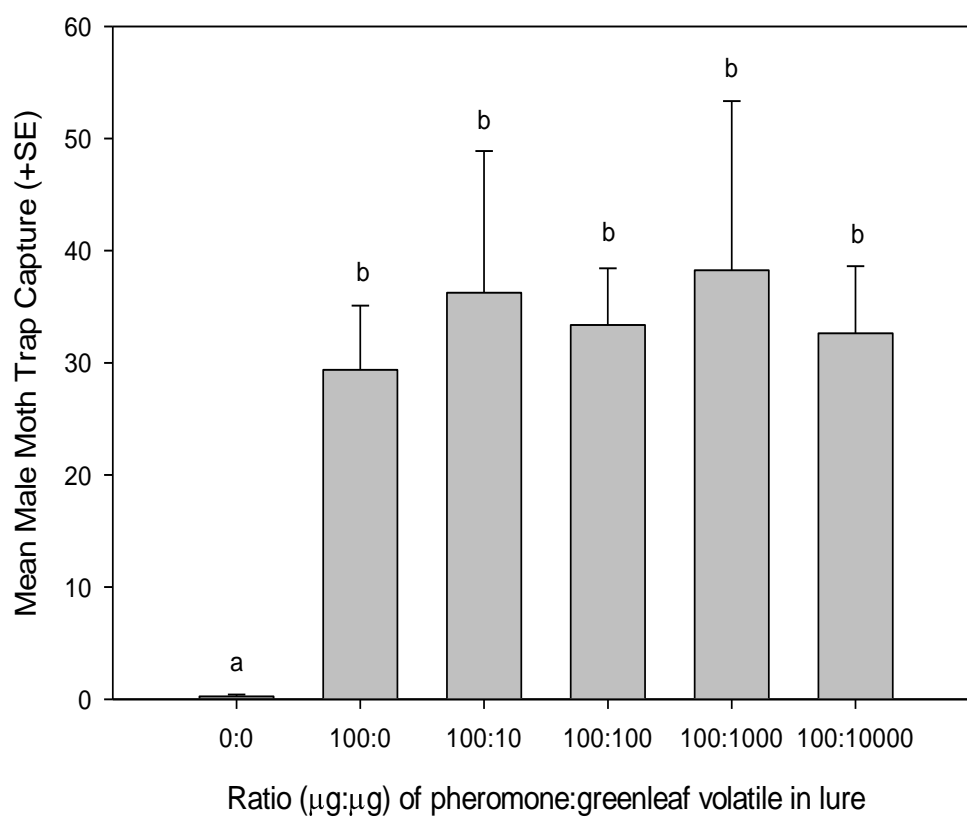


Figure 3.2. Mean (+SE) male moths captured in traps baited with semiochemical treatments consisting of the *Plutella xylostella* pheromone blend (Contech, Inc., Delta, British Columbia, Canada) and various doses of the green leaf volatile (GLV) Z3-hexenyl acetate. $n=8$ sites. Bars with same letters are not significantly different (Tukey's test, $P>0.05$).

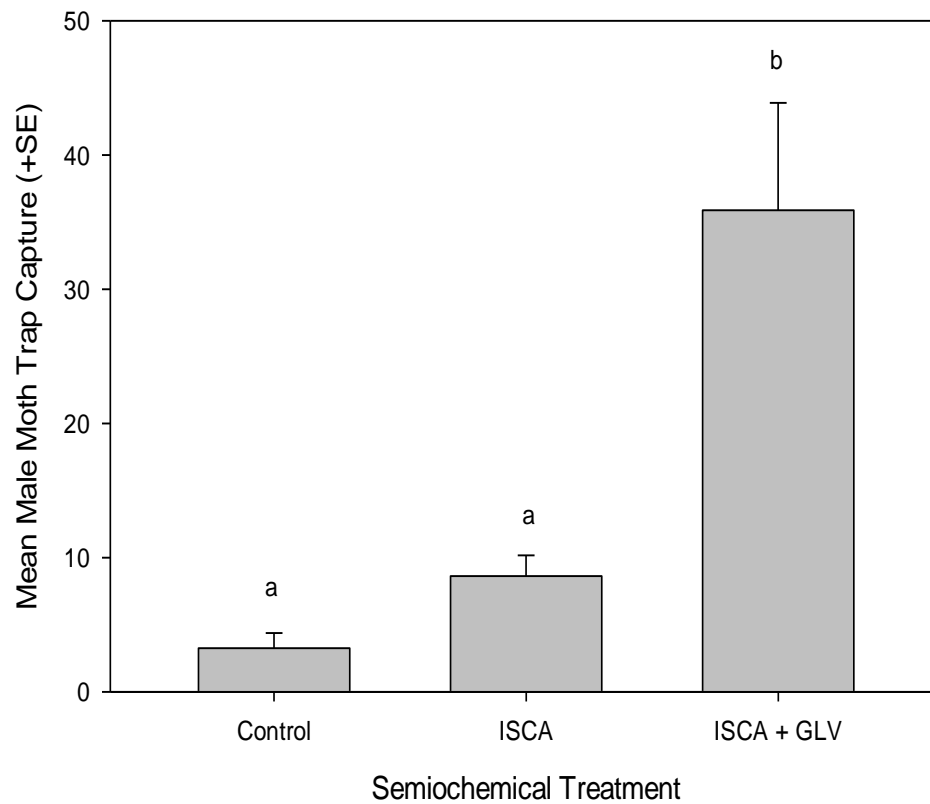


Figure 3.3. Mean (+SE) male moths captured in traps baited with semiochemical treatments consisting of the *Plutella xylostella* pheromone blend (ISCA, Riverside, California, USA) and the green leaf volatile (GLV) Z3-hexenyl acetate. n= 8 sites. Bars labelled with different letters are significantly different (Tukey's $P \leq 0.05$).

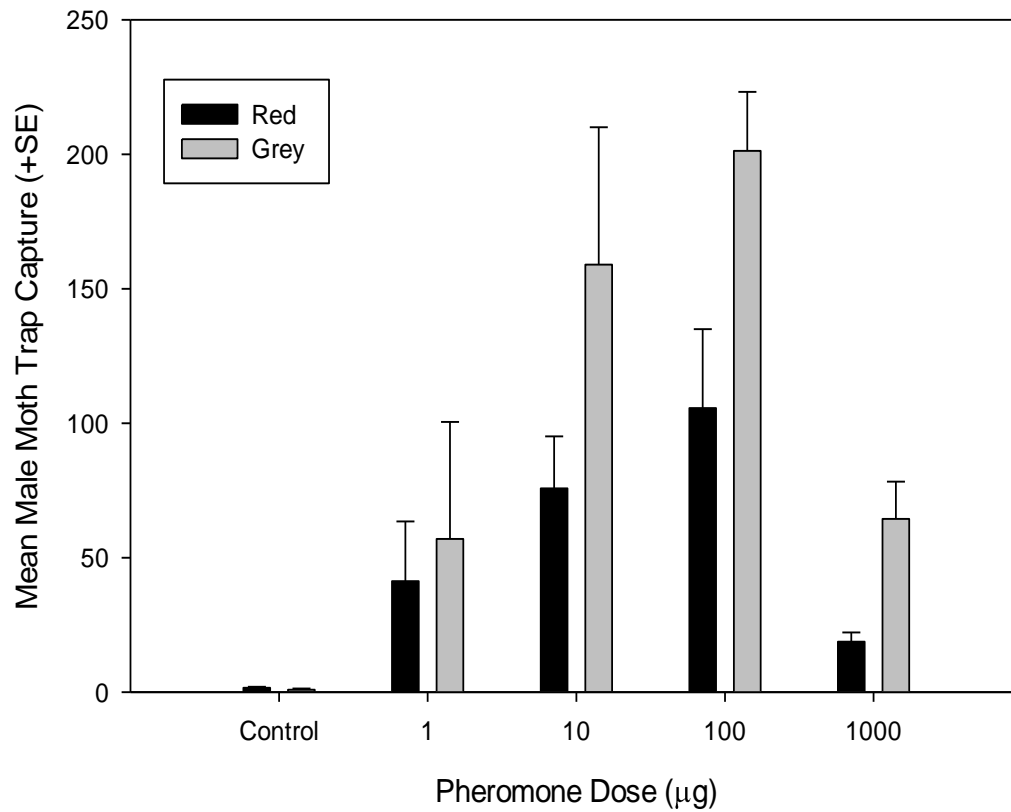


Figure 3.4. Mean (+SE) male moths captured in traps baited with red and grey rubber septa loaded with various pheromone doses of the commercially available *Plutella xylostella* pheromone blend (Contech, Inc., Delta, British Columbia, Canada). n= 8 sites.

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Chapter 4. Concluding Discussion

Plutella xylostella is a difficult pest to manage in brassicaceous crops throughout the world, and canola crops in western Canada are no exception (Palaniswamy et al., 1986; Philip and Mengersen, 1989; Dossdall et al., 2004). Because source populations of *P. xylostella* migrate to the Canadian Prairies, a sensitive detection tool such as sex pheromone-baited traps is needed to detect and predict incipient outbreaks. The goal of these studies was to develop a consistent predictive tool that can relate adult moth trap capture to the population density of the damaging larval stage in the field. In addition, I conducted research on means to improve trap attractiveness to moths, which may serve to enhance the development of a predictive model and generally improve detection and monitoring of *P. xylostella* in the Canadian Prairies.

4.1 A Pheromone-based Predictive Model for *Plutella xylostella*

I found that trap capture of male *P. xylostella* in traps baited with commercially-available sex pheromone lures was not consistently related to larval populations throughout two field seasons: one at moderate (2007) and one at low (2008) population densities. The strongest relationships were during the latter part of the season either when adults and larvae were sampled on the same day or when larvae were from the subsequent sampling collection. These findings are in contrast to the predictive models developed for *P. xylostella* in cabbage and other cole crops, in which moth trap capture forewarned of larval infestations early in the growing season (Baker et al., 1982; Walker et al., 2003). Pheromone-baited

trap capture may be more closely related to larval density for resident moth populations (Baker et al., 1982; Walker et al., 2003) than populations established by migrant moths transported on trajectory winds from infested regions (Doddall et al., 2004; Hopkinson and Soroka, 2010) characteristic of the populations investigated in this study.

Curvilinear relationships in which intermediate moth catch was associated with the highest larval sample densities occurred in several instances when adult and larval samples were significantly correlated. These types of relationships have been observed in other moth species (e.g., Jones et al., 2009) and have been attributed to competition between natural and synthetic pheromone plumes at intermediate population densities (Cardé, 1979). When male moth catch was used to predict larval density in the subsequent sample period, there were significant correlations that were either curvilinear (Figure 2.5) or negative (Figure 2.3). Baker et al. (1982) demonstrated a positive correlation between moth trap capture and larval samples of *P. xylostella* in six out of 15 cabbage fields when larvae were sampled 15-21 days after moth catch in three seasons. Attempts to correlate trap catch with larvae sampled 7-11 days after moth catch resulted in many negative or non-significant correlations (Baker et al., 1982). It is possible that analysis of moth capture with subsequent larval samples in my study may improve with an increased lag period between moth catch and larval sampling. Positive correlations between adult trap catch and larvae sampled 2-3 weeks later were found in four of five fields planted with various brassicaceous crops (Walker et al., 2003). The development of a pheromone-based monitoring system for *P.*

xylostella is hampered by large variation in population densities between fields (Baker et al., 1982). Between-site variation would be expected to be greater in canola fields of western Canada in which *P. xylostella* populations occur mostly by migration (Dosdall et al., 2004; Hopkinson and Soroka, 2010) because deposition of moths from air currents would not be expected to occur uniformly but rather would depend on stochastic environmental factors. This may preclude the use of pheromone trap capture to develop economic thresholds for this insect.

Pheromone-baited traps are a better indicator of *P. xylostella* presence compared to sweep net sampling. The pheromone-based monitoring system currently used by the Prairie Pest Monitoring Network (PPMN) is useful to determine time of moth arrival on trajectory winds throughout western Canada (Hopkinson and Soroka, 2010). However, pest managers and producers may need to inspect individual fields with sweep nets or individual plant counts to assess local immature populations.

In my study, there were many instances in which high trap capture of male *P. xylostella* was associated with low larval densities in canola fields. This may indicate that natural enemies such as parasitoid and generalist predator populations (Sarfranz et al., 2005) or fungal organisms (Furlong and Pell, 2001) impacted larval populations and may have contributed to the curvilinear relationship in my studies. Further improvements of a predictive model may result from incorporation of pre-imaginal mortality factors (Evenden et al., 1995) through life table analysis of *P. xylostella* in canola agroecosystems in western Canada.

This study illustrates that one pheromone-baited trap per field is adequate to capture a representative number of male *P. xylostella* in canola. This could be a function of overall low population densities during the 2008 growing season when these data were collected. However, trap capture in three pheromone-baited traps per site gave a better indication of subsequent larval densities than six pheromone-baited traps in cabbage fields in eastern North America (Baker et al., 1982). Because sampling only occurred at moderate and low population densities in this study, sampling during seasons of high populations/outbreaks may provide more indicative relationships to develop a predictive model based upon moth trap capture and immatures in the field (Evensen et al., 1995).

Pheromone-based monitoring for *P. xylostella* in western Canada is currently a good detection tool of moth arrival but continued research is necessary to consider the feasibility of developing a predictive model for this pest over the large geographic area of the Canadian Prairies.

4.2 Methods to Improve Attractiveness of Pheromone-baited Traps to *Plutella xylostella* Adults

My study indicates that trap capture in the commercially available pheromone-baited traps currently used in the PPMN is most useful as an indicator of moth activity rather than density. It follows that highly attractive traps would be most useful to detect the patchy distribution of *P. xylostella* migrating into the Prairie Provinces. My study has illuminated several ways in which the attractiveness of pheromone-baited traps can be improved.

Trap height influences *P. xylostella* capture in pheromone-baited traps in cabbage (Reddy and Urs, 1996) and canola (Chisholm et al., 1979). Despite the fact that traps positioned 30 cm above the ground caught more moths in canola than traps positioned higher in the canopy (Chisholm et al., 1979), the current trap height used to monitor *P. xylostella* in the PPMN is ~1.5 m above the soil. My data showed no statistical difference in moth capture in traps positioned at various heights in the canopy. However, numerically more male moths were captured in traps positioned low in the canopy (50 cm) or moved with the canopy height than in traps positioned high in the canopy (1.5 m). Based on the findings of my study and those of Chisholm et al. (1979), traps should be positioned 30-50 cm above the ground to maximize trap capture of *P. xylostella* in canola.

The traps in my studies were placed at the edge of canola fields. Investigations on the spatial distribution of *P. xylostella* with pheromone-baited traps in Ethiopia show that trap placement within the crop indicated a higher male presence than those outside the field in cabbage systems (Ayalew et al., 2008). In contrast, capture of male moths on the field edge and interior were highly correlated in various brassicaceous crops in New Zealand (Walker et al., 2003). Future investigations of the effect of trap placement at various distances into the crop may improve moth trap capture and may provide a better correlation of adults to immatures in canola.

The lure change interval for monitoring *P. xylostella* could be lengthened from the three weeks currently used in the PPMN to six weeks. Traps baited with lures changed at the 6-week interval attracted more male moths compared to the

three-week lure change regime (Figure 2.7). This simple change to the current program will decrease both direct costs of pheromone lures and indirect costs of time spent in the field by pest managers. These data are supported by those of Evenden and Gries (2010) who show that older pheromone lures are more attractive than fresh lures to *P. xylostella* in western Canada. These results are contrary to those reported by Môtus et al. (1997) and Mayer and Mitchell (1999) in which moth trap capture was lower in traps baited with aged lures and illustrate the importance of variation in environmental conditions in pheromone-baited trap capture of *P. xylostella*.

Pheromone traps baited with grey rubber septa lures are more attractive than those with red rubber septa lures at all pheromone doses tested in my study. This differential attractiveness may be driven by variable release rates of pheromone components from the two release substrates (Appendix 6). These results are similar to those reported by Mayer and Mitchell (1999) in which grey lures were more attractive than red to *P. xylostella*. As it appears that grey lures release a lower Z11-16:Ald : Z11-16:Ac ratio (Appendix 6) than red lures, lures formulated with Z11-16:Ac as the major pheromone component may be more attractive to *P. xylostella* than lures releasing Z11-16:Ald as the main component which has been demonstrated in other parts of the world (Suckling et al., 2002; Yang et al., 2007). Pheromone blends with acetate as the major component should be investigated to determine optimal blends to monitor *P. xylostella* in western Canada. The current pheromone dose (100 µg) used in the commercially available pheromone lures in the PPMN attracted more males in this study, fewer

male *P. xylostella* were captured in traps baited with higher and lower doses of the Contech pheromone blend.

Plutella xylostella are known to use both visual and olfactory cues to locate host plants (Couty et al., 2006). However, efforts to use trap colour as a visual cue did not improve attractiveness of semiochemical-baited traps to adult males or females. There was no statistical significance among the number of moths captured in the variously coloured traps indicating that the current trap colour (white) should continue to be used for monitoring.

The addition of the green leaf volatile Z3-hexenyl acetate to the commercial pheromone blend at various doses did not increase moth attractiveness to traps in canola. The addition of Z3-hexenyl acetate to the lures in cabbage systems enhances trap capture of both male and female moths (Reddy and Guerrero, 2000; Dai et al., 2008). The background “noise” of canola may provide enough volatiles to attract moths, compared to cabbage and may be a result of the crop agronomic characteristics (plant density, fertility regime, row-spacing, etc.).

In other pest species, the use of suboptimal pheromone doses in combination with green leaf volatiles increases attraction (Yang et al., 2004; Schmidt-Büsser et al., 2009) over pheromone alone. This phenomenon likely occurred in my study using the suboptimal ISCA pheromone lures (Evenden and Gries, 2010). More moths were captured in traps baited with the ISCA blend + Z3-hexenyl acetate compared to the ISCA blend alone. Studies investigating varying ratios of attractive pheromone blends to the green leaf volatile Z3-hexenyl

acetate or other host volatiles (Dai et al., 2008) may enhance adult moth response in canola.

4.3 Summary

This study was the first to attempt to develop pheromone-baited traps as a predictive tool of *P. xylostella* larval density in canola. Although high between-site variation precluded the development of a predictive tool based on early arriving migrant moths, moth trap capture did predict larval density late in the season following a curvilinear relationship. Further research to understand what factors drive these relationships is warranted and could strengthen the predictive capacity of pheromone-baited traps in the future.

Investigations into other more attractive green leaf volatiles should be pursued to determine if synergistic effects of combining host volatiles with attractive pheromone blends exist for western Canadian populations of *P. xylostella*. Z3-hexenyl acetate is a common plant volatile, but crucifer specialists such as *P. xylostella* adults may respond more favourably to chemical compounds produced exclusively by members of the Brassicaceae (Dai et al., 2008). Some of these host compounds are more volatile and difficult to work with, but may be more attractive and provide an enhanced monitoring tool for both male and female moths.

Based on this work, several immediate improvements can be made to the current pheromone-based monitoring system for *Plutella xylostella* in canola in the Prairie provinces: 1) pheromone should be applied at 100 µg to grey rubber septa lures; 2) lures can be changed at six-week intervals throughout the field

season; and 3) one trap per field positioned 50 cm above the soil surface is sufficient to monitor male moth flight. Further research is required to reassess the most attractive pheromone blend for *P. xylostella* in the Prairie Provinces and to determine if geographic variation exists in moth response to green leaf volatiles.

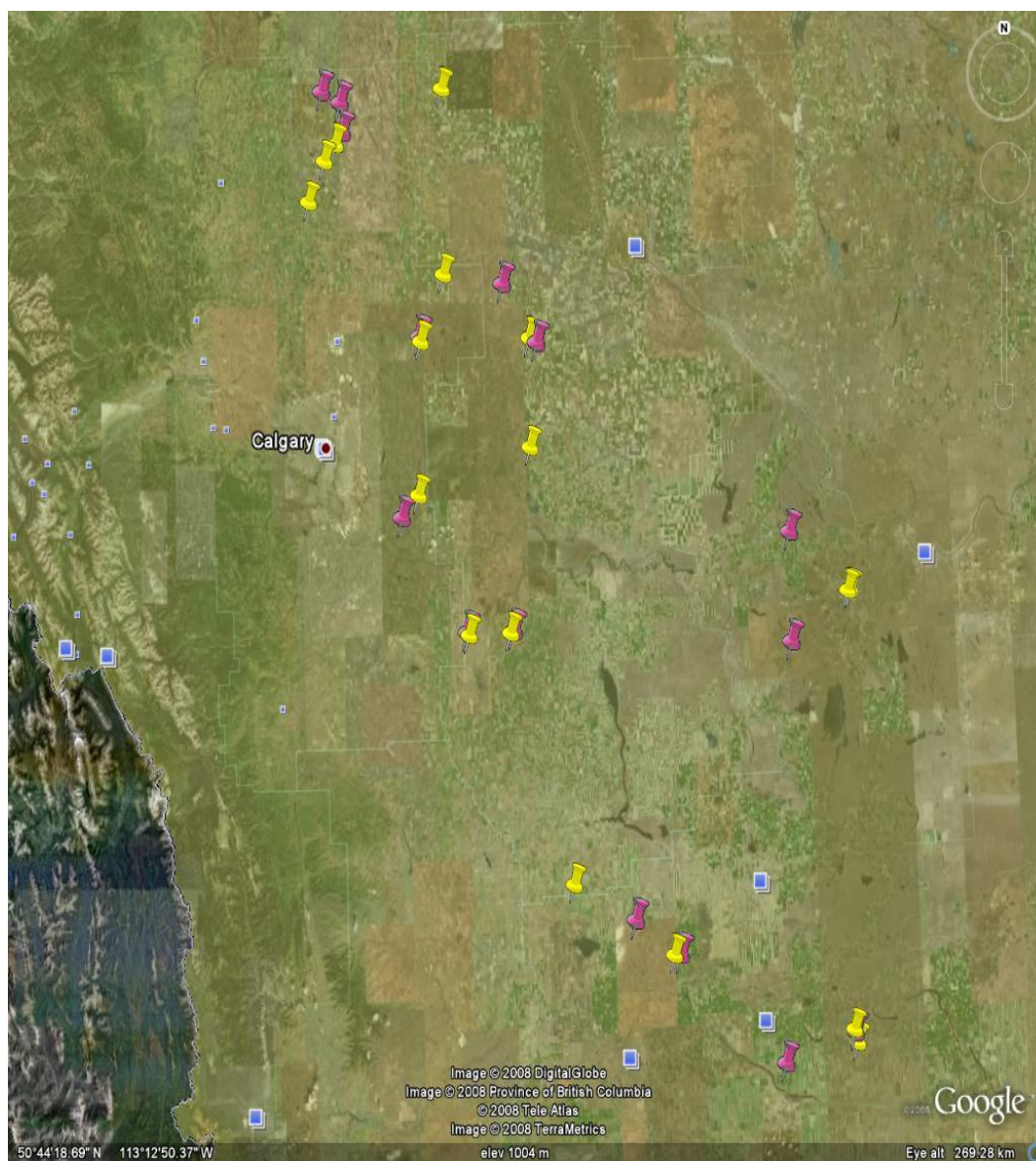
Plutella xylostella is a well-studied insect because it is a key pest of Brassicaceae worldwide. As an outbreak species in the canola crops of western Canada, the development of a predictive model is desirable; however, the biology of *P. xylostella* and annual climatic variations may limit the development of a consistent predictive model for such a large geographic region. As canola acreage continues to grow and the distribution of this species is affected by climate change in western Canada, a proactive approach to develop better predictive tools is of utmost importance to successful canola production in the future.

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Appendix 1.

Site location map of predictive model sites in southern Alberta, Canada, 2007 (yellow) and 2008 (pink).

Appendix 2.

Plutella xylostella Predictive Model Experiments (Chapter 2), detailed field site descriptions, southern Alberta Canada, 2007 and 2008.

Year	Site	North	South	East	West	GPS Coordinates (Latitude)	GPS Coordinates (Longitude)
2007	1	Peas	Canola	Canola	Barley	49°40.165 N	111°54.755 W
2007	2	Canola	Barley	Hay ¹	Canola	49°41.918 N	111°55.441 W
2007	3	Canola	Canola	Wheat	Canola	49°37.767 N	112°13.055 W
2007	4	Wheat	Mustard	Canola	Peas	50°02.892 N	113°04.527 W
2007	5	Hay ¹	Canola	Canola	Barley	49°53.063 N	112°39.969 W
2007	6	Canola	Hay ¹	Canola	Canola	50°39.651 N	111°52.454 W
2007	7	Canola	Canola	Barley	Wheat	50°36.086 N	113°18.927 W
2007	8	Canola	Hay ²	Barley	Wheat	50°35.988 N	113°29.411 W
2007	9	Canola	Canola	Hay ²	Hay ²	50°54.443 N	113°41.502 W
2007	10	Hay ¹	Canola	Barley	Wheat	51°14.498 N	113°40.240 W
2007	11	Barley	Canola	Canola	Wheat	51°23.204 N	113°34.081 W
2007	12	Hay ¹	Canola	Grass ³	Canola	51°33.191 N	114°08.547 W
2007	13	Peas	Canola	Wheat	Barley	51°38.539 N	114°04.344 W
2007	14	Barley	Hay ¹	Barley	Hay ²	51°46.388 N	114°00.112 W
2007	15	Canola	Wheat	Wheat	Barley	51°51.197 N	114°06.425 W
2008	1	Canola	Canola	Canola	Barley	49°37.767 N	112°13.054 W
2008	2	Barley	Barley	Canola	Barley	49°41.254 N	111°55.391 W
2008	3	Canola	Wheat	Canola	Hay ¹	49°52.846 N	112°38.592 W
2008	4	Hay ²	Barley	Canola	Hay ¹	49°57.641 N	112°49.548 W
2008	5	Canola	Barley	Canola	Alfalfa	50°47.419 N	112°07.685 W
2008	6	Canola	Barley	Canola	Canola	50°33.066 N	112°08.109 W
2008	7	Canola	Hay ¹	Canola	Canola	50°36.112 N	113°18.332 W
2008	8	Barley	Hay ¹	Canola	Canola	50°36.079 N	113°30.109 W
2008	9	Hay ²	Grass ⁴	Canola	Wheat	51°13.629 N	113°10.756 W
2008	10	Canola	Barley	Canola	Canola	50°46.043 N	113°46.043 W
2008	11	Hay ²	Wheat	Canola	Barley	51°14.939 N	113°40.754 W
2008	12	Barley	Hay ²	Canola	Hay ²	51°41.998 N	113°59.817 W
2008	13	Canola	Barley	Barley	Barley	51°45.960 N	114°00.309 W
2008	14	Barley	Hay ²	Canola	Hay ²	51°47.453 N	114°05.047 W
2008	15	Canola	Canola	Canola	Canola	51°21.475 N	113°19.199 W

¹-Grass/Alfalfa Mix, ²-Grass Mix, ³-Golf Course-Gun Range, ⁴-Native Grasslands

Appendix 3.

Height Experiment (Chapter 2), field site descriptions and trap heights, for canopy height treatment, southern Alberta, Canada 2008.

Site	Date	Trap Height	North	South	East	West	GPS Coordinates (Latitude)	GPS Coordinates (Longitude)
1	13/5/08	Ground	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	13/5/08	Ground	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	13/5/08	Ground	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	13/5/08	Ground	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	13/5/08	Ground	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	13/5/08	Ground	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	13/5/08	Ground	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	13/5/08	Ground	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W
1	26/5/08	Ground	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	26/5/08	Ground	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	26/5/08	Ground	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	26/5/08	Ground	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	26/5/08	Ground	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	26/5/08	Ground	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	26/5/08	Ground	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	26/5/08	Ground	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W
1	9/6/08	Ground	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	9/6/08	Ground	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	9/6/08	Ground	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	9/6/08	Ground	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	9/6/08	5cm	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	9/6/08	Ground	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	9/6/08	5cm	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	9/6/08	5cm	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W
1	24/6/08	30 cm	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	24/6/08	30 cm	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	24/6/08	15 cm	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	24/6/08	15 cm	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	24/6/08	30 cm	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	24/6/08	30 cm	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	24/6/08	15 cm	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	24/6/08	15 cm	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W

1	9/7/08	80 cm	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	9/7/08	100 cm	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	9/7/08	90 cm	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	9/7/08	90 cm	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	9/7/08	90 cm	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	9/7/08	70 cm	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	9/7/08	100 cm	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	9/7/08	80 cm	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W
1	25/7/08	85 cm	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	25/7/08	105 cm	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	25/7/08	100 cm	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	25/7/08	100 cm	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	25/7/08	90 cm	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	25/7/08	90 cm	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	25/7/08	100 cm	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	25/7/08	90 cm	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W
1	4/8/08	85 cm	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	4/8/08	105 cm	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	4/8/08	100 cm	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	4/8/08	100 cm	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	4/8/08	90 cm	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	4/8/08	95 cm	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	4/8/08	100 cm	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	4/8/08	90 cm	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W
1	18/8/08 ²	85 cm	Canola	Peas	Mustard	Canola	49°37.544 N	111°55.506 W
2	18/8/08 ²	105 cm	Canola	Sugar Beets	Canola	Barley	49°59.387 N	112°50.211 W
3	18/8/08 ²	100 cm	Canola	Barley	Canola	Alfalfa	50°21.258 N	111°47.405 W
4	18/8/08 ²	100 cm	Hay ¹	Hay ¹	Canola	Barley	50°35.915 N	113°19.733 W
5	18/8/08 ²	90 cm	Canola	Hay ¹	Hay ¹	Barley	51°14.508 N	113°12.858 W
6	18/8/08 ²	95 cm	Canola	Barley	Canola	Hay ¹	50°51.823 N	113°43.225W
7	18/8/08 ²	100 cm	Canola	Canola	Canola	Canola	51°31.039 N	112°23.447 W
8	18/8/08 ²	90 cm	Canola	Canola	Canola	Barley	51°48.706 N	112°47.540 W

¹-Grass-Legume Mix, ²-Take-Down Date

Appendix 4.

Ratio of pheromone components used in the commercially available *Plutella xylostella* pheromone blend (ConTech Enterprises Inc., Delta, BC) used in the Prairie Pest Monitoring Network.

	Z11-16: Ac µg	Z11-16: Ald µg	Z9-14: OH µg	Z11-16: OH µg
Target	30	70	10	1
2007	35.83	65.80	7.98	1.40
2008	31.63	69.98	8.27	1.12

GC Purity 2007 = 96.95%

GC Purity 2008 = 98.03%

Appendix 5.

Site descriptions; Green Leaf Volatile and Trap Colour Experiment July 2007 (Chapter 3), site locations geo-referenced near Lethbridge, Alberta, Canada.

Site Number	GPS Coordinate (Latitude)	GPS Coordinate (Longitude)
1	49°36.384 N	112°47.109 W
2	49°36.368 N	112°51.105 W
3	49°40.080 N	112°40.175 W
4	49°41.524 N	112°39.421 W
5	49°44.062 N	112°33.214 W
6	49°44.577 N	112°38.335 W
7	49°45.225 N	112°39.458 W
8	49°45.238 N	112°24.500W

Site descriptions: Green Leaf Volatile and Trap Colour Experiment June 2008 (Chapter 3), site locations geo-referenced near Lethbridge, Alberta, Canada.

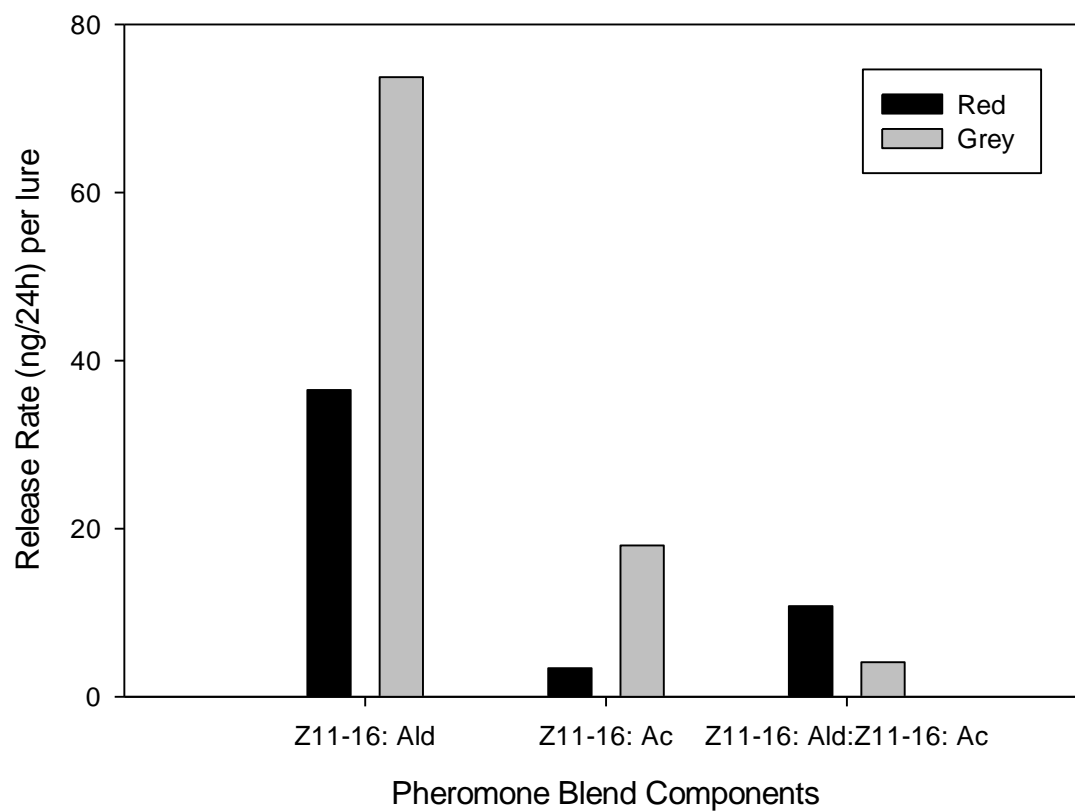
Site Number	GPS Coordinate (Latitude)	GPS Coordinate (Longitude)
1	49°38.257 N	111°56.475 W
2	49°39.080 N	112°13.049 W
3	49°59.870 N	112°54.980 W
4	49°57.778 N	112°54.984 W
5	50°01.129 N	112°51.076 W
6	50° 00.897 N	112°41.702 W
7	49°55.894 N	112°46.310 W
8	49°55.270 N	112°39.981 W

Site descriptions: Green Leaf Volatile Dose Experiment, July 2008 (Chapter 3), site locations geo-referenced near Lethbridge, Alberta, Canada.

Site Number	GPS Coordinate (Latitude)	GPS Coordinate (Longitude)
1	49°36.647 N	112°44.102 W
2	49°33.169 N	112°37.392 W
3	49°37.992 N	112°26.574 W
4	49°41.675 N	112°38.696 W
5	49°45.396 N	112°37.121 W
6	49°47.655 N	112°33.354 W
7	49°45.391 N	112°41.223 W
8	49°41.913 N	112°18.075 W

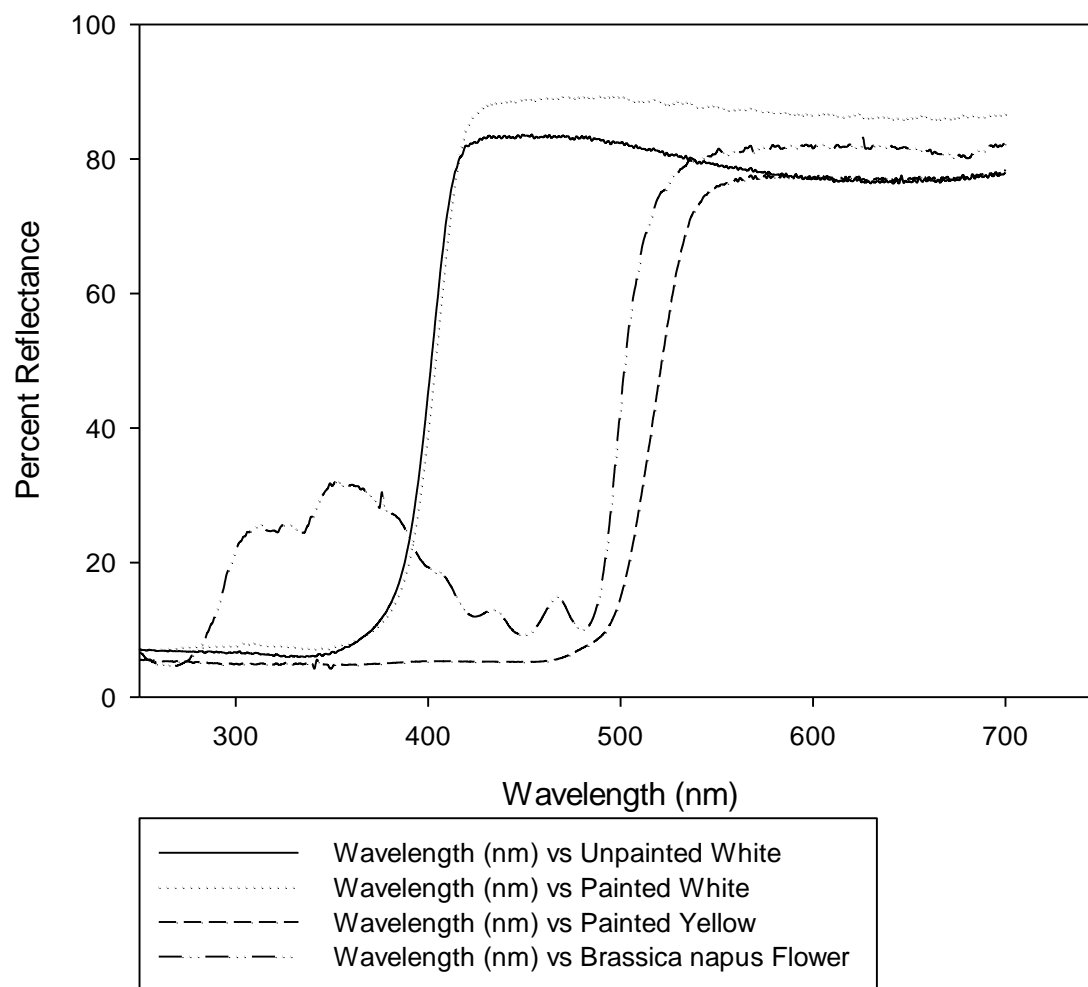
Site descriptions Lure Type and Dose Response Experiment, July 2007 (Chapter 3), site locations geo-referenced near Lethbridge, Alberta, Canada.

Site Number	GPS Coordinate (Latitude)	GPS Coordinate (Longitude)
1	49°36.387 N	112°46.478 W
2	49°40.079 N	112°40.177 W
3	49°41.198 N	112°40.014 W
4	49°43.133 N	112°33.169 W
5	49°40.358 N	112°37.216 W
6	49°46.164 N	112°36.011 W
7	49°45.223 N	112°39.467 W
8	49°47.276 N	112°45.208 W

Appendix 6.

Release rates of Z11-16: Ald, Z11-16:Ac and the blend of Z11-16: Ald:Z11-16: Ac fresh 100 μ g lures from pheromone dose and lure type experiment.

Appendix 7.



Reflectance results for unpainted, painted white and painted yellow traps. *Brassica napus* flower reflectance added for comparison (Tansey et al., 2010).