University of Alberta

Semiochemical-based mass trapping of the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) (Lepidoptera: Sesiidae)

by

Virgiliu Marius Aurelian

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Ecology

Department of Biological Sciences

Fall 2011
Edmonton, Alberta

Dedication

This thesis is dedicated to my grandfather Dumitru-Jean Aurelian and my grade 5 Biology teacher Ştefan Neagu, who are the ones responsible for lighting up the fire of passion for studying insects, and to my lovely wife Larisa whose unconditional love, support and dedication were my *ignis perpetua* that kept me grounded into the present.

Abstract

Semiochemical-based mass trapping was tested against the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)), an invasive apple pest in British Columbia. Two yellow traps baited with apple clearwing moth sex pheromone, (*Z*,*Z*)-3,13 octadecadienyl acetate, and Concord grape juice, respectively were tested. Mass trapping reduced moth capture in assessment traps more reliably with pheromone than juice mass traps. The optimal trap density was 50 – 100 traps / ha for pheromone and at least 100 traps / ha for juice traps. The main effect of pheromone-based mass trapping was disruption of male moth orientation. Moth catches and mating status of captured females in juice assessment traps in plots treated with pheromone mass traps did not differ from the control. Catches in the two trap types were correlated and juice traps can monitor moth flight. Traps targeting the apple clearwing moth captured non-target arthropods that were more diverse in organic than in conventionally-managed orchards.

Acknowledgements

Many wonderful people to whom I will always be grateful contributed to this present work. The most significant contribution comes from my supervisor Dr. Maya L. Evenden and my co-supervisor Dr. Gary J. R. Judd because they gave me the opportunity to pursue this degree under their direction and patience. I feel indeed fortunate to have such dedicated researchers who provided guidance and constructive criticism whenever I required their help. I truly learned a lot from them. Their editing skills were invaluable and hence, much appreciated to the progress of my degree.

Drs. Felix Sperling and Nadir Erbilgin provided an excellent feedback on the progress of my research during our annual committee meetings. Their input was always appreciated.

Tiffany Fransbergen and Larisa N. Aurelian were wonderful assistants who greatly enhanced the quality of my research. They endured long hours in the field and worked at peak performance under the scorching sun above and the soggy, mosquito-infested grass below. Without them repetitive tasks such as counting insects, emptying traps and replacing pheromone lures would have driven one on the path to insanity.

My lab mates fostered productive, intellectual discussions during our weekly lab meetings and provided useful and much needed constructive criticism of my research as my degree progressed *da capo al fine*. I am indebted to all of them. A special thank you goes to Joelle Lemmen and her husband, Andrew, who

provided accommodation in Edmonton until I found a place of my own. Without their kindness I would have experienced first hand the golden days of hoboism. Boyd Mori, Caroline Whitehouse and Devin Goodsman provided excellent and much needed help with learning a new and very interesting programming language.

I would also like to thank all the apple growers who showed interest in my work and readily granted access to their apple orchards: Bob Bengag, Tony Dawson, Fred Danenhower, Trevor & Debbie Evans, Manjinder Gill, Wilfred Mennell, Wayne Ritchie, Greg Sanderson, Rob Dawson, Ron & Andrea Schneider and Nirmal Sidhu. I am greatly indebted to SunRype for providing the very large amount of Concord grape juice used in all of my experiments.

This research was financed by an Agriculture and Agri-Food Canada grant to Dr. Gary J. R. Judd from the Pacific Agri-Food Research Station, Summerland branch, BC, by a Department of Biological Sciences teaching assistantship and a Faculty of Graduate Studies and Research Queen Elizabeth II scholarship at the University of Alberta, Edmonton, AB.

Table of Contents

Chapter 2. Development of semiochemical-based mass trapping of the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) (Lepidoptera: Sesiidae) in apple orchards in the Southern Interior of BC, Canada

Chapter 3. Mechanisms of pheromone-based mass trapping of the apple clearwing moth, *Synanthedon myopaeformis* (Borkhausen) (Lepidoptera: Sesiidae)

Chapter 4. Diversity and abundance of arthropod by-catch in semiochemical-baited traps in conventionally- and organically-managed apple orchards

Chapter 5. Concluding discussion

List of Tables:

List of Figures

Chapter 1. Introduction

1.1. Clearwing moths

Clearwing moths (Lepidoptera: Sesiidae, except Tinthiinae) are a well defined group currently placed in the Cossoidea-Sesioidea assemblage (Cossoidea *sensu lato*) (Regier et al. 2009; Mutanen et al. 2010). Clearwing moths are divided into three subfamilies, with the highest species diversity in the tropical and equatorial climates (McKern et al. 2008). Most clearwing species are typically diurnal and heliophilic with only a few species active at dusk or at night (Popescu-Gorj et al. 1958; Eichlin & Duckworth 1988). Flight activity is generally restricted near their larval host plants. Although adults of some species (e.g. *Synanthedon vespiformis* (Linnaeus), *S. polygoni* (Hy. Edwards), *Albuna pyramidalis* (Walker)) are known to frequent flowers (Popescu-Gorj et al. 1958; Eichlin & Duckworth 1988), most adult clearwings do not feed even though a proboscis may be present (Greenfield & Karandinos 1979). Most holarctic species have approximately a two-month flight period in temperate regions and adults are found from beginning of May until end of September (Spatenka et al. 1999; Aurelian, V. M. unpublished data). Adult clearwing moths are best known as mimics of various aculeate wasp species, although the aposematic colouration of some (e.g. *Euhagena nebraskae* Hy. Edwards and *Synanthedon polygoni* (Hy. Edwards)) might indicate that some species are chemically defended.

Like most moths, clearwings depend on long-range pheromonal communication for mate location. However, visual cues may also be important for mate location as the males of some species discriminate between variously coloured pheromone-baited traps (Timmons & Potter 1981; Buda & Karalius 1993). The chemical composition of sex pheromones of most sesiids is not known, but responses of many species to similar sex attractants indicate that clearwing moths probably use a narrow range of pheromones consisting of C-18 diene alcohols, acetates and aldehydes with unsaturation points at the 2 or 3 and 13 carbon positions (Priesner et al. 1986; Szocs et al. 1989; El-Sayed 2008). Specificity is probably achieved through the ratio of components released by females and the presence of inhibitory components that act heterospecifically (Szocs et al. 1990; Mozuraitis et al. 2006). Some cross attraction does occur between various species as calling female ash borers (*Podosesia syringae* (Harris)) attract con- and heterospecific males (Nielsen & Balderston 1973). Other pre-reproductive isolating mechanisms include temporal separation of pheromone release in sympatric species (Greenfield & Karandinos 1979; Bergh et al. 2006). Male hair pencils may play a role in short range courtship communication through production of visual or olfactory cues (Kimura & Honda 1999; Hillier & Vickers 2004).

Most clearwing moths have a one-year life cycle in warm climates and a two or three-year life-cycle in more northern regions. Larvae are oligophagous or monophagous borers in stems, roots and trunks of various herbaceous plants and trees (Eichlin & Duckworth 1988; Spatenka et al. 1999). Among the more notable exceptions to this feeding life style are some tropical seed borers (Harms & Aiello 1995; McKenna & McKenna 2006; Nereida 2005) and two insectivorous species that feed exclusively on scale insects (Bradley 1956; Duckworth 1969). The boring behaviour of clearwing moth larvae has resulted in several species being designated as pests. Examples of native North American clearwing moth pests include the peach tree borer (*Synanthedon exitiosa* (Say)), strawberry crown borer (*S. bibionipennis* (Boisduval)), squash vine borer (*Melittia cucurbitae* (Harris)), sequoia pitch moth (*S. sequoiae* (Hy. Edwards)), Douglas-fir pitch moth (*S. novaroensis* (Hy. Edwards)), American hornet moth (*Sesia tibialis* (Harris)), raspberry crown borer (*Pennisetia marginata* (Harris)) and ash borer (*Podosesia syringae* (Harris)). Three more clearwing moths introduced from Europe via infected plant stock have also become important pests in North America: the currant borer (*Synanthedon tipuliformis* (Clerck)), the European hornet moth (*Sesia apiformis* (Clerck)) and the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)).

1.2. The apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen))

The apple clearwing moth, also known as the small red-belted clearwing in parts of Europe (Van Frankenhuyzen 1978) is an introduced species in Canada, first discovered in Cawston, British Columbia (BC) in 2005 (Philip 2006). It is native to Eurasia (Spatenka et al. 1999) and was most likely introduced to North

America via infested root stock from Central Europe (Phillip 2006). The apple clearwing moth is considered an important pest of commercial apple trees (*Malus domestica* Borkhausen) (Dickler 1976; Blaser & Charmillot 1984; Al-Antary et al. 2004) and occasionally pear trees (*Pyrus* spp.) (Baggiolini & Antonin 1976) and can cause significant economic crop loss (Dickler 1976). Mountain ash (*Sorbus* sp.), hawthorn (*Crataegus* sp.), common sea-buckthorn (*Hippophae rhamnoides* Linnaeus) and *Prunus* sp. are also recorded as minor host plants of the apple clearwing moth throughout Europe (Spatenka et al. 1999).

Young apple trees grafted on dwarfing root stock seem to be the favoured hosts of the apple clearwing moth. Trees under three years of age are not attacked, although older trees are attacked repeatedly (Buleza et al. 1990). According to Ateyyat (2006) dwarfing rootstocks are preferentially infested over non-dwarfing rootstocks because of the higher numbers of available entry points at the rootstock/scion union. Dwarfing rootstocks further promote pathogen infestation (Harris 1991; Deckers 1994; Leskey & Bergh 2005). Strapazzon & Granata (1984) found that larval density of apple clearwing moth in infested trees is directly proportional to the diameter of cankers caused by unidentified fungi. Baggiolini & Antonin (1976) and Bolay et al. (1976) found a close association between *Cytospora* sp. fungal infection levels and apple clearwing moth larval densities on pear trees. It is possible that the apple clearwing moth has a symbiotic relationship with the fungus (Gibson & Hunter 2010) as *Cytospora* increases the survival rate of neonate larvae (Bolay et al. 1976).

Apple clearwing moths preferentially attack certain apple cultivars (Mutsu, Marigold and Stayman) over others (Red Delicious and Granny Smith) (Ciglar & Masten 1977; Dickler 1976) and preliminary observations in the Southern Interior of BC indicate that in organic apple orchards Ambrosia, Gala and Granny Smith varieties sustain heavier infestation levels than Fuji or Sunrise varieties.

In the more northern latitudes, the apple clearwing moth life cycle typically takes two years to complete (Dickler 1976; Injac & Tosevski 1987). Apparently, some larvae feeding in the graft region of apple trees can complete their life cycle in one year, while those feeding in the branches of the same trees usually take two years to finish their development (Dickler 1976).

First and second instar larvae feed superficially within the bark while older instars feed on the vascular tissues between the bark and cambium. Active infestation can be determined by the presence of frass (Figure 1.1, Hay 1968; Solomon 1977). Larval galleries are irregularly shaped but generally perpendicular to the tree axis. The developmental threshold of larvae is unknown, but considered to be 10°C (Judd 2008), below which larvae stop feeding and become quiescent. Feeding normally resumes in early spring but larvae will begin feeding whenever they are brought into the laboratory and placed at spring-like temperatures during winter. Pupation occurs in May in an elongated cocoon constructed from silk, frass and pieces of bark (Figure 1.2). Each abdominal segment of a clearwing moth pupa has a double row of adminticula (Popescu-Gorj et al. 1958) (defined as chitinous spines) used to aid with adult emergence. The terminalia (last abdominal segments), however, have only one row of adminticula, such that male pupae have two single rows of adminticula on their terminalia while female pupae have three single rows of adminticula on their terminalia (Figure 1.3). Another reliable characteristic that can be used to separate pupae by sex is the location of the genital pores (segment 8 in females and segment 9 in males) (Popescu-Gorj et al. 1958).

The first adults appear in late May or early June, depending on the accumulation of degree days. According to Judd (2008), 50% emergence occurs at 300 degree-days after the first male is captured in a pheromone trap (Biofix) baited with a 1 mg standard monitoring lure. Adult apple clearwing moths (Figure 1.4) are long-lived and produce offspring over an extended period (Kutinkova et al. 2006). Males respond to synthetic pheromone lures from morning until dusk (Pühringer 2009). Temperature directly correlates with male flight activity and below 10° C moths stop flying (Buleza et al. 1990). The major component of the female pheromone is (*Z*,*Z*) 3,13 octadecadienyl acetate (Judd et al. 2011). Minor components do exist in the female effluvium but they did not increase trap catches of conspecific males (Judd et al. 2011) These compounds may be important in the inhibition of heterospecifics as occurs in other clearwing species (Mozuraitis et al. 2006). Preliminary observations of postmating behaviour indicate that apple clearwing moth females will move up and down a tree prior to oviposition of 1-2 eggs at a time in bark cracks, pruning wounds or burr knots of host apple trees. Eggs are small (~130 μm in length), ovoid, brownish in colouration and covered with numerous hexagonal units making up the chorion (Figure 1.5).

In Europe, both male and female apple clearwing moths are strongly attracted to various flowers such as black elderberry (*Sambucus nigra* Linnaeus) (Injac & Tosevski 1987) and danewort (*S. ebulus* Linnaeus) (Popescu-Gorj et al. 1958). Personal observations indicate that in BC both sexes frequent a wide variety of flowers, such as showy milkweed (*Asclepias speciosa* Torrey), white sweet clover (*Melilotus albus* Medikus), wild peas (*Pisum* sp.), ornamental lilies (*Lilium* sp.) and wild mustard (*Sisymbrium* sp.). In apple orchards with low floral diversity, apple clearwing moths feed on alternative food sources such as aphid honey dew (Figure 1.6). In addition to floral nectar and aphid honeydew, apple clearwing moth adults are also attracted to various combinations of apple or pear juices (Blaser & Charmillot 1984; Kilic et al. 1988) and to grape juice (Judd 2008). Van Frankenhuyzen & Wijnen (1979) used a combination of molasses and red wine to attract adults. A bait solution consisting of molasses, 10% acetic acid or pear juice and 20% geraniol is highly attractive to both sexes (Ademard & Monnet 1984). Combinations of acetic acid and pear ester are attractive to both apple clearwing and codling moths (*Cydia pomonella* (Linnaeus)), another important pest in apple orchards (Tóth et al. 2010). According to Judd (2008), male and female apple clearwing moths are strongly and preferentially attracted to fresh grape juice over orange juice, apple juice and a sugar water control. Visual cues also appear to be important since yellow pheromone-baited traps had higher trap catches when compared to blue, black and red painted traps in BC populations (Judd 2008). Feeding attractants can be successfully employed to determine local phenologies of both sexes of apple clearwing moths and acetic acid or esters maximize trap capture (Orban et al. 2009).

1.3. Pheromone-based management techniques

Isolation and identification of moth sex pheromones allows for pheromone-based tools to be adopted for Integrated Pest Management (IPM). Pheromone-based management tactics include the use of synthetic copies of pheromones for monitoring pest populations and for direct control through tactics such as mating disruption, mass trapping and attract-and-kill.

Pheromone-based monitoring is a widely used tool in many Integrated Pest Management (IPM) programmes in various cropping systems (Witzgall et al. 2010). Sex pheromones can be used to bait traps to detect the presence of a pest species (Barak & Harein 1982; Kehat & Dunkelblum 1993; Witzgall et al. 2010) or be employed to determine the phenology of pest species in a cropping area (Biever & Hostetter 1989; Harris et al. 1997; Gibb et al. 2005) in order to time control measures (Glen & Brain 1982; Reddy & Guerrero 2001). Pheromone-based monitoring can also be developed as a predictive tool to associate pheromone-trap capture with immature stages of the insect or damage caused to the crop (McBrien et al. 1994; Evenden et al. 1995; Damos & Savopoulou-Soultani 2010) and contribute to the development of an economic threshold (Bechinski et al. 1989; Knight & Light 2005a; Mudavanhu et al. 2011).

Synthetic sex pheromones can also be used in integrated pest management to directly control pest populations (El-Sayed et al. 2006; Witzgall et al. 2010). There are three major forms of pheromone-based control: mating disruption, attract-and-kill and mass trapping. Mating disruption is the most widely used pheromone-based control tactic and the primary option available to organic growers in North American agricultural settings. Mating disruption works through the release of large amounts of synthetic sex pheromone from multiple sources into the atmosphere (Cardé & Minks 1995; Miller et al. 2006a). Male moths can be affected by this treatment through a variety of mechanisms such as neurophysiological effects on the peripheral or central nervous system, camouflage of the female's pheromone plume and false-trail-following (Bartell 1982; Cardé & Minks 1995). Neurophysiological effects occur as a result of adaptation of male antennal receptor neurons or habituation of neurons in the central nervous system to pheromone, resulting in the male becoming unresponsive to the female's signal (Yamanaka et al. 2003; Judd et al. 2005). Camouflage of the female's plume can occur if the synthetic pheromone masks the pheromone plumes produced by calling females (Byers 2007). False-trail-following occurs if the synthetic pheromone plumes competitively attract males so that they waste time and energy responding to synthetic pheromone sources instead of searching for calling females (Miller et al. 2006a, 2006b). The intended consequence of a mating-disruption treatment is that females remain unmated or experience a delay in mating in the cropping area and their subsequent fecundity is reduced (Suckling et al. 2005). The mating-disruption tactic works best for isolated pest populations at low densities (Judd et al. 2005) and is negatively affected by hilly terrain (Trematerra 1993). Also, mating disruption may be best suited to pests with one to several generations / growing season. For insects with biennial life cycles (like the apple clearwing moth) mating disruption has to be applied consecutively for at least two years to control the entire population. This tactic is also affected by crop structure and environmental factors such as wind (Cardé & Minks 1995; Teixeira et al. 2010). Season-long control by mating disruption depends on the longevity and stability of the pheromone formulation.

Attract-and-kill works by attracting insects with an attractant and killing the attracted insects with a killing agent (Mitchell 2002; Evenden et al. 2005). The commercially developed attract-and-kill formulations are a combination of sex pheromones and pyrethroid insecticides (Krupke et al. 2002; Evenden & McLaughlin 2005). Attract-and-kill requires less pheromone and less insecticide compared to mating disruption and insecticide treatments, respectively. The insecticide can also be mixed with host-plant volatiles (kairomones) so both sexes (Camelo et al. 2007) or even immatures (Martel et al. 2007) can be attracted and removed from the population, thereby maximizing its efficiency at controlling pest populations. The biggest disadvantage of attract-and-kill is that the insect must be attracted to the source in order to be exposed to the killing agent. In general, the effectiveness of attract-and-kill is related to the insecticide exposure time, which in turn is directly proportional to the attractiveness of the semiochemicals used in the formulation (Evenden & McLaughlin 2004). Therefore the full pheromone blend or pheromone plus host kairomones must be used in the formulation and this blend may be difficult to maintain under field conditions for long periods (McDonough et al. 1992; Vargas et al. 2005). This management tactic is often not an option for organic growers, because synthetic insecticides cannot be used in organic orchards or fields (Pimentel et al. 2005).

Mass trapping controls pest populations through removal in semiochemical-baited traps positioned throughout the cropping area (El-Sayed et al. 2006). Mass trapping is compatible with IPM in organic orchards. According to Yamanaka et al. (2003) mass trapping can be expensive in some cases due to the initial purchase of many durable traps. However, such traps can be used over multiple years without replacement and high-yield bucket traps, such as Unitraps^{TM} do not require constant maintenance (Voerman & Van Deventer 1984). Several other capturing devices can be utilized in addition to the high capacity traps. Delta traps baited with sex pheromones and sticky bottoms have been successfully employed in mass trapping of various pests (Madsen & Carty 1979; Nassef et al. 1999). Mass trapping with pheromone-baited oil traps showed promise in the suppression of the pink bollworm (*Pectinophora gossypiella* (Saunders)) populations in cotton plantations (Mafra-Neto & Habid 1996). Water traps are useful in mass trapping pests of stored products (Bacon et al. 1976; Ryne et al. 2002). Mass trapping of flies is achieved with a light coloured flat sheet baited with food attractants and an insecticide as in the case of fruit flies (Broumas et al. 2002) or spherical, dark red spheres as in the case of apple maggot flies (*Rhagoletis pomonella* (Walsh)) (Drummond et al. 1984). Whiteflies (Hemiptera: Aleyrodidae) can be mass trapped with yellow sticky cards (Yano 1987; Gu et al. 2008). Lindgren funnel traps baited with aggregation pheromones or food-derived volatiles are highly attractive to various economically important beetle species (Peng & Williams 1991) and have been used to mass trap ambrosia bark beetles in dryland sorting areas (Lindgren & Fraser 1994). Trap-logs are widely used in the mass trapping of forest pests such as bark beetles (Raty et al. 1995; Faccoli & Stergulc 2008).

An effective mass trapping tactic is dependent on trap and bait parameters (Broumas et al. 2002). Trap parameters include trap density, saturation point (capacity) and trap colour. Bait parameters include bait attractiveness and dosage.

Trap density is by far the most important trapping parameter. A high trap density may not necessarily translate into high percentage of damage reduction (Jamieson et al. 2008). For example, damage caused by the spruce bark beetle (*Ips typographus* (Linnaeus)) was reduced by at least 82% between mass trapping-treated and untreated infested forest stands, regardless of the trap density tested (Faccoli &Stergulc 2008). In some cases higher trap densities remove higher numbers of adults from population. Trematerra (1993) found that 6 to 12 traps / hectare, each baited with 10 mg of sex attractant targeting the apple clearwing moth removed the highest numbers of males, although the author did not quantify the overall tree damage reduction. High trap densities (one trap every two trees) targeting the Chinese tortrix (*Cydia trasias* (Meyrick)) resulted in a 72% reduction in next generation larvae and 65% to 92% reduction in leaf petiole damage (Zhang et al. 2002). However, the authors used a suboptimal pheromone blend and did not test the effectiveness of mass trapping at other trap densities. Yongmo et al. (2005) reported that 25 traps / hectare, each baited with 1.5 mg sex attractant reduced the number of next generation tea tussock (*Euproctis pseudoconspersa* (Stand)) larvae up to 51%. According to Byers (2007) a small number of traps with high pheromone doses can be as effective at removing males as many traps with low pheromone doses as long as the sex pheromone used is as attractive as conspecific calling females.

A second trap parameter important in mass trapping is trap saturation point. Saturation point, or trap capacity is an important factor in limiting trap captures of high pest densities (Elkinton 1987; Harris et al. 1996). Saturation point can be expressed in terms of depth or surface area. In the case of moths, high capacity funnel traps are preferred to low saturation sticky traps (Voerman & Van Deventer 1984; Trematerra 1993). In some cases, a less expensive alternative to funnel traps are the sex pheromone-baited water traps (Ryne et al. 2002). Freshly cut tree logs sprayed with insecticides and baited with blends of aggregation pheromones and host volatiles is an inexpensive, yet efficient way to control bark beetle populations (Raty et al. 1995; Faccoli & Stergulc 2008) because they provide a larger trapping area than commercial traps. Funnel traps with a 32 cm trapping diameter captured significantly more tea tussock (*Euproctis pseudoconspersa* (Strand)) males than traps with a 20 cm trapping diameter (Yongmo et al. 2005).

A third trap parameter is spectral reflectance. Vision is an important characteristic of orienting behaviour in diurnal insects. Green and yellow Delta traps captured significantly more currant borer males than other colours (Suckling et al. 2005). Interestingly, currant borers can perceive UV light (350 nm wavelength), but only wavelengths in the visible spectrum (wavelength of about 500 nm) increase attraction to pheromone sources (Karalius & Būda 2007). White-coloured funnel traps captured significantly more jasmine moth (*Palpita unionalis* (Hübner)) males than brown traps, but were only marginally better than yellow or green funnel traps (Athanassiou et al. 2004). According to Judd (2008), spectral reflectance of the funnel is particularly important in the attraction of apple clearwing males to pheromone-baited Unitraps™. Red bucket traps baited with aggregation pheromone of the palm weevil (*Rhynchophorus ferrugineus* Olivier) attracted significantly more adults than any other coloured traps (Al-Saoud et al. 2010). Yellow water traps captured more pollen beetles (*Astylus atromaculatus* Blanchard) than white or blue water traps, although the difference was only marginally significant (Van den Berg et al. 2008). Mexican fruit flies (*Anastrepha ludens* (Loew)) are attracted to yellow traps reflecting wavelengths in the 500 – 580 nm range (Robacker et al. 1990). Bark beetles showed greater attraction to dark-coloured than to light-coloured traps baited with aggregation pheromone (Strom et al. 2001; Strom & Goyer 2001), indicating that contrast between trap reflectance and background reflectance may be more important than the specific wavelengths of the trap themselves (Allan & Stoffolano 1986).

One of the most important variables that affect the behaviour and subsequent capture of target pests is bait attractiveness. For example, Fu et al. (2002) found that male Chinese tortrix moths (*Cydia trasias* (Meyrick)) respond optimally by flying and alighting near the pheromone source only when a ternary pheromone blend was presented to them. There was no significant difference in the number of males attracted to the ternary blend and calling females. Zhang et al. (2007) re-analyzed the male-produced pheromone of the spruce bark engraver (*Ips duplicatus* (Sahlberg)) and determined that the addition of a newly characterized, minor, male-produced aggregation pheromone compound resulted in significantly more beetles captured in traps baited with the ternary blend than in traps baited with binary blends. A five-component pheromone blend was as attractive to codling moth (*Cydia pomonella* (Linnaeus)) males as female gland extracts (El-Sayed et al. 1999). In some cases, however, addition of minor components does not increase trap attractiveness (Judd et al. 2011).

Attraction to pheromone sources can be further enhanced by adding host plant volatiles. The addition of α-pinene, a known pine bark volatile, to the binary pheromone blend of the southern pine beetle (*Dendroctonus frontalis* Zimmermann) resulted in higher mean trap catches of both the southern pine beetle and western pine beetle (*D. brevicornis* LeConte) (Hofstetter et al. 2008). A similar trend was observed by the addition of pear ester to the codling moth pheromone (Knight et al. 2005). A control strategy that incorporates a feeding attractant is appealing for the management of actively feeding pests because the active removal of females may result in a more efficient control of pest populations when compared to traditional sex pheromone-based mass trapping (Reddy et al. 2006; Stringer et al. 2008). Volatile feeding attractants and especially esters (El-Sayed et al. 2005) are attractive to both male and female moths (Light et al. 2001; Judd 2008; Knight 2010; Tóth et al. 2010). Noctuids and pyralids are particularly responsive to floral volatiles (Guédot et al. 2008) or fermented fruit (El-Sayed et al. 2005). Traps baited with phenylacetaldehyde and benzaldehyde, two commonly encountered floral volatiles, capture large numbers of male and female alfalfa loopers (*Autographa californica* (Speyer)) (Landolt et al. 2001) and soybean looper (*Thysanoplusia orichalcea* (Fabricius)) moths (Stringer et al. 2008).

A second bait parameter important in mass trapping is bait dose. In some species, the number of adults captured is directly proportional to bait loading (Fisher et al. 1985; Branco et al. 2006). Odour plumes emanating from high dose lures travel farther downwind and consequently have a higher range of attraction than those from low dose lures (Schlyter 1992; Dodds & Ross 2002; Branco et al. 2006). The more insects removed, the better the control (Byers 2007). However, there can be an upper threshold of maximal responsiveness to semiochemical cues, beyond which the numbers of insects orienting to the source will decline. For example, the response of oriental fruit moths (*Grapholita molesta* (Busck)) to sex pheromone-baited traps is curvilinear (Baker et al. 1981). Once an upper pheromone concentration threshold is reached males stop responding and aggregate around traps (Baker & Roelofs 1981; Yamanaka et al. 2003). The upper trap catch threshold varies with the insect species targeted. This maximal response is set by physiological constraints, such as saturation of the pheromone receptors or deactivation enzymes with pheromone molecules (Rospars et al. 2007; Prestwich et al. 1989). For example, the highest trap captures of oriental fruit moths occur at intermediate pheromone doses tested (Evenden & McLaughlin 2004). Suckling et al. (2005) baited Delta sticky traps with various pheromone concentrations targeting currant borer adults (*Synanthedon tipuliformis* (Clerck)) and determined that the highest trap catch occurred when lures were loaded with 10 mg of the synthetic pheromone blend. A similar dose-dependent response was noted for apple clearwing moth males (Judd 2008). Codling moth males respond maximally to traps when lures are loaded with 0.1 to 1 mg sex pheromones (Kehat et al. 1994) and when lures are loaded with 10 to 50 mg kairomones (Knight & Light 2005b).

Trap and bait parameters interact with one another and give rise to further variables, the most important of which is trapping interference. Trapping interference occurs when the odour plumes from one trap interact with those of an adjacent trap and prevent orientation to the trap (Wall & Perry 1978; McMahon et al. 2010). Similar to the mechanism of false-trail-following in pheromone-based mating disruption, trapping interference can result in localized aggregation of males around traps (Yamanaka et al. 2003). Studies of trapping interference are uncommon in the scientific literature but they can provide useful information on bait characteristics such as the range of attraction for various insect pests (Schlyter 1992). Trapping interference can be kept to a minimum as long as adjacent traps are separated by at least two attractive radii (Dodds & Ross 2002). The attractive radius (or range of attraction) is the maximum distance over which an insect shows a directed response to an attractive source such as a calling female or semiochemical-baited trap (Wall & Perry 1987).

Polygyny and initial population density also affect the efficacy of mass trapping. Polygyny, or multiple mating in males, appears widespread in moths, although the full extent of this reproductive strategy has not been reviewed yet. Since a male can fertilize multiple females over 90% of male moths (Knipling & McGuire 1966) or 80% of both male and female bark beetles (Fahse & Heurich 2011) must be removed from the population in order to keep populations under control. High pest densities negatively affect the effectiveness of mass trapping (El-Sayed et al. 2006). For example, mass-trapping experiments done on high densities of *Euproctis pseudoconspersa* (Strand), a Chinese tussock moth, resulted in a pest density reduction of only 51% in the first year (Yongmo et al. 2005). At high population densities the chance encounter between receptive males and females increases and can render semiochemical-based strategies such as mass-trapping ineffective at controlling populations (Jones 1998). Competition between traps and high densities of calling females can significantly decrease male trap captures and can therefore, render large scale mass trapping of various pests ineffective at controlling populations (Croft et al. 1986; Unnithan & Saxtena 1991). Nevertheless, in such cases mass trapping can still prevent an impending outbreak as long as traps remove 50% of the adult population (Weslien 1992). According to Carvalho & Mexia (2003) mass trapping efficacy improves as cigarette beetle (*Lasioderma serricorne* (Fabricius)) density declines following a density-dependent hyperbolic curve.

In a theoretical study on the efficiency of mass trapping and mating disruption, Yamanaka (2007) suggested that both management options can achieve the same level of pest density reduction. Field studies on the effect of attract-and-kill formulations on the control of light brown apple moth (*Epiphyas* *postvittana* (Walker)) indicated that active removal of males from the population accounts for only 50% of the reduction seen in assessment traps (Suckling & Brockerhoff 1999). The other 50% of the reduction in trap catch in assessment traps is a consequence of point source competition (Charmillot et al. 1996). If formulations use suboptimal pheromone doses, calling females may outcompete the point sources and render semiochemical-based tactics ineffective at controlling populations (Evenden & McLaughlin 2004). Thus, at high pest densities active removal of adults through mass trapping or attract and kill may be more advantageous than mating disruption because a lower percentage of available males can result in a lower percentage of matings (Byers 2007) and consequently, in a higher percentage of population suppression.

Another important, albeit relatively unexplored aspect of a mass trapping tactic is the attractiveness of the individual mass trapping traps to non-target arthropods. Both pheromone- and kairomone-baited traps used in the management of various pest species are also attractive to a wide array of non-target arthropods, including pollinators, predators and parasitoids. For example, bumblebees (*Bombus* sp.) and honeybees (*Apis mellifera* Linnaeus) (Apidae) are a common by-catch in yellow-coloured funnel traps (Herman et al. 1994; Weber et al. 2005). In general kairomone-baited traps attract non-target arthropods due to their complex chemical profiles. Traps baited with methyl eugenol, a known kairomone of the Oriental fruit fly (*Bactrocera dorsalis* (Hendel)), or a mixture of methyl eugenol and decaying Oriental fruit flies attracted a large number of non-target species including 187 species endemic to the study area (Leblanc et al. 2009).

Most specimens recovered belonged to Diptera and Lepidoptera. Mass trapping systems should incorporate trap (or bait) modifications in order to minimize trap attractiveness to non-target groups.

There are many more articles published on mating disruption than on mass trapping (El-Sayed et al. 2006; Yamanka 2007), leading to the logical conclusion that the amount of research effort put into the development of mass trapping lags behind research into mating disruption. Although research on mass trapping is limited to date, the results are promising (Reddy & Urs 1997; Broumas et al. 2002; Yongmo et al. 2005; Jamieson et al. 2008) and its potential to control high pest densities in North America deserves further consideration and research.

1.4. Pheromone-based management strategies for control of the apple clearwing moth

Before the present work, several researchers attempted to control the apple clearwing moth with semiochemical-based tactics of mating disruption and mass trapping. The efficiency of the two control tactics was variable, most likely due to a lack of replication, improper statistical analysis, low pheromone doses or trap densities tested and variable pest densities examined.

Mating disruption of the apple clearwing was effective at controlling moth populations in small, isolated apple orchards in Greece (Kyparissoudas & Tsourgianni 1993). In this setting, captures in assessment traps were reduced by

91% following a 3-year control effort (Kyparissoudas & Tsourgianni 1993). Mating-disruption treatment resulted in 81% reduction in larval infestations in apple orchards in Germany (Stüber & Dickler 1987). After a three-year mating disruption campaign in BC, apple clearwing moth populations continue to be high enough to warrant research on an alternative means of control that is also compatible with organic practices (Judd 2008).

Previous studies on mass trapping of apple clearwing moth relied on traps baited with the major pheromone component (*Z*,*Z*)-3,13-octadecadienyl acetate (Judd et al. 2011), a highly attractive compound to many species of clearwing moths (Voerman et al. 1978; Taft & Snow 1991; Aurelian, V. M. unpublished). In a non-replicated study mass trapping with 12 pheromone-baited, high-capacity traps / ha provided a maximum catch while minimizing trap interference (Trematerra 1993). In another non-replicated study on apple clearwing moths, Önuçar & Ulu (1999) found 57% and 69% reduction in pupal and larval densities, respectively in plots treated with pheromone-baited mass trapping traps applied at a density of one trap / tree as compared to non-treated control plots. Bosch et al. (2001) conducted a three-year mass trapping study using traps baited with low pheromone doses (1 mg) in two apple orchards with high (trapping density of 5 traps / ha) and low (trapping density of 9 traps / ha) infestations of apple clearwing moth. Trapping efficiency was calculated between 52% and 94%, depending on the severity of infestation (Bosch et al. 2001).

1.5. Thesis objectives

In order to design an effective mass trapping strategy for the apple clearwing moth in British Columbia, I will examine the following four objectives:

(1) Determine the trap density (expressed as traps / ha) of pheromone and kairomone-baited traps required to significantly interfere with orientation of apple clearwing moths to assessment traps and result in moth removal from the population;

(2) Determine whether kairomone or pheromone mass traps alone or together interfere with orientation of adults to either of the two volatile cues;

(3) Determine whether active removal of males in mass-trapping traps contributes to a reduction in the response of apple clearwing moths to assessment traps and is therefore an important mechanism of action in pheromone-based mass-trapping programs; and

(4) Determine whether the apple clearwing moth traps capture a significant amount of non-target arthropod by-catch in organic and conventionally managed apple orchards and whether the by-catch can be used to examine arthropod communities in organic vs conventional apple orchards.

1.6. Literature cited

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Figure 1.1. Damage to apple trees due to feeding of apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) larvae in the burr-knot area. Reddish-brown fecal pellets (frass) are diagnostic of active infestations.

Figure 1.2. Apple clearwing moth cocoon attached to the underside of apple tree bark. Compared to other clearwing species the cocoon is thin and cannot be easily removed from its substrate.

Figure 1.3. Apple clearwing moth pupae. The black arrows indicate the single rows of adminticula on the terminalia. (A) Male pupae have two single rows of adminticula on their terminalia. (B) Female pupae have three single rows of adminticula on their terminalia.


A
B

Figure 1.4. Apple clearwing moth adults *in copula*. The male (lower right) can be distinguished from the female (upper left) by the white abdominal venter, narrow abdomen and large, fan-shaped hair pencils at the end of abdomen.

Figure 1.5. False-coloured Scanning Electron Micrograph of an apple clearwing moth egg.

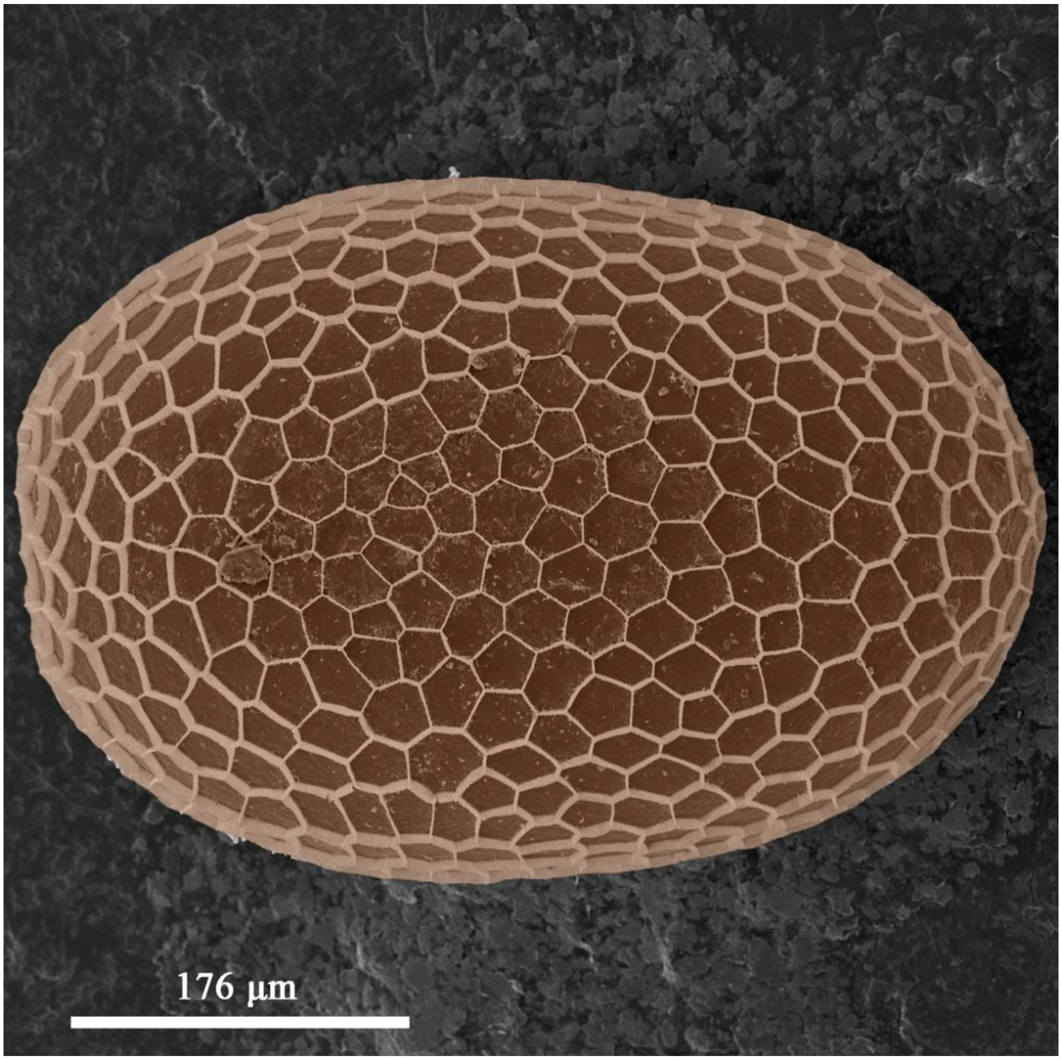
176 µm

Figure 1.6. Apple clearwing moth adults feeding on rosy apple aphid (*Dysaphis plantaginea* (Passerini))-secreted honeydew in a flower-deprived, conventionally managed apple orchard in the Southern Interior of BC.

Chapter 2: Development of semiochemical-based mass trapping of the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) (Lepidoptera: Sesiidae) in apple orchards in the Southern Interior of BC, Canada

2.1. Introduction

Mass trapping is one of the oldest pest control tactics. Classical mass trapping relies on semiochemical-baited traps to attract and remove insects from a pest population and is used to control a large number of insect pests in a variety of agricultural crops (Wong et al. 1972; Reddy & Urs 1997; Leskey et al. 2009; Trematerra & Gentile 2010) and forest systems (McLean & Borden 1979; Bakke & Lie 1989; Weslien 1992; Schlyter et al. 2001). The success of mass trapping is variable, likely due to an insufficient understanding of the parameters affecting trapping success (El-Sayed et al. 2006). These parameters include, but are not limited to, 1) the attractiveness of the lure in relation to other cues in the agroecosystem (Unnithan & Saxena 1991; Sauer & Karg 1998); 2) polygyny and population heterogeneity (Knight 2007); 3) trapping efficiency determined by the attractive radius of the traps and the trapping density (Byers et al. 1989; Schlyter 1992; Branco et al. 2006) and 4) spectral reflectance of traps to trap diurnal insects that use visual cues (Vernon & Gillespie 1990; Van den Berg et al. 2008; Al-Saoud et al. 2010).

Theoretical modelling of sex pheromone-based mass trapping suggests that over 90% of males in a population must be removed in order to reduce pest density below the established economic threshold (Knipling & McGuire 1966). Such a high trap capture is required because removal of only males from a population may not adequately control polygynous species in which males mate with multiple females. One way to circumvent this issue would be to use traps baited with lures that are attractive to both sexes. For example, trapping 50% of a bark beetle population in the early stages of outbreak is enough to prevent outbreak because both sexes are captured in traps (Weslien 1992). In many moth species actively-feeding adults can be successfully trapped with floral or food volatiles (Light et al. 2001; Landolt & Higbee 2002; Tóth et al. 2010). Removal of females can reduce the numbers of eggs laid and thereby directly affect the density of offspring (Camelo et al. 2007).

In Europe the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) is an important pest of commercial apple trees (*Malus domestica* Borhkausen) (Dickler 1976; Blaser & Charmillot 1984; Al-Antary et al. 2004) and pear trees (*Pyrus* sp.) (Baggiolini & Antonin 1975). Apple clearwing moths can also infest hawthorn (*Crataegus* sp.) and mountain ashes (*Sorbus* sp.), albeit to a lesser degree (Spatenska et al. 1999). The apple clearwing moth was recently discovered in North America infesting apple rootstocks in the Southern Interior of British Columbia (BC) and London, Ontario Canada (Philip 2006) and Washington State (LaGasa et al. 2009). Larval feeding under bark can result in the loss of tree vigour and fruit yield (Dickler 1976; Castellari 1987). In temperate regions, larvae overwinter in different stages of development, resulting in a biennial life cycle (Dickler 1976; Castellari 1987). Pupation occurs in early- to mid-May and eclosion of adults begins in late-May to early-June in the Southern Interior of BC (Judd 2008).

Semiochemical-based tools have potential in the management of the apple clearwing moth (Stüber & Dickler 1988; Kyparissoudas & Tsourgianni 1993; Trematerra 1993; Bosch et al. 2001). The compound (*Z*,*Z*)-3,13 octadecadienyl acetate ((*Z*,*Z*) 18:Ac) attracts male apple clearwing moths (Voerman et al. 1978; Buleza et al. 1990) and has recently been identified as the female-produced sex pheromone (Judd et al. 2011). Adult apple clearwing moths frequent flowers (Popescu-Gorj et al. 1958; Injac & Tosevski 1987; also personal observations) and are attracted to a wide range of food baits, ranging from mixtures of apple or pear juices (Audemard & Monnet 1984; Blaser & Charmillot 1984), molasses and red wine (Van Frankenhuyzen 1978) to pear ester (Tóth et al. 2010), boiled grape juice (Ozkan et al. 1986) and fresh grape juice (Judd 2008).

Pheromone-based control of apple clearwing moth has been tested in several studies. In Europe mating disruption was successfully employed to control apple clearwing populations (Stüber & Dickler 1988; Kyparissoudas & Tsourgianni 1993) but preliminary trials in BC have been less successful most likely because of high population densities (Judd 2008). Pheromone-based mass trapping has also been studied in Europe to control the apple clearwing moth with various degrees of success (Trematerra 1993; Bosch et al. 2001).

The aim of the present study is to examine some parameters of semiochemical-based mass trapping to assist in its development as a pest management tool to control the apple clearwing moth in BC. According to Judd

(2008) both sexes of apple clearwing moth are highly attracted to fresh grape juice in yellow bottle traps. Here, I test whether the removal of both sexes from the population through capture in kairomone and pheromone-baited traps improves the efficacy of mass trapping with pheromone or kairomone alone. I investigate the impact of trap density and bait type on mass trapping efficacy as measured by trap capture in pheromone and kairomone-baited assessment traps.

2.2. Materials and Methods

Study sites

Apple orchards under conventional management (i.e. synthetic pesticide spray applications) in Cawston, BC (49.15 N & -119.74 W) were chosen as experimental sites to test the effect of semiochemical-baited mass trapping on apple clearwing moth populations. Each orchard accommodated one replicate per mass trapping experiment. Orchards were superspindle-type plantings with an average tree density of 5444 / ha, except for one orchard that was a spindle-type planting with a density of 1202 apple trees / ha.

Semiochemical-baited traps

The sex pheromone-baited traps consisted of yellow Unitraps (AgBio Inc., Westminster, CO, USA) baited with grey halobutyl rubber septa (West Co., Lyonville, PA, USA) impregnated with 1 or 10 mg of the sex pheromone of the apple clearwing moth, (*Z*,*Z*)-3,13 octadecadienyl acetate ((*Z*,*Z*) 18:Ac) (Pherobank, Wageningen, The Netherlands; > 95% isomeric purity) diluted in HPLC grade hexane (Aldrich Chemical Co., Milwaukee, WI, USA). Kairomone-baited traps consisted of 2L pop bottles (Bottle Depot, Summerland, BC) spray painted yellow (Krylon Fusion for Plastic®, Home Hardware, Penticton, BC) and fitted with two large opposing windows (13 cm x 13 cm) cut into them (Figure 2.1). One 473 ml (16 oz) transparent plastic cup (Fabri-Kal® PK16S-C polypropylene deli containers, Canadian Wholesale Club, Penticton, BC) was placed in the bottom of each bottle trap and filled with 300 ml fresh Concord grape juice (SunRype™, Kelowna, BC) as a kairomone bait that both attracted and captured moths (Figure 2.1).

Experimental design

Mass trapping experiments followed a small-plot protocol (Novak & Roelofs 1985; Evenden et al. 1999) with each orchard divided into four 40 x 40 m (0.16 ha) plots positioned at least 20 m from the edge of the orchard with an inter plot distance of 40 m. Interplot distance followed other small plot protocol studies (Evenden et al. 1999). According to Trematerra (1993), trap interference is minimized if adjacent traps baited with apple clearwing moth pheromone (10 mg) are separated by at at least 40 m. For each experiment, trap density treatments were randomly assigned following a Randomized Block Design. Within experimental plots, mass traps were positioned ~ 1.5 m above the ground at varying treatment densities, depending on the experiment (Figure 2.2). Experiments were conducted in pairs (pheromone-baited versus kairomone-baited traps) within the same orchards, thereby providing an indirect means of comparing pheromone-baited mass trapping with kairomone-baited mass trapping at the same density. Mass-trapping treatments were assessed by comparing moth capture in one centrally-located pheromone-baited and two kairomone-baited assessment traps separated by 15 – 20 cm in each replicated plot. Moths captured in kairomone-baited traps were counted semi-weekly (in order to avoid trap saturation) and separated by sex. Moths captured in pheromone-baited traps were counted weekly. Each experiment was replicated four times across space and was one week in length. Traps were cleaned and pheromone lures were replaced after each experiment. Kairomone baits were replaced on a semi-weekly basis.

Mass-trapping experiments

Three pairs of experiments conducted in the summer of 2009 tested the hypothesis that the density of pheromone or kairomone-baited traps will influence the efficacy of mass trapping as assessed by the mean moth capture in assessment traps and total removal of moths in mass traps (Table 2.1). Experiments 1 and 2 compared the efficacy of mass trapping achieved with 2, 4 and 8 traps / plot (13, 25 and 50 traps / ha) early (end of June/early July, 2009) and late (late July/early August, 2009) in the flight season, respectively. Pheromone mass traps in both experiments were baited with 10 mg of (*Z*,*Z*) 18:Ac and kairomone-baited traps contained 300 ml fresh Concord grape juice. Assessment traps were baited with 10 mg and 1 mg of (*Z*,*Z*) 18:Ac in Experiments 1 and 2, respectively. Assessment traps baited with 1 mg of pheromone more closely approximates the calling females than the 10 mg pheromone dose. Kairomone-baited assessment traps were baited with 300 ml fresh grape juice in both experiments.

Experiment 3 tested a greater range of trap densities consisting of 4, 8 and 16 traps / plot (25, 50 and 100 traps / ha) at peak flight in mid to late July, 2009 (Table 2.1). Pheromone- and kairomone-baited mass traps were baited with 10 mg of (*Z*,*Z*) 18:Ac or 300 ml of fresh grape juice, respectively. Pheromone and kairomone assessment traps were baited with 1 mg of (*Z*,*Z*) 18:Ac and 300 ml of fresh grape juice, respectively.

Experiment 4 was conducted in mid-July 2010 (Table 2.1) and tested the hypothesis that a combined mass trapping with both kairomone and pheromone-baited traps would increase the efficacy of mass trapping over use of either lure type alone. This experiment allowed for direct comparison between mass trapping with pheromone or kairomone mass traps. Mass-trapping treatments consisted of 4 pheromone-baited traps / plot (25 traps / ha), 4 kairomone-baited traps / plot (25 / ha) and a combination of 4 pheromone-baited and 4 kairomone-baited traps / plot (a total of 50 traps / ha) as compared to a non-trapped blank control (Figure 2.3). In the treatment in which both pheromone and kairomone-baited traps were used, traps were positioned in pairs separated by 10-15 cm. Pheromone mass traps were baited with 10 mg (*Z*,*Z*) 18:Ac and kairomone-baited traps were baited with

300 ml fresh grape juice. Pheromone and kairomone assessment traps were baited with 1 mg (*Z*,*Z*) 18:Ac and 300 ml grape juice, respectively.

Statistical analyses

For each experiment, Generalized Linear Models were used to determine if the total number of moths / plot captured in mass traps varied with trap density and orchard. The mean number of males captured in assessment traps was assessed using a GLM that contained trap density, assessment trap type, orchard and density*trap type interaction terms as explanatory variables. In a separate model, the number of moths captured in kairomone-baited assessment traps was specified as the dependent variable and moth sex, trap density, orchard and sex*density interaction terms were specified as independent variables. In order to account for over-dispersion of the data, each model was fitted with error terms having a negative binomial distribution. All analyses were conducted using R statistical package (R Development Core Team 2010). P-values for each GLM were generated from resulting Analysis of Deviance tables. Treatment means were compared using Tukey's HSD multiple-comparison test with adjusted P-values ($\alpha = 0.05$). For the GLM models with significant interaction terms individual P-values were extracted from the negative binomial ANCOVA tables.

2.3. Results

Catches in mass-trapping traps

In Experiments 1-3, there was an overall trend toward an increase in the total number of moths captured in mass traps as the trap density increased (Figures 2.4, 2.5 and 2.6). Using trap densities of 2, 4 and 8 traps / plot in Experiments 1 and 2 there was no significant ($P > 0.1$) effect of trapping density on the mean-total number of males removed in the pheromone-baited mass traps (Figures 2.4A and 2.5A). There was a significant ($P < 0.001$) effect of trap density on the total number of moths removed in kairomone-baited mass traps (Figures 2.4B, 2.5B). In Experiment 1, conducted early in the season, the number of moths removed with kairomone-baited mass traps increased with density from 2 to 8 traps / plot and each trap density was significantly different from the others tested (Figure 2.4B). Significantly more ($P < 0.001$) males were recovered from the kairomone-baited mass-trapping traps than females (Figure 2.4B). In Experiment 2 conducted late in the season, the number of male and female moths removed in kairomone-baited mass traps was highest at a density of 8 traps / plot which was significantly more than the number removed in plots with 2 traps / plot but not significantly larger than the number of males removed in plots with 4 traps / plot (Figure 2.5B). Significantly more ($P = 0.003$) females were recovered from the kairomone mass trapping traps than males (Figure 2.5B).

Different results were obtained in Experiment 3 at the higher trap densities of 4, 8 and 16 traps / plot (Figure 2.6). There was a significant ($P < 0.001$) effect of trapping density on the number of males removed in the pheromone-baited mass traps (Figure 2.6A). As per the lower trap densities in Experiments 1 and 2, trap density significantly ($P < 0.001$) affected the number of moths removed in the kairomone-baited mass traps (Figure 2.6B). Similar numbers ($P = 0.35$) of males and females were recovered from the kairomone mass trapping traps (Figure 2.6B).

Catches in assessment traps

In pheromone mass trapping plots, assessment traps positioned at the centre of treated plots captured significantly fewer ($P = 0.01$) males than in the control plots (Figures 2.7A, 2.8A) independent of the specific trapping density treatment. Kairomone- and pheromone-baited assessment traps positioned in plots treated with pheromone mass traps in Experiments 1 and 2 were equally attractive ($P = 0.4$ and $P = 0.6$, respectively) to males (Figures 2.7A, 2.8A). Early in the season (Experiment 1) fewer females ($P < 0.001$) were recovered from the kairomone-baited assessment traps than males (Figure 2.7A). This result was reversed late in the season (Experiment 2), when fewer males ($P = 0.02$) were recovered from the kairomone-baited assessment traps than females (Figure 2.8A).

Moth capture in assessment traps in kairomone mass trapping plots was not significantly different ($P = 0.2$) between control and treated plots for Experiment

1 (Figure 2.7B) but was significantly different (P = 0.02) for Experiment 2 between control and the four and eight traps / plot (Figure 2.8B). The 10 mg pheromone-baited pheromone assessment traps captured significantly (P < 0.001) more males than the 300 ml grape juice-baited kairomone assessment traps early in the season (Figure 2.7B), but a similar number (P = 0.06) of males was recovered from the 1 mg pheromone-baited and 300 ml grape juice-baited assessment traps late in the season (Figure 2.8B). Significantly (P < 0.001) fewer females than males were captured in kairomone assessment traps early in the season, while a similar (P = 0.18) number of both sexes was captured in the late season experiment.

In Experiment 3, when the greatest range of mass-trapping densities was tested, significantly (P < 0.001) fewer males were captured in assessment traps positioned in plots treated with 4, 8 and 16 pheromone-baited traps / plot compared to the control (Figure 2.9A). Male moth captures in pheromone-baited assessment traps approached zero at 8 and 16 traps / plot and were significantly (P < 0.001) lower than captures in kairomone-baited assessment traps in plots treated with 8 and 16 pheromone-baited mass traps / plot (Figure 2.9A). There was no difference in the number of males captured in pheromone- and kairomone-baited assessment traps positioned in plots with 4 pheromone-baited mass traps / plot or in the control (P = 0.14, Figure 2.9A).

Capture of moths in assessment traps in the kairomone mass trapping experiment at the higher trap density examined in Experiment 3 (Figure 2.9B) was markedly different than assessment trap catch in the pheromone mass trapping counterpart (Figure 2.9A). In the kairomone mass trapping plots, there was no significant ($P = 0.1$) difference in the number of moths captured in assessment traps across the three mass trapping densities tested when compared to the control (Figure 2.9B). However, fewer ($P < 0.001$) males were captured in kairomone assessment traps than in pheromone assessment traps (Figure 2.9B). Kairomone-baited assessment traps captured fewer ($P < 0.001$) females than males in kairomone mass trapping plots (Figure 2.9B).

In Experiment 4 significantly more ($P < 0.001$) males were removed from plots treated with pheromone mass traps than kairomone mass traps or a mixture of both trap types despite the fact that there were numerically more traps positioned in the combined lure treatment plots (Figure 2.10A). In the two-lure plots there was no difference ($P = 0.99$) in the total number of males captured in pheromone and kairomone mass traps (Figure 2.10A). Kairomone-baited mass traps in mixed plots captured significantly ($P < 0.001$) more males than plots baited only with kairomone mass traps. Fewer ($P = 0.002$) females were captured in kairomone mass traps in mixed plots than in kairomone alone mass trapping plots.

Assessment traps positioned in plots treated with pheromone, kairomone or both types of mass traps in Experiment 4 captured significantly fewer ($P < 0.001$) males than in the non-treated control (Figure 2.10B). More males were captured in the pheromone-baited assessment traps than in the kairomone-baited assessment traps ($P = 0.007$) in all plots except for mixed mass trap plots, for which the situation was reversed (Figure 2.10B). There was a significant (P <

0.001) interaction between the assessment bait used and mass trapping treatment, such that pheromone-baited assessment traps captured significantly fewer males in mixed mass trapping plots than kairomone-baited assessment traps in control plots (Figure 2.10B). The number of males captured in kairomone assessment traps did not fluctuate significantly in treated plots and control. When both males and females captured in kairomone assessment traps were considered, the effect of mass trapping treatment was not significant ($P = 0.07$) (Figure 2.10B). Across all mass trapping treatments tested, fewer females than males were captured in kairomone assessment traps.

The sex ratio in catches of apple clearwing moths was dynamic and changed as the season progressed. Catches were male-biased early in the season, approached 50:50 close to peak flight and became female-biased late in the season (Figure 2.11).

2.4. Discussion

In the present study I found that when sex pheromones and kairomones were offered together more males responded to the kairomone mass traps and fewer males responded to pheromone mass traps when compared to either stimulus alone. One possible explanation is that the kairomone and pheromone plumes interact with one another and enhance the attractiveness of kairomone traps. Similarly, kairomone-baited codling moth traps captured more males when males were pre-exposed to codling moth pheromone, but no significant increases in pheromone trap catch were observed when males were pre-exposed to kairomone (Yang et al. 2005). Ochieng et al. (2002) found that there is a synergistic interaction between host volatiles and sex pheromone of corn earworm (*Helicoverpa zea* Boddie). When the stimuli are presented simultaneously (i.e. at the same time) there is an increased firing rate by the pheromone-tuned olfactory receptor neurons that arborize in the male-specific macroglomerular complex in addition to action potentials triggered by other olfactory receptor neurons that arborize in ordinary glomeruli (Ochieng et al. 2002). The exact mechanism behind the enhanced response to sex pheromone in the presence of plant volatiles is not well understood, although low doses of host volatiles appear to behave in a manner similar to minor pheromone components by optimizing the firing rate of pheromone-tuned olfactory receptor neurons (Bäckman et al. 2000). The presence of host volatiles can also activate (or inhibit) the interneurons that link the pheromone-specific glomeruli to the ordinary glomeruli in the male brain in a combinatorial manner (Trona et al. 2010). Furthermore, one non-pheromonal component can stimulate multiple ordinary glomeruli and one ordinary glomerulus can respond to several non-pheromonal components (Carlsson et al. 2002). This plasticity in response to host or food volatiles may indicate an adaptive response to novel volatile emission sources. It is also possible that more males were attracted to the two stimuli together and more orienting insects would be captured in the moderately attractive trap but far more efficient kairomone trap than when the two traps are widely separated (McMahon et al. 2010).

Females show markedly different responses than males in response to the combined stimuli. Fewer females are captured in kairomone traps if these traps are placed next to pheromone traps. Currently, it is not known if apple clearwing moth females detect their own pheromone, but female response to sex pheromone has been reported in other moth species. Female grape root borers (*Vitacea polistiformis* (Harris)) are repelled by their own pheromone, move extensively in pheromone-based mating disruption treated plots (Pearson & Meyer 1996), and will even move away from habitat permeated with sex pheromone (Pearson & Schal 1999) in order to decrease intraspecific competition and maximize reproductive success (Pearson et al. 2004). Exposure to pheromone results in a cessation of calling behaviour of beet armyworm (*Spodoptera exigua* (Hübner)) females (Yang et al. 2009).

Trapping interference can generally be avoided if traps are separated by at least two attractive radii (Dodds & Ross 2002). It follows then that at the density at which total moth catches do not significantly increase further, an approximate range of attraction can be calculated as being half of the shortest intertrap distance. In general, the range of attraction depends on the pheromone dose, species being targeted and environmental conditions, such as wind movement and ambient temperature. For example, 1 mg codling moth (*Cydia pomonella* (Linnaeus)) lures have a range of attraction of 10 m (Grieshop et al. 2010), while 6 mg fall webworm (*Hyphatria cunea* (Drury)) lures captured over 90% of the released males within 30 m (Zhang & Schlyter 1996). In Experiments 1 and 3, the range of attraction for the 10 mg apple clearwing moth pheromone lures is at least 20 m. In a similar study on apple clearwing moth mass trapping in Europe, the lowest trapping interference occurred when the intertrap distance was 40 m (Trematerra 1993). Interestingly, in Experiment 3 done at peak flight the range of attraction appears to be less than 5 m. This observation is likely an artefact of an increased trap catch probability for a given time period at higher moth densities, since the range of attraction depends on the release rate of semiochemical and the physiological sensitivity of the insect and not on the population density. Therefore, at the onset and end of moth flight a few high capacity traps (13 to 25 traps / ha) baited with high doses (10 mg) of sex pheromone need to be deployed whereas at peak flight when moth densities are particularly high 100 traps / ha should be deployed in order to optimize mass trapping efficacy. It follows that up to 100 pheromone-baited traps / ha should be deployed for the entire duration of the moth season. Similar mass trapping studies on the apple clearwing moth concluded that an average trapping density of 12 traps / ha, each baited with 10 mg pheromone is effective at controlling populations (Trematerra 1993). In this case however, traps were placed higher in the tree canopy, about 2.5 m above ground and moth densities present in treated plots were likely lower than those present in the Southern Interior of BC. Although I did not measure damage reduction in my experiments, Bosh et al. (2001) reported a 44% decrease in the number of apple clearwing moth pupal exuviae after two years of mass trapping at a low trap density with a low pheromone dose that presumably minimized trap interference.

A high trapping density of at least 100 traps / hectare ought to be used if mass traps are baited with kairomone lures as my data show that trap interference for kairomone-baited traps is low. Although kairomones have a higher volatility than clearwing sex pheromones due in part to lower molecular weight (Butler & McDonough 1979), the attractive radius of the trap appears to be consistently lower than sex pheromone in this system. A lack of trap interference between any of the kairomone-baited mass trap densities tested indicates that the range of attraction for the 300 ml grape juice to apple clearwing moths is less than 5 m. Towards the end of flight it appears that trapping interference is present in kairomone mass trapping at densities of 50 traps / ha. However, this may not be a true trapping interference but rather an artefact of decreased kairomonal response of male moths close to the end of the flight, because at the beginning of the flight, when adult moth density is also low, no such trapping interference is detected. There is little information on the range of attraction to kairomone lures but in general kairomones have a short range of activity. Zhang & Schlyter (2003) showed that the range of inhibition of traps baited with volatiles extracted from non-host trees, detected by *Ips typographus* adults, is around 2 m.

The advantage of trapping females in kairomone-baited traps would likely offset the smaller range of attraction of kairomone lures as compared to pheromone-based mass traps. Although food-based kairomones released from both flowers (Haynes et al. 1991; Landolt et al. 2001; Stringer et al. 2008) and fruit (Reddy et al. 2006; Landolt & Guédot 2008) are attractive to males and females of several moth species, there have been no studies to date on kairomone-based mass trapping of moth pests. There is one study on the effect of kairomone-based attract-and-kill on adult alfalfa looper (*Autographa californica* (Speyer)) populations (Camelo et al. 2007). Feeding stations were baited with an attractive mixture of phenylacetaldehyde and β-myrcene and coated with 7% permethrin as a killing agent. The removal of both males and females significantly lowered population densities as measured by kairomone and pheromone monitoring traps by at least 80% compared to untreated plots, especially in areas where food sources were scarce.

A second advantage of female removal in mass trapping programs is that traps do not need to be as efficient as when only males are removed from the population. For example, mass traps baited with aggregation pheromones targeting both male and female *Ips typographus* (Linnaeus) bark beetles need to remove only 50% of the beetle population in order to prevent outbreaks (Weslien 1992). Since a female can lay hundreds of eggs over its entire lifespan removal of females will directly affect next generation densities.

Assessment traps positioned in the centre of each plot provide a measure of communication disruption to semiochemical cues due to mass trapping treatments as compared to untreated control plots. Fewer males were captured in pheromone-baited assessment traps in pheromone treated plots when compared to the control plots. This is most evident in Experiments 2 & 3 in which mass traps are baited with the high pheromone dose of 10 mg and assessment traps are baited with the less competitive 1 mg lure. Reduction of male capture in 10 mg baited assessment traps positioned in pheromone mass trapping plots in Experiment 1 may be associated with competition instead of camouflage of the plume (Miller et al. 2006a, 2006b). At the 50 and 100 traps / ha, the trap catch in pheromone assessment traps approached zero even at peak flight. This is known as trap shutdown in the mating disruption literature and is one of the tools used to determine the effectiveness of semiochemical-mediated communication disruption (Mitchell et al. 1997).

In conclusion, I showed that a non-competitive bait such as a food-based kairomone may be useful in the assessment of populations under pheromone-treated mass trapping or mating disruption. There was less fluctuation in the numbers of males captured in kairomone traps independent of the mass trapping densities tested. In mating disruption studies kairomone-baited traps provide a more objective assessment of pheromone-based mating disruption than pheromone monitoring traps (Ryne et al. 2006; Knight 2010) because (1) they attract male moths via a different mechanism of action than sex pheromones (de Bruyne & Baker 2008) and, consequently, their plumes are not camouflaged by the pheromone dispensers, (2) females can also be captured and their mating status assessed in order to determine the degree of communication disruption (Knight 2007) and (3) action thresholds can be developed in orchards treated with sex pheromones (Knight & Light 2005).

An analysis of the sex ratio of moths captured in kairomone-baited traps indicates that pheromone-baited traps targeting male moths are most effective at early to peak flight. Later in the season when females are more prominent in the population, at least 100 kairomone-baited traps / ha should be deployed at peak flight and left until the end of flight. Bucket traps baited with 10 mg apple clearwing moth sex pheromone should be separated by at least 40 m in order to minimize trapping interference. The range of attraction of kairomones is much smaller (less than 5 m) than the range of attraction of pheromones, so deployment, baiting and emptying of kairomone traps (if no bucket traps are available) may become expensive and labour intensive. Nevertheless, traps baited with kairomone remove large numbers of females from the population and can also be used as an objective assessment tool on the effectiveness of pheromone-based pest control such as mass trapping or mating disruption.

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Table 2.1. The mass trapping experiments conducted in the Southern Interior of British Columbia.

Exp. #	Bait	Assessment	Treatments	Date
1	Pheromone	10 mg pheromone, 300 ml grape juice	0, 2, 4 and 8 traps / plot (0, 13, 25 and 50 traps / ha)	29 June – 06 July 2009
	Kairomone			
2	Pheromone	1 mg pheromone, 300 ml grape juice	0, 2, 4 and 8 traps / plot (0, 13, 25 and 50 traps / ha)	28 July – 03 August 2009
	Kairomone			
3	Pheromone	1 mg pheromone, 300 ml grape juice	0, 4, 8 and 16 traps / plot (0, 25, 50 and 100 traps / ha)	13 – 30 July 2009
	Kairomone			
4	Both	1 mg pheromone, 300 ml grape juice	Control, 4 pheromone traps / plot, 4 kairomone traps / plot and mixed (4 pheromone + 4 kairomone traps / plot)	13 – 20 July 2010

Figure 2.1. Apple clearwing moth traps used in mass trapping experiments. (A) High capacity commercially-available yellow pheromone-baited Unitraps®. (B) Custom-designed yellow kairomone-baited bottle trap.


A
10 PCS4-2
B
JCS4-1
JCS4-1

Figure 2.2. Schematic arrangement of the different mass trapping trap densities tested in 2009. Experimental and control plots were 40 x 40 m (0.16 ha) and were separated by 40 m "buffer" plots. Black & grey dots represent the mass traps, baited with either 10 mg apple clearwing moth sex pheromone or 300 ml fresh Concord grape juice. Black circles represent pheromone assessment traps baited with 10 mg apple clearwing moth sex pheromone (Experiment 1). White circles represent pheromone assessment traps baited with 1 mg apple clearwing moth sex pheromone (Experiments 2 & 3). Grey dots represent kairomone assessment traps baited with 300 ml fresh Concord grape juice.

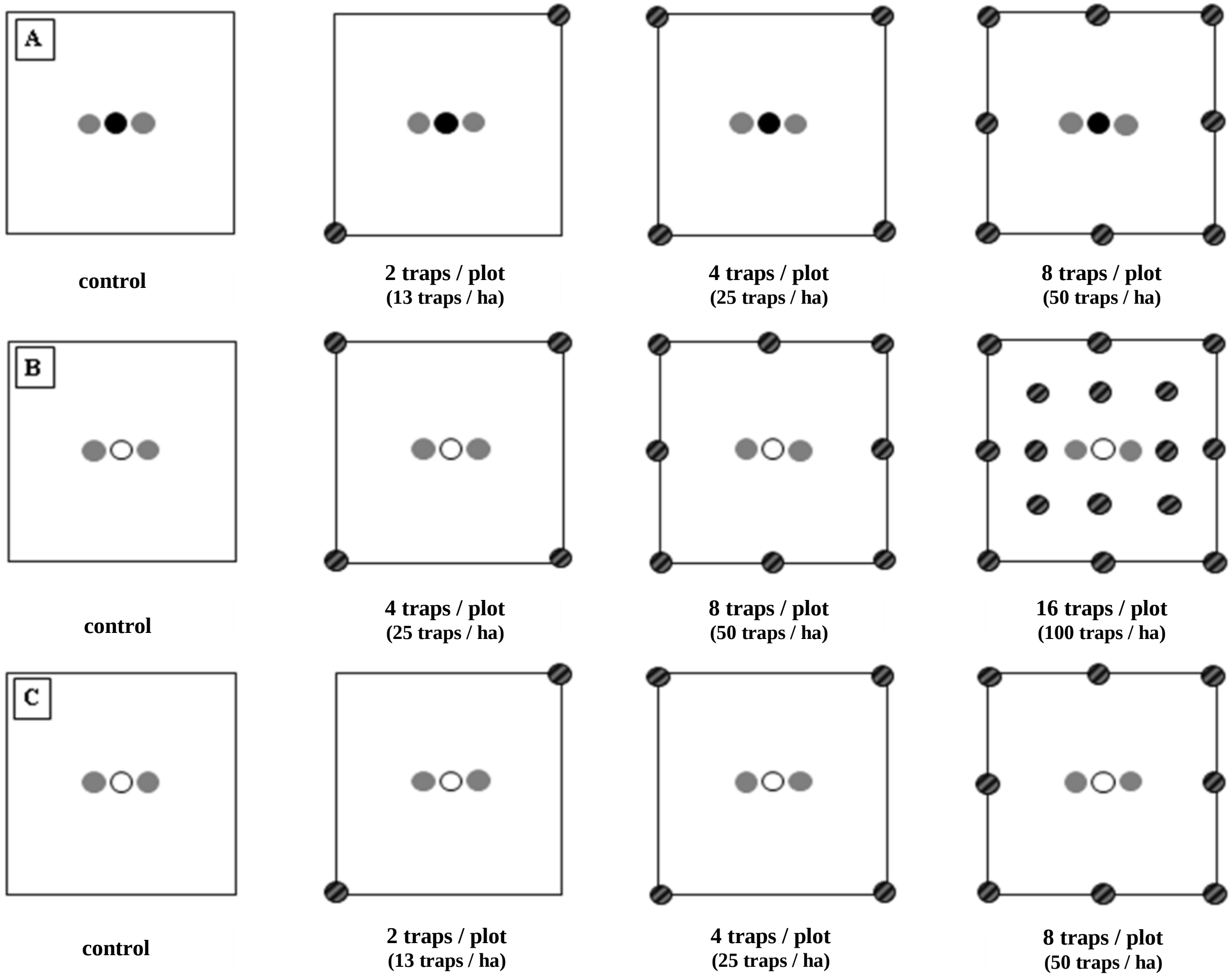
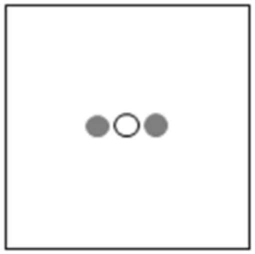
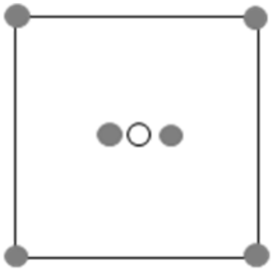

A
control
2 traps / plot
(13 traps / ha)
4 traps / plot
(25 traps / ha)
8 traps / plot
(50 traps / ha)
B
control
4 traps / plot
(25 traps / ha)
8 traps / plot
(50 traps / ha)
16 traps / plot
(100 traps / ha)
C
control
2 traps / plot
(13 traps / ha)
4 traps / plot
(25 traps / ha)
8 traps / plot
(50 traps / ha)

Figure 2.3. Schematic representation of the semiochemical-baited mass trapping treatments tested in Experiment 4 in 2010. Experimental plots and control were 40 x 40 m (0.16 ha) and were separated by 40 m "buffer" plots. Black dots represent the pheromone mass traps baited with 10 mg apple clearwing moth sex pheromone. White circles represent pheromone assessment traps baited with 1 mg apple clearwing moth sex pheromone. Grey circles represent kairomone-baited mass trapping (perimeter) and assessment (middle of plots) traps baited with 300 ml fresh Concord grape juice.

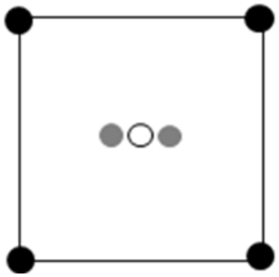


control



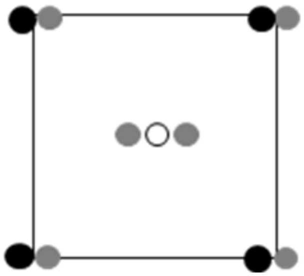
4 kairomone traps / plot

(25 traps / ha)



4 pheromone traps / plot

(25 traps / ha)



combination
(4 pheromone traps / plot +
4 kairomone traps / plot)

(50 traps / ha)

Figure 2.4. Effect of trap density on the total number of apple clearwing moth adults captured in Experiment 1. (A) pheromone-baited mass trapping traps. (B) kairomone-baited mass trapping traps. Whiskers represent data that falls within 1.5 interquartile ranges of the median (horizontal lines). Dots represent outliers that fall between 1.5 and 3 interquartile ranges from the median. Bars marked with different small letters indicate statistical significance among treatments and capital letters indicate statistical significance between males and females captured in kairomone-baited mass traps (α = 0.05).

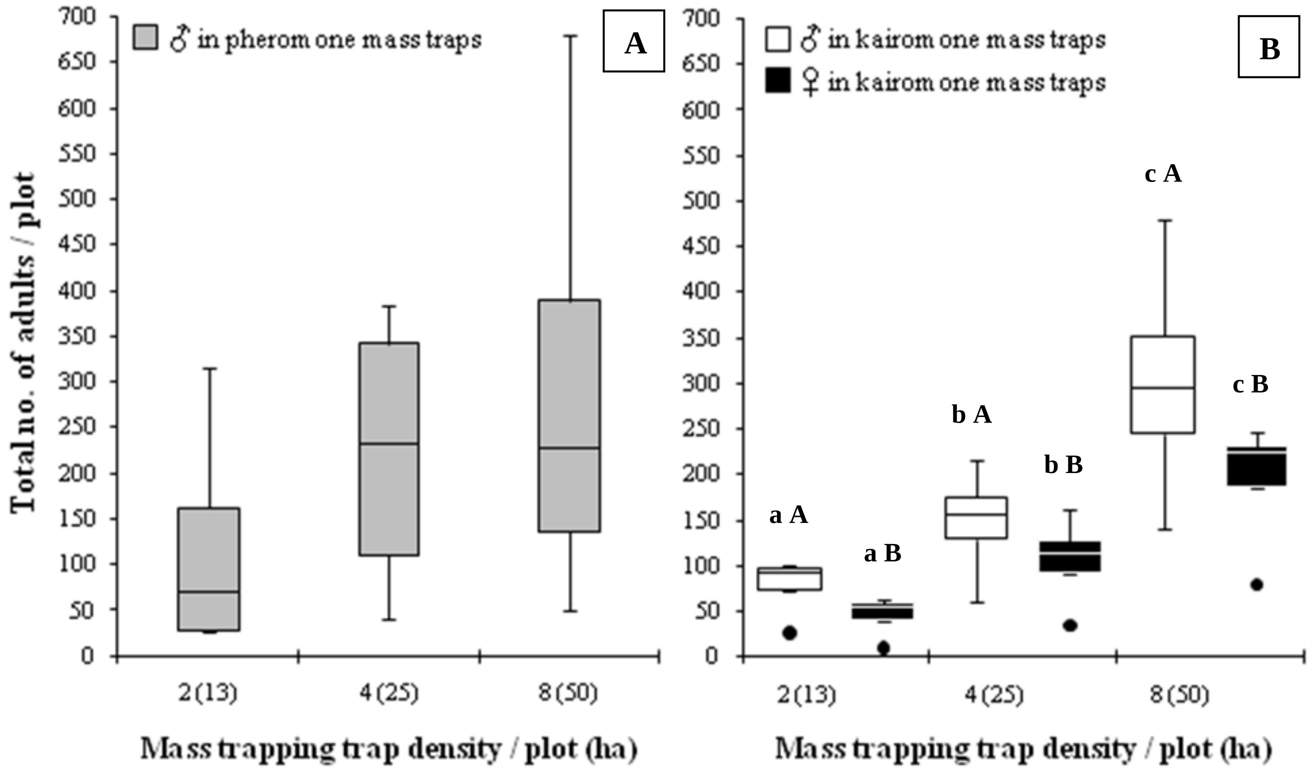

A
▨ ♂ in pheromone mass traps
Total no. of adults / plot
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
2 (13)
4 (25)
8 (50)
Mass trapping trap density / plot (ha)
B
☐ ♂ in kairomone mass traps
■ ♀ in kairomone mass traps
a A
a B
b A
b B
c A
c B
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
2 (13)
4 (25)
8 (50)
Mass trapping trap density / plot (ha)

Figure 2.5. Effect of trap density on the total number of apple clearwing moth adults captured in Experiment 2. (A) pheromone-baited mass trapping traps. (B) kairomone-baited mass traps. Whiskers represent data that falls within 1.5 interquartile ranges of the median (horizontal lines). Dots represent outliers that fall between 1.5 and 3 interquartile ranges from the median. Bars marked with different small letters indicate statistical significance among treatments and capital letters indicate statistical significance between males and females captured in kairomone-baited mass traps ($\alpha = 0.05$).

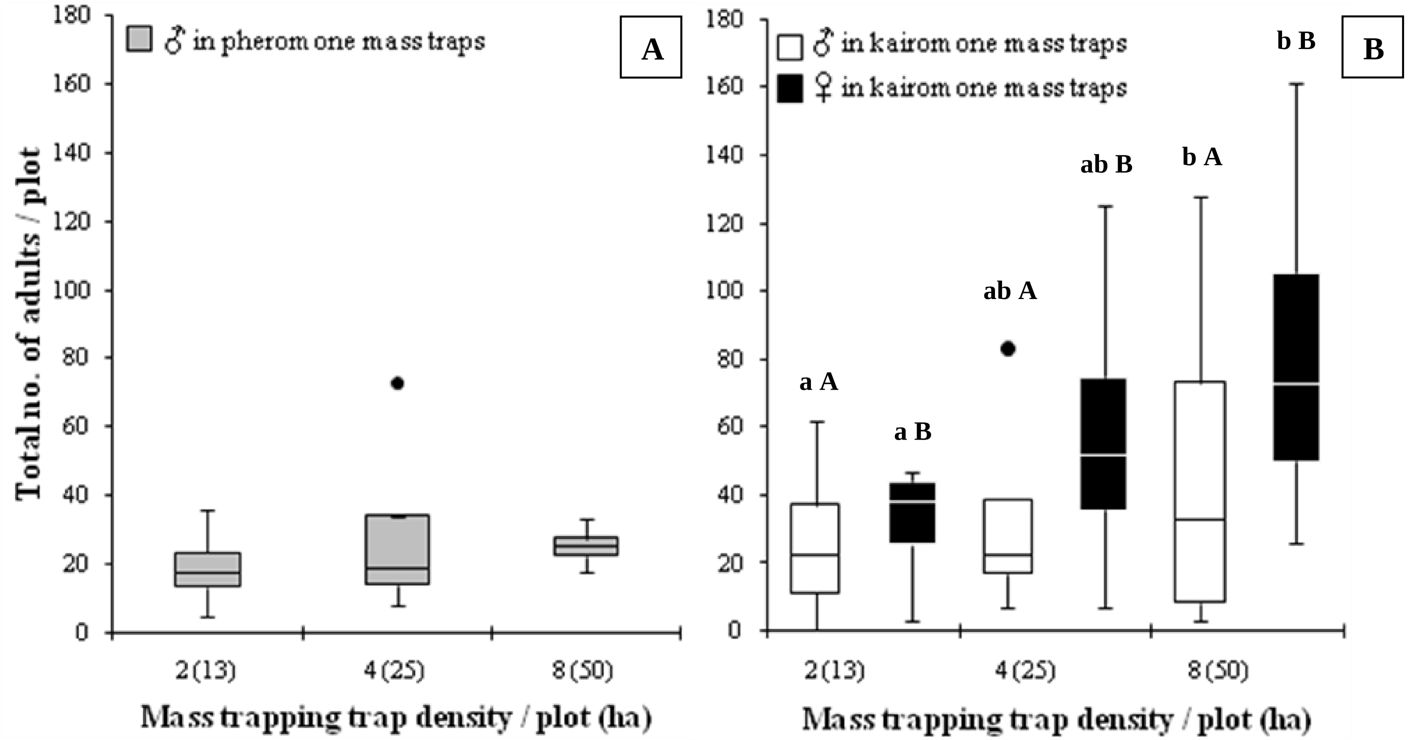

A
B
Total no. of adults / plot
0
20
40
60
80
100
120
140
160
180
🟫 ♂ in pheromone mass traps
☐ ♂ in kairomone mass traps
■ ♀ in kairomone mass traps
a A
a B
ab A
ab B
b A
b B
2 (13)
4 (25)
8 (50)
Mass trapping trap density / plot (ha)

Figure 2.6. Effect of trap density on the total number of apple clearwing moth adults captured in Experiment 3. (A) pheromone-baited mass trapping traps. (B) kairomone-baited mass traps. Whiskers represent data that falls within 1.5 interquartile ranges of the median (horizontal lines). Bars marked with different small letters indicate statistical significance among treatments and capital letters indicate statistical significance between males and females captured in kairomone-baited mass traps (α = 0.05). Dots represent outliers that fall between 1.5 and 3 interquartile ranges from the median.

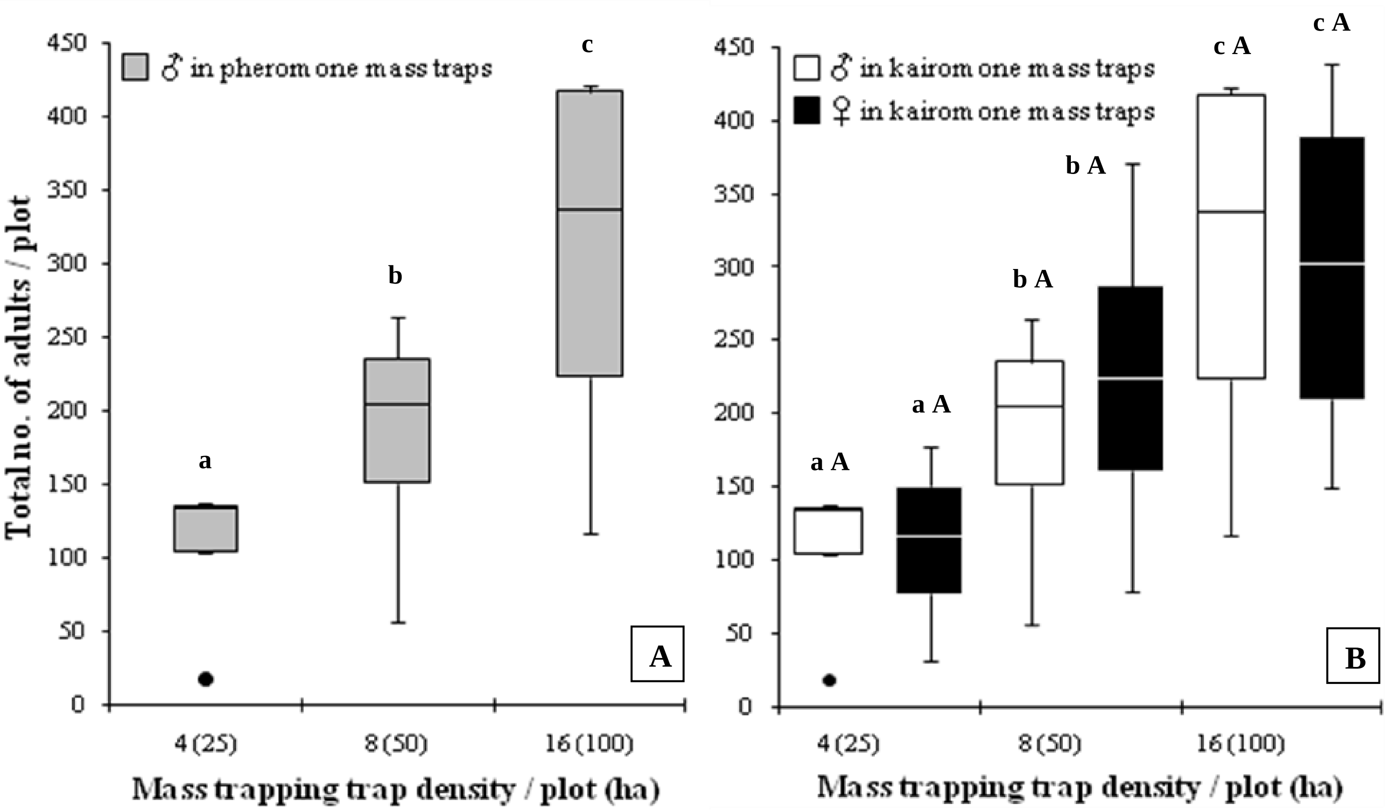

Total no. of adults / plot
450
400
350
300
250
200
150
100
50
0
▨ ♂ in pheromone mass traps
a
b
c
A
4 (25)
8 (50)
16 (100)
Mass trapping trap density / plot (ha)
☐ ♂ in kairomone mass traps
■ ♀ in kairomone mass traps
a A
a A
b A
b A
c A
c A
B
4 (25)
8 (50)
16 (100)
Mass trapping trap density / plot (ha)

Figure 2.7. Effect of mass trapping trap density on the mean numbers of apple clearwing moth adults captured in in assessment traps in Experiment 1. Whiskers represent data that falls within 1.5 interquartile ranges of the median. Dots represent outliers that fall between 1.5 and 3 interquartile ranges from the median (horizontal lines). (A) The surrounding mass traps were baited with pheromone lures. There was a significant difference in the number of males captured in assessment traps located in control vs. mass trapping plots ($P = 0.01$). There was no significant difference in the number of males captured in pheromone and kairomone assessment traps ($P = 0.38$). Significantly more males were captured than females ($P < 0.001$). (B) The surrounding mass traps were baited with kairomone lures. There was no significant difference in the number of males captured between control and mass trapping densities tested ($P = 0.2$). More males were captured in pheromone assessment traps than in kairomone assessment traps ($P < 0.001$). More males were captured than females ($P < 0.001$).

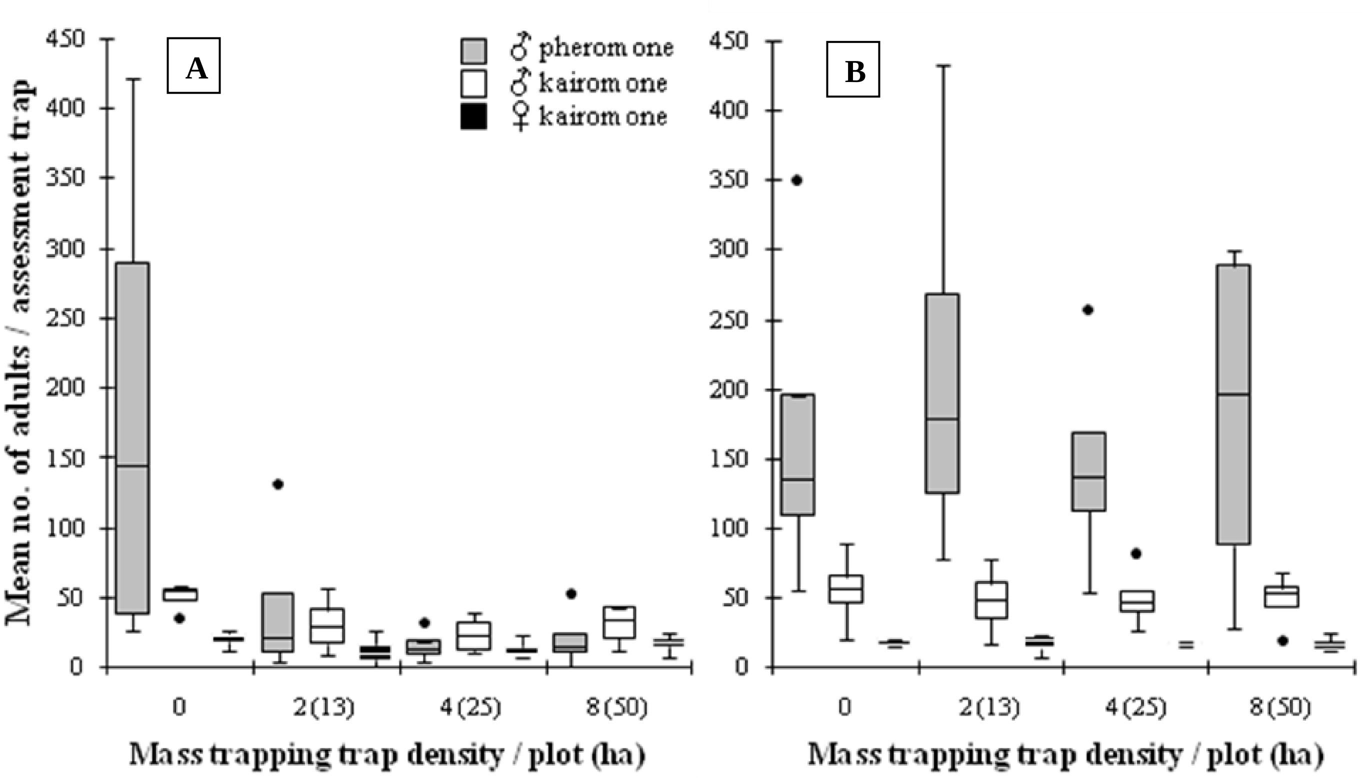

A
B
♂ pheromone
♂ kairomone
♀ kairomone
Mean no. of adults / assessment trap
450
400
350
300
250
200
150
100
50
0
0
2 (13)
4 (25)
8 (50)
Mass trapping trap density / plot (ha)
Mass trapping trap density / plot (ha)

Figure 2.8. Effect of mass trapping trap density on the mean number of apple clearwing moth adults captured in assessment traps in Experiment 2. Whiskers represent data that falls within 1.5 interquartile ranges of the median (horizontal lines). Dots represent outliers that fall between 1.5 and 3 interquartile ranges from the median. (A) The surrounding mass traps were baited with pheromone lures. There was a significant difference in the number of males captured in assessment traps located in control vs. mass trapping plots ($P < 0.001$). There was no significant difference in the number of males captured in pheromone and kairomone assessment traps ($P = 0.58$). Significantly more females were captured than males ($P = 0.02$). (B) The surrounding mass traps were baited with kairomone lures. There was a significant difference in the number of males captured between control and mass trapping densities tested ($P = 0.025$). There was no significant difference in the number of males captured in pheromone and kairomone assessment traps ($P = 0.06$). Similar numbers of males and females were captured in kairomone assessment traps ($P = 0.18$).

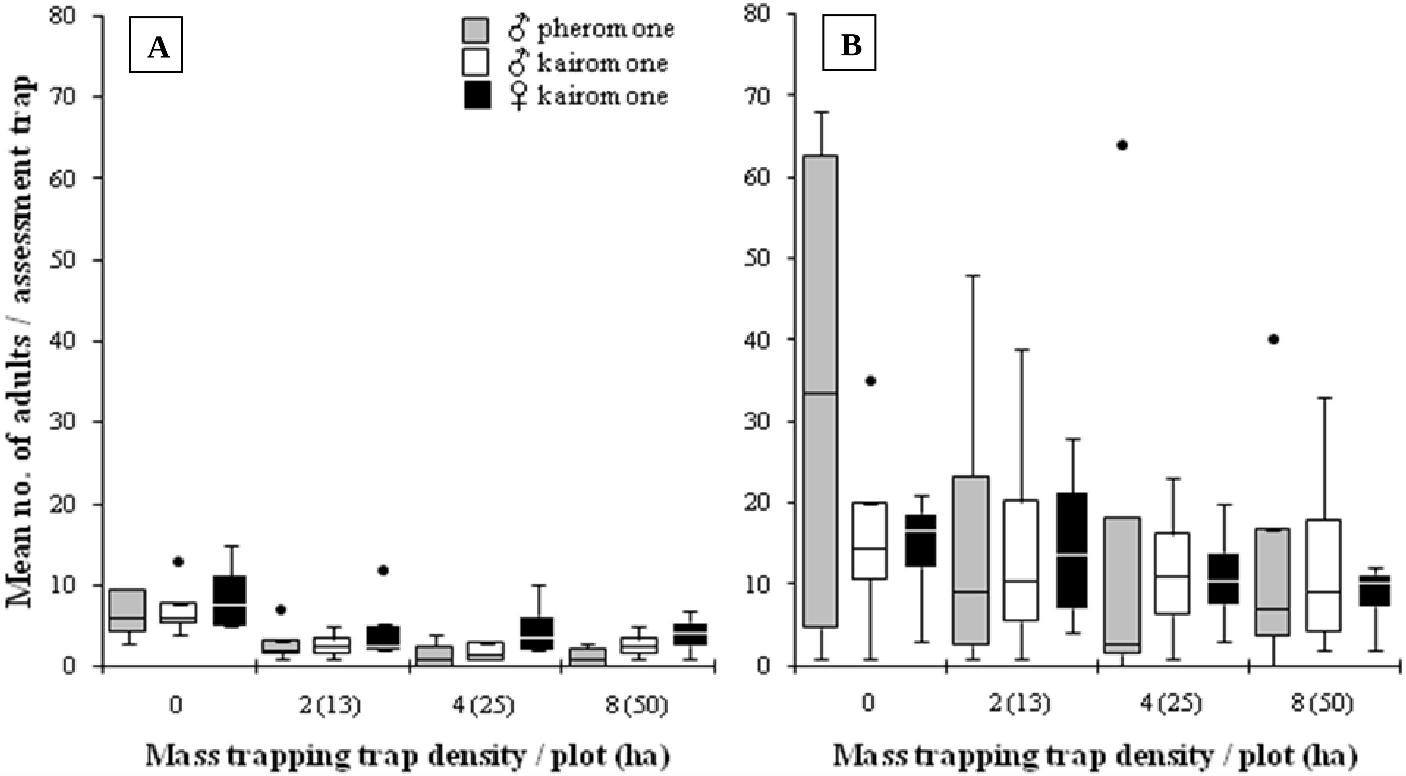
A
B
♂ pheromone
♂ kairomone
♀ kairomone
Mean no. of adults / assessment trap
0
10
20
30
40
50
60
70
80
0
2 (13)
4 (25)
8 (50)
Mass trapping trap density / plot (ha)

Figure 2.9. Effect of mass trapping trap density on the mean numbers of apple clearwing moth adults captured in assessment traps in Experiment 3. Whiskers represent data that falls within 1.5 interquartile ranges of the median. Dots represent outliers that fall between 1.5 and 3 interquartile ranges from the median (horizontal lines). (A) The surrounding mass traps were baited with pheromone lures. There was a significant difference in the number of males captured in assessment traps located in control vs. mass trapping plots ($P < 0.001$). There was a significant interaction between bait and trap density tested, with captures in pheromone assessment traps approaching zero as mass trapping density increased ($P < 0.001$). Significantly more males were captured than females ($P < 0.001$). (B) The surrounding mass traps were baited with kairomone lures. There was no significant difference in the number of males captured between control and mass trapping densities tested ($P = 0.12$). More males were captured in pheromone than in kairomone assessment traps ($P < 0.001$). More males were captured in kairomone assessment traps than females ($P < 0.001$).

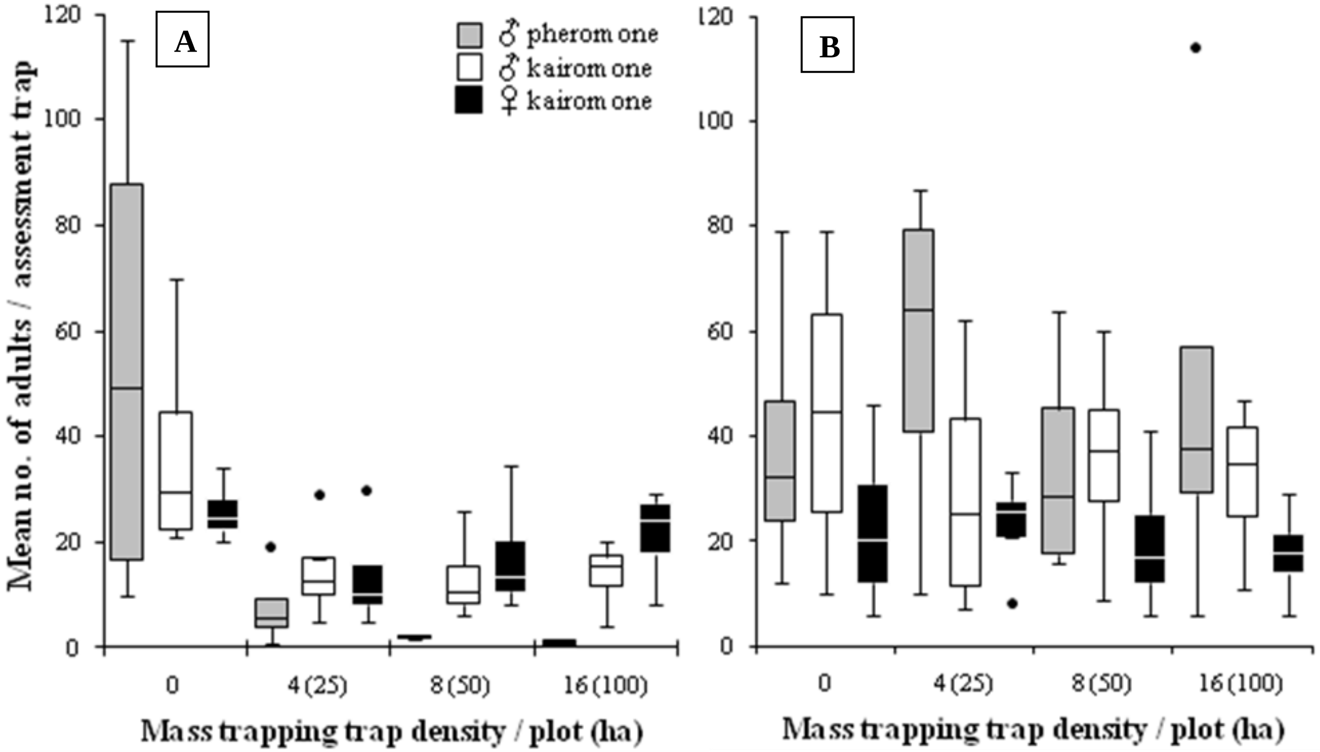

A
B
♂ pheromone
♂ kairomone
♀ kairomone
Mean no. of adults / assessment trap
0
20
40
60
80
100
120
0
4 (25)
8 (50)
16 (100)
Mass trapping trap density / plot (ha)

Figure 2.10. Effect of semiochemical-baited mass trap bait type on the numbers of apple clearwing moth captured in Experiment 4. (A) Mass traps. There is a significant difference in the number of males captured in kairomone, pheromone and mixed plots ($P < 0.001$). More females were captured in kairomone plots than in mixed plots ($P < 0.001$) (B) Assessment traps. There was a significant difference in the number of males captured between control and treatments tested ($P < 0.001$). There was a significant interaction between bait and treatment tested, with fewer males captured in pheromone assessment traps in mixed plots than in kairomone-baited assessment traps in control ($P < 0.001$). More males were captured in kairomone assessment traps than females ($P < 0.001$). There was also a significant interaction between sex and treatment tested, with more males captured in kairomone assessment traps in mixed plots than females in control ($P = 0.007$).

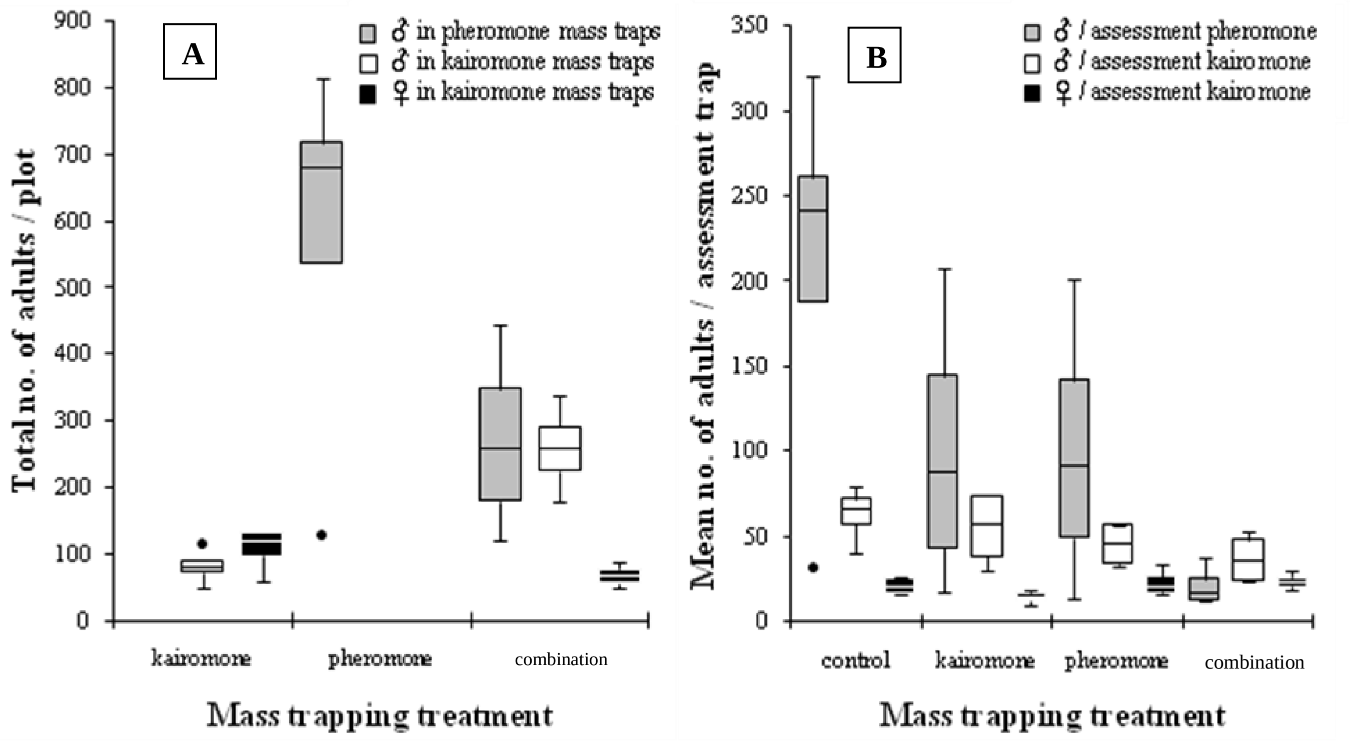
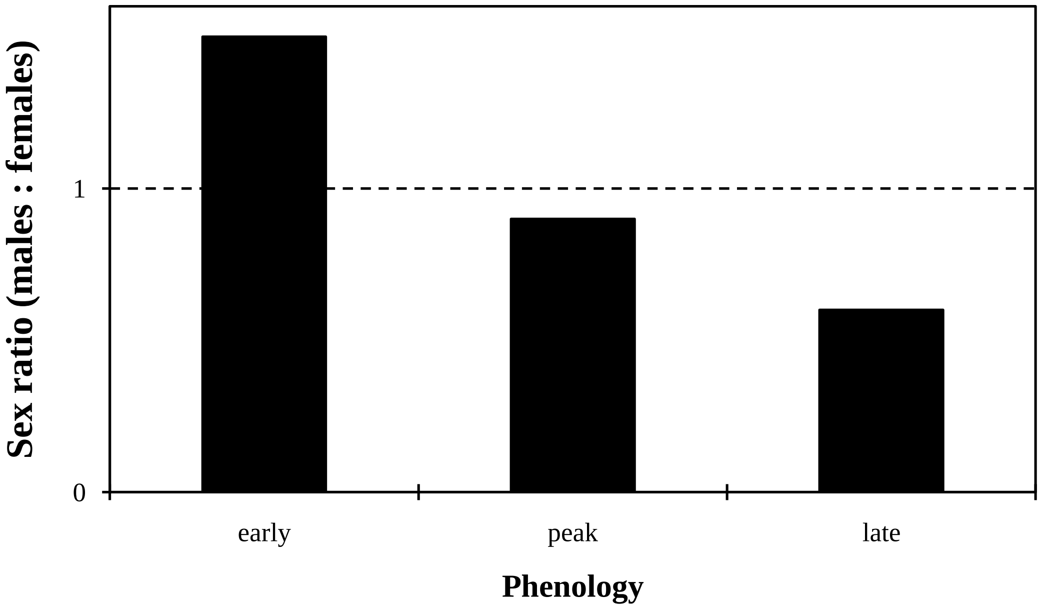

A
♂ in pheromone mass traps
♂ in kairomone mass traps
♀ in kairomone mass traps
Total no. of adults / plot
0
100
200
300
400
500
600
700
800
900
kairomone
pheromone
combination
Mass trapping treatment
B
♂ / assessment pheromone
♂ / assessment kairomone
♀ / assessment kairomone
Mean no. of adults / assessment trap
0
50
100
150
200
250
300
350
control
kairomone
pheromone
combination
Mass trapping treatment

Figure 2.11. Sex ratio as determined by the total number of males captured divided by the total number of females captured in kairomone-baited mass traps sampled for one week during the early (Experiment1), late (Experiment 2) and peak (Experiment 3) flight of the apple clearwing moth in 2009. The dashed line indicates a 50:50 sex ratio.


Sex ratio (males : females)
1
0
early
peak
late
Phenology

Chapter 3. Mechanisms of pheromone-based mass trapping of the apple clearwing moth, *Synanthedon mypaeformis* (Borkhausen) (Lepidoptera: Sesiidae)

3.1. Introduction

Many insects use olfaction alone or combined with other sensory modalities for intra- and interspecific communication to mediate behaviours such as mating, host location, oviposition and defense (Corbet 1985; Cardé & Bell 1995). Within the Lepidoptera, the use of female-produced pheromones to mediate mate location is a primitive trait (Roelofs et al. 2002) and this behaviour is enhanced by simultaneous detection of host- plant chemicals by male moths in some species (Light et al. 1993; Landolt & Phillips 1997; Reddy & Guerrero 2004). The reliance of moths on sex pheromones for mate location and response to host-plant volatiles for host location makes chemical communication a good tool for pest management. Semiochemicals are used in pest management to monitor and directly control moth pests (Dent 2000; El-Sayed et al. 2006; Witzgall et al. 2010). The most commonly used semiochemical-based pest management control tactics are mating disruption and mass trapping (Byers 2007; Yamanaka et al. 2007).

Mass trapping is the first and oldest semiochemical-based pest management tactic (Steiner 1952). The principle behind it is straightforward: if a large enough proportion of insects is trapped and removed from the population then fewer matings should occur in the treated cropping area and offspring density and damage should decrease (El-Sayed et al. 2006). In order to maximize the efficacy of the mass-trapping tactic, the bait and the trap must be attractive and retain the target pest, respectively. Parameters that affect the trap effectiveness may include trap capacity, colour and trapping density in the cropping area (El-Sayed et al. 2006). The trap bait must be applied at a dose that is highly attractive and lasts a reasonable period of time under field conditions (Suckling 2000; El-Sayed et al. 2006). Interactions between trap and bait parameters generate other variables that are important to the efficacy of mass-trapping treatments, the most important of which are the range of attraction (Schlyter 1992; Byers 1999; Dodds & Ross 2002) and the potential for trapping interference (Wall & Perry 1978; McMahon et al. 2010). The range of attraction is directly proportional to the bait attractiveness and dose used, although the relationship will vary by target species and is not necessarily linear (Baker et al. 1981; Branco et al. 2006). The interaction of odour plumes generated from multiple traps under a given trap density and semiochemical dose characterizes trapping interference. In order to minimize trap interference, adjacent traps must be separated by at least two attractive radii (Dodds & Ross 2002). The attractive radius is the maximum distance over which an insect shows directed flights towards an attractant source such as calling female or semiochemical trap (Wall & Perry 1987). Trapping interference can result in localized confusion of orienting adults similar to a response expected in mating disruption treated environments (Yamanaka et al. 2003).

Most mass trapping-based pest management programs use pheromone-baited traps to attract and remove male moths from the pest population (El-Sayed et al. 2006). The major disadvantage of classical, male-based mass trapping is that over 90% of the male population must be removed in order to reduce a pest population below an economic threshold (Knipling & McGuire 1966). Nevertheless, pheromone-based mass trapping is an effective pest management tactic in several cropping systems. For example, mass trapping of the Chinese tortrix moth, *Cydia trasias* (Meyrick) resulted in 72% reduction in the next generation larvae (Zhang et al. 2002). Traps baited with aggregation pheromones attractive to both male and female *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae) reduced beetle damage by over 80% (Faccoli & Stergulc 2008). In terms of moths, mass trapping can be improved by using kairomones attractive to both sexes in order to decrease the density of both males and females in the population (Light et al. 2001; Horton & Landolt 2002; Yang et al. 2004).

Mating disruption has been more extensively studied and is more widely used operationally than mass trapping (El-Sayed et al. 2006; Yamanaka 2007). Mating disruption is achieved through application of large amounts of sex pheromone that interferes with mate-finding between males and females, subsequently reducing mating and oviposition (Cardé & Minks 1995; Miller et al. 2006a). Unlike mass trapping, the mechanism of how this interference is achieved is not immediately obvious (Bartell 1982). The mechanisms by which mating disruption works fall into four major categories: false-trail-following, camouflage, desensitisation and neurophysiological effects (Miller et al. 2006a). False-trail-following is the primary mechanism operating in most mating disruption trials (Gut et al. 2007). Males orienting to pheromone dispensers waste their time and energy either flying or clustering around the dispensers instead of searching for calling females (Miller et al. 2006a, 2010; Trona et al. 2009). Camouflage is a masking of the females' plume and is thought to be most effective when the pheromone released from the dispenser most closely mimics the natural blend (Bosa et al. 2006; Miller et al. 2006a). Desensitisation of antennal receptors can occur in response to pheromone treatment of the cropping area through overstimulation and inactivation of the antennal receptors which subsequently arrests orientation behaviour (Baker et al. 1989; Judd et al. 2005; Stelinski et al. 2008). Neurophysiological effects include habituation of the central nervous system processing of chemical cues (Miller et al. 2006a; Stelinski et al. 2006). Recently, neurophysiological mechanisms using high doses of pheromone inhibitors have shown potential in mating depression (Leskey et al. 2009), especially in bark beetle-mating systems (Miller et al. 1995; Zhang & Schlyter 2004; Fettig et al. 2009; Etxebeste & Pajares 2010). In most mating disruption programs, a combination of these mechanisms is thought to be responsible for decreased pest densities in the next generation (Evenden et al. 2000; Miller et al. 2006a, 2006b). All of these mechanisms may lead to delayed mating which reduces oviposition and pest density (Lingren et al. 1988; Jones & Aihara-Sasaki 2001).

Assessing the efficacy of pheromone-based mass trapping or mating disruption often relies on comparing the number of individuals captured in pheromone-baited traps in treated vs. control plots. A lack of insect catches in assessment traps positioned in the treated plots, known as “trap shutdown”, is often used as an indicator of effective communication disruption (Mitchell et al. 1997). Direct assessment of insect mating behaviour can be achieved through dissection of sentinel females positioned in treated and control plots (Thomas & Burnip 1991; Evenden et al. 1999) or collection and dissection of feral females if the treated area is large enough to minimize immigration effects. An alternative approach might be to employ assessment traps baited with attractants that do not directly compete with the pheromone control treatment. For example, traps baited with food-based kairomones placed in the middle of the treated plots attractive to both males and females can be used to determine any treatment effects on current insect populations and on the female mating status (Chapter 2; Knight & Light 2005b; Knight 2010).

The apple clearwing moth, *Synanthedon myopaeformis* (Borkhausen) (Lepidoptera: Sesiidae) is a diurnal moth recently introduced into North America from Europe via imported apple rootstock (Philip 2006). Larvae feed under the bark of apple trees, preferentially infesting the graft area of dwarfing rootstocks (Ateyyat 2006). This feeding can negatively affect tree vigour and fruit yield (Dickler 1976; Castellari 1987). In a temperate climate apple clearwing moths mainly have a biennial life cycle with a single annual flight (Injac & Tosevski 1987; Spatenka et al. 1999).

The purpose of this study is to examine the potential mechanisms of action of pheromone-based mass trapping by determining if removal of male moths through trap capture is necessary to disrupt pheromone-based mating behaviour of the apple clearwing moth using high and low pheromone lure loads. In one of the few studies that compared the efficacy of mass trapping to a mating disruption treatment Kitamura & Kobayashi (1985) concluded that removal of *Spodoptera litura* (Fabricius) males is not necessary for population control. I compared the catches of apple clearwing adults in assessment traps in plots treated with pheromone-baited mass trapping utilizing open and closed traps. Open traps retain attracted male moths while closed traps do not, but in both plots moths are similarly attracted to pheromone sources. Plots with closed traps simulate a mating-disruption treatment. In order to minimize the effect of moth immigration and emigration, pheromone treatments were assessed in large orchard plots. The population densities were assessed before and between each sequential replicate to ensure that population density was similar among the variously-treated plots at each time interval. Assessment of moth activity in treated and control plots during and between replicates was conducted with both competitive (pheromone) and non-competitive (kairomone) assessment traps. Dissection of feral female moths captured in kairomone-baited traps gives a direct measure of mating interference due to pheromone treatments.

3.2. Materials and Methods

Study site

A large organically-managed apple orchard near Keremeos, BC (49.21 N & -119.84 W) was chosen as a study site. The orchard consisted of three large sections: two 2.5 ha superspindle-type apple plantings at an average density of 5438 apple trees / ha; and a 2.2 ha middle section planted with grapevines. The orchard was located on hilly terrain. One orchard section sustained heavy apple clearwing moth infestations (as assessed by Judd, G. J. R. unpublished) and consisted of mostly Gala and Pink Lady apple varieties. The other orchard section consisted of Ambrosia and Orin apple varieties and had much lower apple clearwing moth densities.

Experimental design

Each of the two apple orchard sections was divided into three plots for use in two separate experiments. Experiments differed in the dose of pheromone used in mass trapping traps (1 mg vs. 10 mg), were conducted in parallel and replicated through time in 2010. In each experiment, the middle plot in each section was 0.5 ha in size and was designated as the non-treated control. The other two plots were 0.9 ha in size and were assigned to the mass trapping treatments. Both experiments tested the hypothesis that active removal of male moths from the population is necessary to disrupt communication between males and females Pheromone lures in one treated plot were positioned in open traps to remove males and trap catch was compared to a similarly treated plot in which traps were plugged with custom-made aluminium stoppers to prevent removal of moths from the population. Treatment of experimental plots, consisted of 20 pheromone-baited mass traps (25 traps / ha) deployed in a grid pattern ~ 1.5 m above the ground (Figure 3.1). Each experiment was replicated 6 times and each replicate consisted of two phases: a 4-day, non-baited pre-assessment phase during which lures and lure baskets were removed from all mass traps, followed by a 4-day assessment phase during which mass traps were baited with fresh pheromone lures positioned in clean lure baskets. The purpose of the pre-assessment phase was to allow moth populations to recover and minimize the potential variance that temporal replication might add. Trap catch was collected at the end of the fourth day. At the end of each replicate mass-trapping treatments (open vs. closed traps) were switched between replicates to minimize positional effects on each treatment.

Moth population density was assessed both prior to (pre-assessment phase) and after (testing phase) each treatment application. In each plot, moth capture was compared in four centrally-located pheromone-baited traps separated from each other by ~ 30 m and two kairomone-baited assessment traps separated from each other by ~8 m. Two pheromone assessment traps were baited with 1 mg apple clearwing moth sex pheromone and the other two were baited with 10 mg sex pheromone. Moths captured in assessment traps were counted every four days. Females recovered from kairomone-baited assessment traps were dissected to determine their mating status based on the presence of a spermatophore in the *bursa copulatrix*. Traps were cleaned with soap and water and 70% ethanol and pheromone lures and kairomone baits were replaced after each replicate. Adult phenology was monitored on a weekly basis in control plots after the completion of the experiments until no more moths were captured in all assessment trap types (02 September 2010). In the case of kairomone traps bait was replaced and moths were counted twice / week but counts were summed on a weekly basis.

The sex pheromone-baited assessment traps consisted of yellow Unitraps (AgBio Inc., Westminster, CO, USA) baited with grey halobutyl rubber septa (West Co., Lyonville, PA, USA) impregnated with 1 or 10 mg of the sex pheromone of the apple clearwing moth (Pherobank, Wageningen, The Netherlands; >95% isomeric purity) diluted in HPLC grade hexane (Aldrich Chemical Co., Milwaukee, WI, USA). Kairomone traps were used for assessment of treatments only, followed the design described in Chapter 2 and were baited with 300 ml fresh Concord grape juice (SunRype^{TM}, Kelowna, BC).

Statistical analyses

For each experiment, a General Linear Mixed Effects Model applied to square root transformed data was used to determine if there was a significant difference in the mean number of moths captured in each type of assessment trap / plot for each mass-trapping treatment tested relative to the non-mass-trapped control plot. The model was fitted with an autocorrelation procedure of order 1 and time was specified as a random factor. A second Generalized Least Squares model of untransformed data using best fit lines was produced in order to illustrate significant treatment dynamics as the season progressed. In order to account for autocorrelation of time series and non-linearity of the count data, this second model was also fitted with an autocorrelation procedure of order 1. Time was considered a random factor and fitted to a quadratic curve. Variance correction was applied in order to stabilize the unequal variances of datasets. For each dataset I used the Delta method (Lyons 1991; Oehlert 1992) to calculate normal approximations of the 95% confidence bands. All analyses and time series graphs were conducted using R statistical package (R Development Core Team 2010). Overall treatment effect graphs consist of means of non-transformed data fitted with Poisson error bars (Figures 3.3A, 3.3B). Contingency table analysis tested whether the frequency of females with 0, 1 and 2 or more spermatophores is independent of the mass trapping treatment from which females were recovered. Phenology curves were produced using weekly pooled catches / trap type from the control plots.

3.3. Results

Assessment traps positioned in the centre of each plot provided a good measure of communication disruption to semiochemical cues due to mass trapping treatments with and without moth removal when compared to the untreated control plot. However, there were differences in trap catch between the different trap types tested. Pheromone-baited assessment traps were in direct competition with mass trapping traps whereas kairomone-baited traps provided a relative measure of insect activity using a lure that did not compete with the mass traps.

When 1 mg pheromone lures were used to bait mass traps, treatment with either open or closed pheromone-baited mass traps significantly ($P < 0.001$) reduced the number of adults captured in pheromone assessment traps relative to traps in the control plot (Figure 3.2 testing phase). Throughout Experiment 1, open and closed mass-trapping treatments had a similar impact on the number of adults captured in assessment traps-(Figure 3.3). The entire mass trapping period corresponded to about 60% of the seasonal flight period (Figure 3.4). Low moth catches late in the adult flight as well as high moth catches at peak flight did not alter the comparative effectiveness of treatments as measured by the 1 and 10 mg pheromone assessment traps (Figure 3.3). Both open and closed mass traps continued to produce low trap catches when compared to the untreated control. The type of assessment bait used in Experiment 1 had a significant ($P < 0.001$) effect on the number of males captured in both the pre-assessment and treatment application phases (Figure 3.2 testing phase). Assessment traps baited with 10 mg of apple clearwing moth pheromone captured the largest number of males whereas kairomone traps baited with 300 ml Concord grape juice captured the lowest number of males. Kairomone traps captured similar ($P = 0.55$) numbers of males and females. Before the experiment and between replicates during the pre-assessment phase, no treatment effects (P = 0.07) on the mean number of adults captured in the 1 mg pheromone-baited assessment traps were observed (Figure 3.2, pre-assessment phase) but there was a significant (P = 0.03) difference in the mean number of males captured in the 10 mg pheromone-baited assessment traps across the different treatments tested and control. Kairomone traps captured equal numbers of males and females (P = 0.31).

When experimental plots were treated with 10 mg lures (Experiment 2), different results were obtained. A similar (P = 0.11) number of moths was captured in assessment traps positioned in plots treated with mass-trapping treatments and the untreated control. During the treatment phase of this experiment, there was no significant (P = 0.12) effect of bait type on the total numbers of males captured in assessment traps (Figure 3.5 testing phase). Kairomone traps captured similar numbers of males and females (P = 0.37) during the treatment phase of the second experiment. In the pre-assessment phase, there was no significant (P = 0.23) treatment effect on the number of moths captured in the various plots (Figure 3.5 pre-assessment phase). As expected, there was a significant bait effect (P < 0.001) as more males were captured in pheromone- than kairomone-baited assessment traps during the pre-assessment phase. Kairomone assessment traps captured equal numbers of males and females (P = 0.97).

Mating status was assessed for all females captured in kairomone-baited assessment traps in both experiments. For Experiment 1, there were no treatment effects (χ^2 = 1.95, df = 4, P = 0.75) on the mating status of females captured in kairomone assessment traps (Figure 3.6A). Experiment 2 produced similar (χ^2 = 1.80, df = 4, P = 0.77) results (Figure 3.6B). The majority of the dissected females (72.5% in Experiment 1 and 68.8% in Experiment 2) were mated and had only one spermatophore in the *bursa copulatrix*, regardless of plot treatment.

3.4. Discussion

The overall purpose of this study was to determine whether active removal of males or pheromone communication disruption between males and females is the main mode of action in pheromone mass trapping of the apple clearwing moth in plots treated with low and high pheromone doses. Theoretical studies by Byers (2007) predict that pheromone-based mass trapping is a better alternative to pheromone-based mating disruption because the former results in removal of males from the population and therefore lowers the probability of mate finding. Pheromone-baited traps should reduce-the probability of mating if synthetic plumes are preferred over calling females (Byers 2007). This preferential response to traps is a consequence of highly attractive pheromone blends released at a higher concentration compared to calling females (Miller et al. 2006b).

The effect of pheromone treatment on male apple clearwing moths will most likely be impacted by several factors including pheromone dose, population density and male moth behaviour. Teixeira et al. (2010) showed that the mechanism of action in pheromone-based mating disruption treated plots targeting peach tree borer males (*Synanthedon exitiosa* (Say)) is dynamic and can switch from false-trail-following in young, naive males to sensory adaptation in older, pre-exposed males. Treatment with the 1 mg lures in mass traps in the current study competed with the 1 mg pheromone-baited assessment traps and evoked false-trail-following (Bartell 1982; Miller et al. 2006a, b) to mass-trapping traps, as reflected by a reduction of male moth capture in assessment traps. In comparison, the plumes emanating from the assessment traps baited with the high dose 10 mg lures should be more detectable by male moths in the background of plumes from the low dose mass trapping traps resulting in higher trap capture in these assessment traps. Although significantly fewer males were captured in both high and low dose pheromone assessment trap types in the treated plots as compared to the untreated control plots, more males were captured in 10 mg assessment traps than 1 mg assessment traps regardless of plot treatment. This lends support to the hypothesis that plumes emanating from 10 mg-baited assessment traps are less well camouflaged (Bartell 1982; Cardé 1990) than plumes from the 1 mg-baited traps. Dose-dependent response to pheromone has been demonstrated previously for the apple clearwing moth (Judd 2008) although trap catch may not increase linearly with pheromone dose (Obeng-Ofori & Coaker 1990; Branco et al. 2006). Schlyter (1992) showed that the range of attraction to a pheromone-baited trap is dependent on the pheromone concentration because higher pheromone loads have higher release rates (Franklin & Grégoire 2001), resulting in plumes with larger active spaces (Roelofs 1978).

In the first experiment, treatment with either open or closed mass traps baited with 1 mg lures resulted in a significant reduction in the number of males captured in pheromone-baited assessment traps in treated vs. control plots. This finding indicates that the mechanisms by which mass trapping is acting in this experiment are by disruption of male moth orientation and competitive attraction and not by removal of males through trap capture. Other experiments on tree fruit pests using pheromone-based attract-and-kill formulations have also found that mate finding is impaired by pheromone treatments with or without the killing agent (Evenden & McLaughlin 2004). Similarly, treatment of cotton fields with pheromone with and without insecticide resulted in equal suppression of mating success of *Spodoptera litura* (Fabricius) (Kitamura & Kobayashi 1985) and *S. littoralis* (Boisduval) (Downham et al. 1995) (Lepidoptera: Noctuidae). In contrast, trap catch of male light brown apple moth, *Epiphyas postvvittana* (Walker) (Lepidoptera: Tortricidae) in assessment traps positioned in orchard plots treated with either an open or caged pheromone-based attracticide, where males could not contact the toxicant, showed that 50% of trap suppression was due to removal of males by insecticide exposure (Suckling & Brockerhoff 1999). The importance of male moth removal from a population may be dependent on population density and therefore, I assessed apple clearwing moth density before all replicates in both experiments. During the pre-assessment phase of this study, when treatment plots were not baited with pheromone lures, assessment traps in the experimental plots captured similar numbers of moths to the control, except for the 10 mg-baited pheromone assessment traps in Experiment 1. Nevertheless, moth densities among plots were similar throughout the experiment in the two different types of assessment traps. Treatment of cotton with pheromone formulations with or without insecticide was equally effective against the pink bollworm, *Pectinophora gossypiella* (Sanders) (Lepidoptera: Gelechiidae) at low population densities but the formulation that contained the toxicant was more effective at high population densities (Conlee & Staten 1981). In my first experiment, the population density of apple clearwing moths was high and removal of males still did not enhance the effect of pheromone alone as measured by the pheromone-baited assessment traps at both doses.

Interestingly, results from the second, high dose experiment that compared open and closed mass trapping traps baited with the high pheromone dose lures were very different than those of the low dose experiment. In the high dose experiment, male moth capture in pheromone-baited assessment traps baited with both 1 and 10 mg lures positioned in the treatment plots did not differ from the non-treated control plots as opposed to the low dose experiment. However, a comparison of trap capture in these plots between the pre-assessment and treatment phases of the experiment indicate that trap capture was reduced in all plots, including the control as a result of pheromone treatment. One explanation may be that the large amount of pheromone dispensed in the mass trapping-treated plots affected the control plot through movement of pheromone among plots. According to Milli et al. (1997) wind direction and speed can alter the structure of the pheromone cloud in a pheromone-treated orchard. For example, wind entering an apple orchard under mating disruption can produce gaps of no pheromone up to 15 m wide and can push the pheromone cloud 60 m downwind without any decrease in concentration. The net result is attraction of adults far downwind and increased infestation at the crop edges (Moser et al. 2009). Another possibility is that the high level of pheromone released in the mass trapping plots in the second experiment could have attracted males away from the control into the treated areas and contributed to the lack of statistical significance among treatments. A high trapping density coupled with a high pheromone dose, as was used in the second experiment, should maximize trapping interference and reduce moth catches in assessment traps. In comparison to the pre-assessment phase of the experiment, the 10 mg mass trapping treatments did camouflage the assessment traps, including those in the adjacent untreated control plots. Non-target effects of pheromone treatment on control plots is sometimes unavoidable in the design of pheromone-based control experiments (Novak & Roelofs 1985).

In addition to interference with male behaviour pheromone treatments can also interfere with female behaviour. In an area permeated with high doses of pheromone, beet armyworm (*Spodoptera exigua* (Hübner)) females can stop calling (Yang et al. 2009) or in the case of grape root borer (*Vitacea polistiformis* (Harris)) increase their emigration rate away from the treated area (Pearson & Schal 1999). On the other hand, females of other moth species such as the squash vine borer (*Melittia cucurbitae* (Harris)) are attracted to areas permeated with sex pheromones (Pearson 1995). Weissling & Knight (1996) showed that codling moth females exposed to codlemone increase their rate of pheromone release. Grape root borer females showed extensive movement in a mating disruption plot and 54% were able to find a male and mate (Pearson & Meyer 1996).

My approach to assess pheromone mass trapping with non-competitive kairomone-based assessment traps revealed that there was likely no change in population density as a result of mass-trapping treatment in both mass-trapping experiments. The numbers of males and females captured in kairomone-baited traps were remarkably consistent among plots and between the pre-assessment and treatment phases in both experiments. Non-competitive traps may be a more objective population assessment tool in pheromone-treated plots than pheromone-baited traps, partly because kairomone-baited traps attract moths via a different mechanism of action than pheromone-baited traps (de Bruyne & Baker 2008). This result is similar to that of Knight & Light (2005b, 2005d) who showed that traps baited with pear ester, a kairomone attractive to codling moth, *Cydia pomonella* (Linnaeus) adults, captured large numbers of codling moth males and females in plots treated with pheromone-based mating disruption. Pear ester-baited traps produced maximal catches when adjacent traps were separated by at least 10 m, which would indicate a range of attraction of around 5-7 m (Knight & Light 2005d). Kairomone-baited traps likely have a smaller range of attraction than pheromone-baited traps and may provide a better estimate of within plot moth activity, especially in small-plot experiments (Chapter 2). The kairomone-baited assessment traps used in this study have a range of attraction of less than 5 m (Chapter 2).

Another benefit of kairomone traps in population assessment of pheromone-based control tactics is their ability to attract and capture females. Captured females can be further dissected and mating status examined in order to confirm whether the treatment applied had a significant effect on mating behaviour (Knight & Light 2005c). In the current study, there was no difference in the number of males and females captured in kairomone traps which can either be attributed to an overall 1:1 sex ratio or similar attractiveness of the bait to both sexes. In spcies exhibiting protandry, such as the apple clearwing moth can still maintain an overall 50:50% sex ratio as long as the emergence rate of females continues to exceed their mortality rate for a longer period of time than the corresponding male emergence and mortality rates. Muralimohan & Srinivasa (2010) showed that populations of *Opisina arenosella* Walker have an overall 50:50 sex ratio, even though the sex ratio changes from male-biased early in the season to female-biased late in the season. Apple clearwing moth phenology curves are suggestive of a variable sex ratio, with protandry occuring initially, followed by an equal sex ratio at peak flight, which then becomes female-biased as the flight curve tapers off. Emergence of apple clearwing moths in field cages indicates that both sexes have similar emergence curves, albeit with a slight temporal separation (Judd 2008). The flight phenology curve obtained in this study showed that females likely live longer or retain behavioural responses for a longer period than males. Female codling moths show a more dose-dependent relationship than males to kairomones (Hern & Dorn 1999) and more or less specific blend ratios are required to evoke strong neural activity in the female antennal lobe (Najar-Rodriguez et al. 2010).

Females may also be differentially attracted to kairomone-baited traps based on mating status (Knight & Light 2005b, d). Most kairomones used in pest management exploit cues used by females to locate oviposition sites (Foster & Harris 1997). In the current study, kairomone-baited traps recovered more mated females than virgin females. There was no significant difference in the proportion of virgin, singly or multiply-mated females recovered in kairomone-baited traps positioned in mass trapping plots with open or closed traps as compared to control plots. These findings indicate that pheromone-based mass trapping did not interfere with mating behaviour as compared to control plots. Recovery of few virgin females in pheromone-treated plots does not necessarily mean that pheromone treatment is ineffective at population control. In mating disruption studies of codling moth that resulted in adequate population control, only between 5 (Light et al. 2001) and 20% (Knight & Light 2005b) of females recovered in kairomone-baited traps were virgin. The effectiveness of mass trapping on subsequent oviposition and larval infestation remains to be studied in this system.

Most apple clearwing moth females appear to be mated only once and only between 2 and 5% of females captured in kairomone traps were virgin regardless of plot treatment. Of the small proportion of multiple-mated females recovered in my study, the most common were females mated twice and the rarest were females mated six times (less than 0.05%). Knight (2007) reported that pheromone permeation negatively affected the ability of codling moth females to mate more than once due to the effect of pheromone on delaying rather than eliminating mating behaviour. A similar phenomenon could have occurred in the pheromone-baited mass trapping plots in this study but assessment of egg laying and offspring survival are required to determine if mating delay can contribute to population control in this species as it has been shown in other Lepidoptera (Jones & Aihara-Sasaki 2001).

3.5. Literature cited

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Figure 3.1. Schematic arrangement of the different pheromone plot treatments tested in 2010. Experimental plots were 0.9 ha and control was 0.5 ha. Grey dots represent the mass traps, baited with either 1 (Experiment 1) or 10 (Experiment 2) mg apple clearwing moth sex pheromone. “X” indicates plugged traps (the treatment that mimics mating disruption). White and black circles represent pheromone assessment traps baited with 1 and 10 mg apple clearwing moth sex pheromone, respectively. Dark grey dots represent kairomone assessment traps baited with 300 ml fresh Concord grape juice.

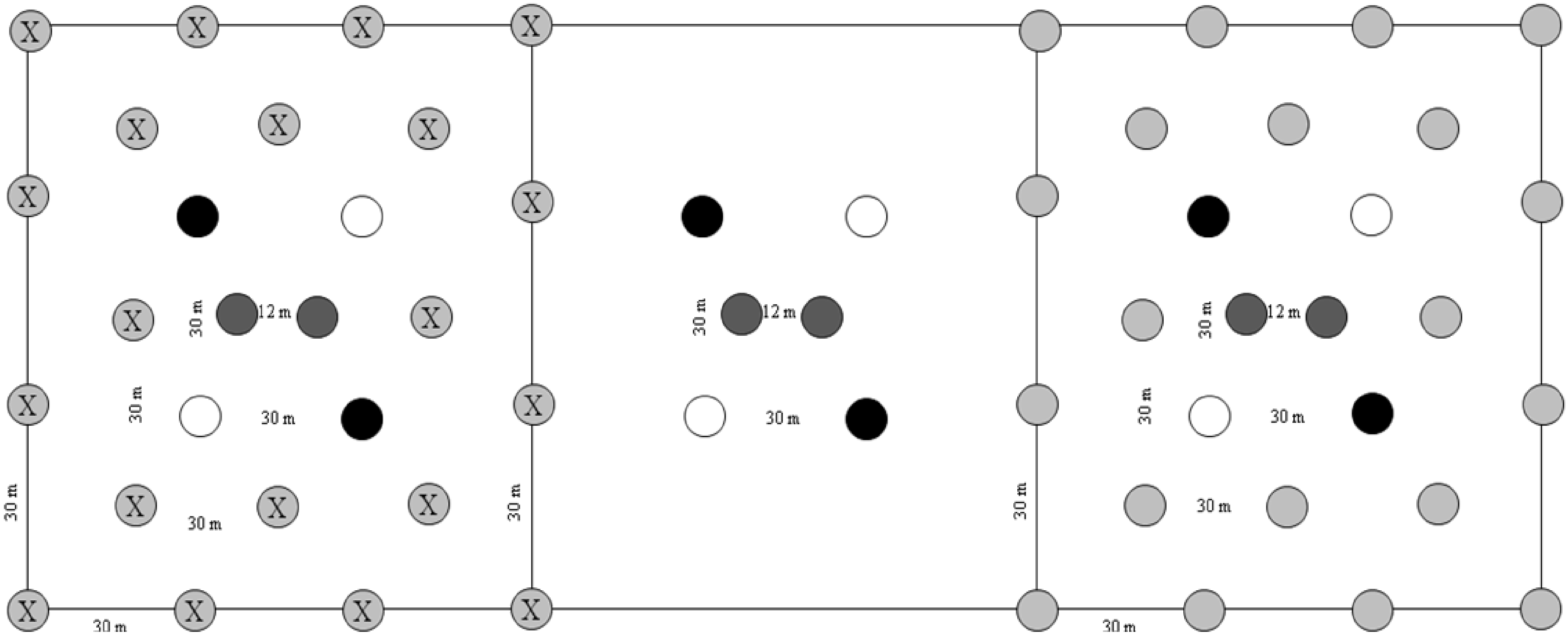

X
30 m
12 m
30 m
30 m
30 m
30 m
30 m
30 m
12 m
30 m
30 m
30 m
12 m
30 m
30 m
30 m
30 m

Figure 3.2. The mean number of apple clearwing moths captured in assessment traps ± Poisson Error (P.E.) bars in the pre-assessment phase before pheromone treatment and during the experiment when plots were treated with 1 mg pheromone mass traps. Bars labelled with different lowercase letters indicate statistical significance between the different plot treatments for each bait type and uppercase letters indicate statistical significance between the different bait types (or between males and females) ($\alpha = 0.05$).

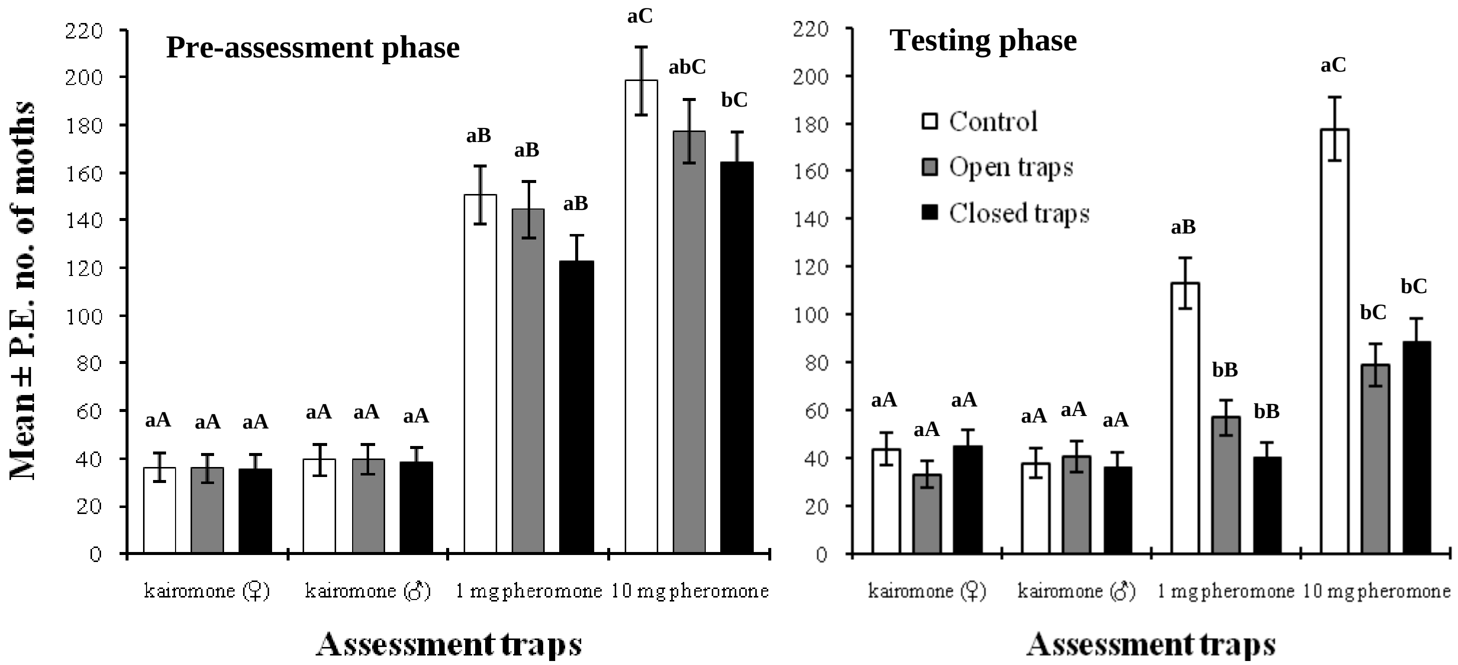

Pre-assessment phase
Testing phase
Mean ± P.E. no. of moths
0
20
40
60
80
100
120
140
160
180
200
220
aA
aA
aA
aA
aA
aA
aB
aB
aB
aC
abC
bC
aA
aA
aA
aA
aA
aA
aB
bB
bB
aC
bC
bC
Control
Open traps
Closed traps
kairomone (♀)
kairomone (♂)
1 mg pheromone
10 mg pheromone
Assessment traps

Figure 3.3. Effect of treatment with 1 mg pheromone in open and closed traps over time in Experiment 1 as determined by best fit curves with 95% shaded confidence bands. Darker grey areas represent overlap regions. Replicate 1 started on 28 June and Replicate 6 started on 03 August 2010. There was a significant difference between treatments and control from the onset of experiment, but not between the different pheromone treatments. Assessment traps were baited with (A) 1 mg and (B) 10 mg of apple clearwing moth pheromone.

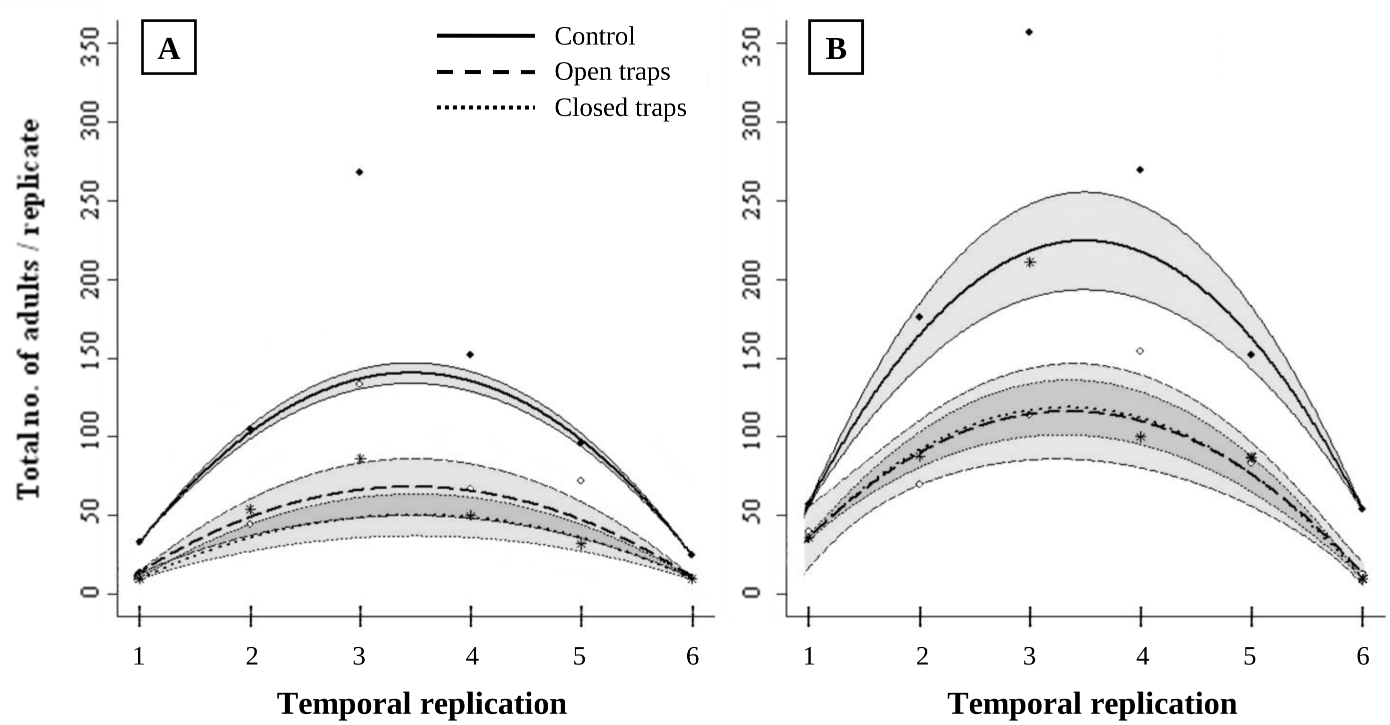

A
B
Control
Open traps
Closed traps
Total no. of adults / replicate
0
50
100
150
200
250
300
350
1
2
3
4
5
6
Temporal replication
Temporal replication

Figure 3.4. Local phenology of the apple clearwing moth flight as determined by pooled weekly counts in assessment traps in control plots for Experiments 1 and 2. Monitoring started on 21 June and ended on 09 September 2010.

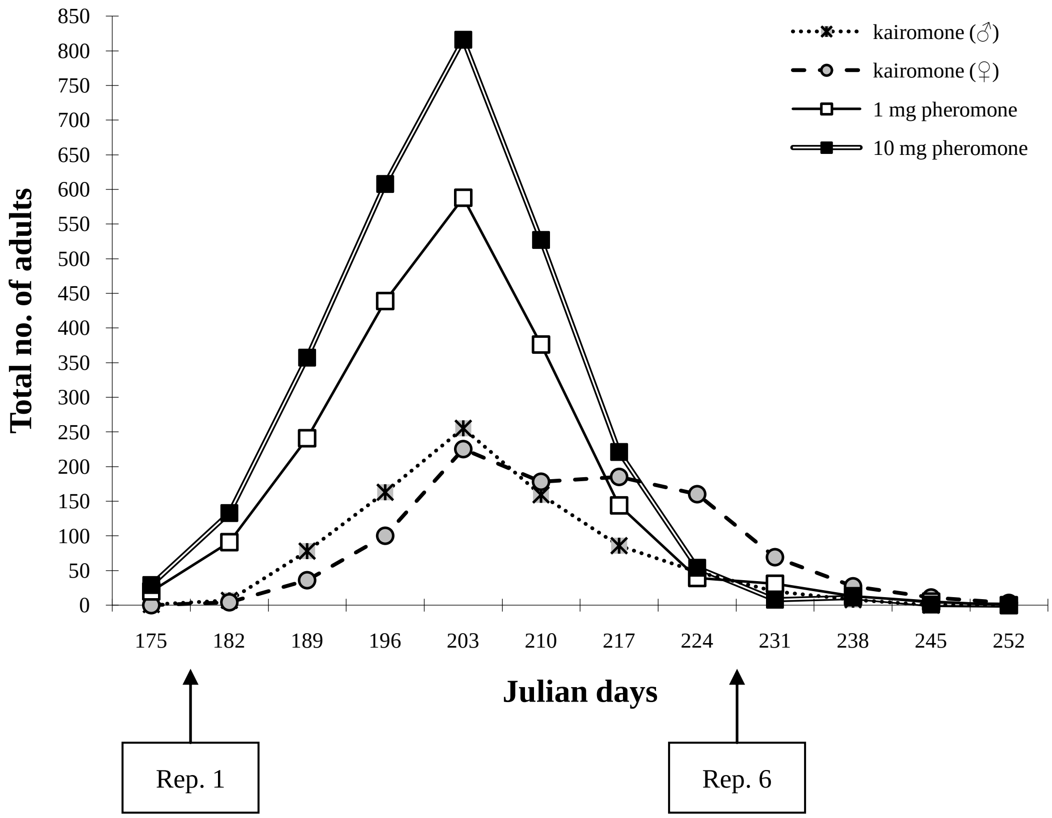

850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Total no. of adults
175
182
189
196
203
210
217
224
231
238
245
252
Julian days
kairomone (♂)
kairomone (♀)
1 mg pheromone
10 mg pheromone
Rep. 1
Rep. 6

Figure 3.5. The mean number of apple clearwing moths captured in assessment traps ± Poisson Error (P.E.) bars in the pre-assessment phase before pheromone treatment and during the experiment when plots were treated with 10 mg pheromone mass traps. Bars labelled with different lowercase letters indicate statistical significance between the different plot treatments for each bait type and uppercase letters indicate statistical significance between the different bait types (or between males and females) ($\alpha = 0.05$).

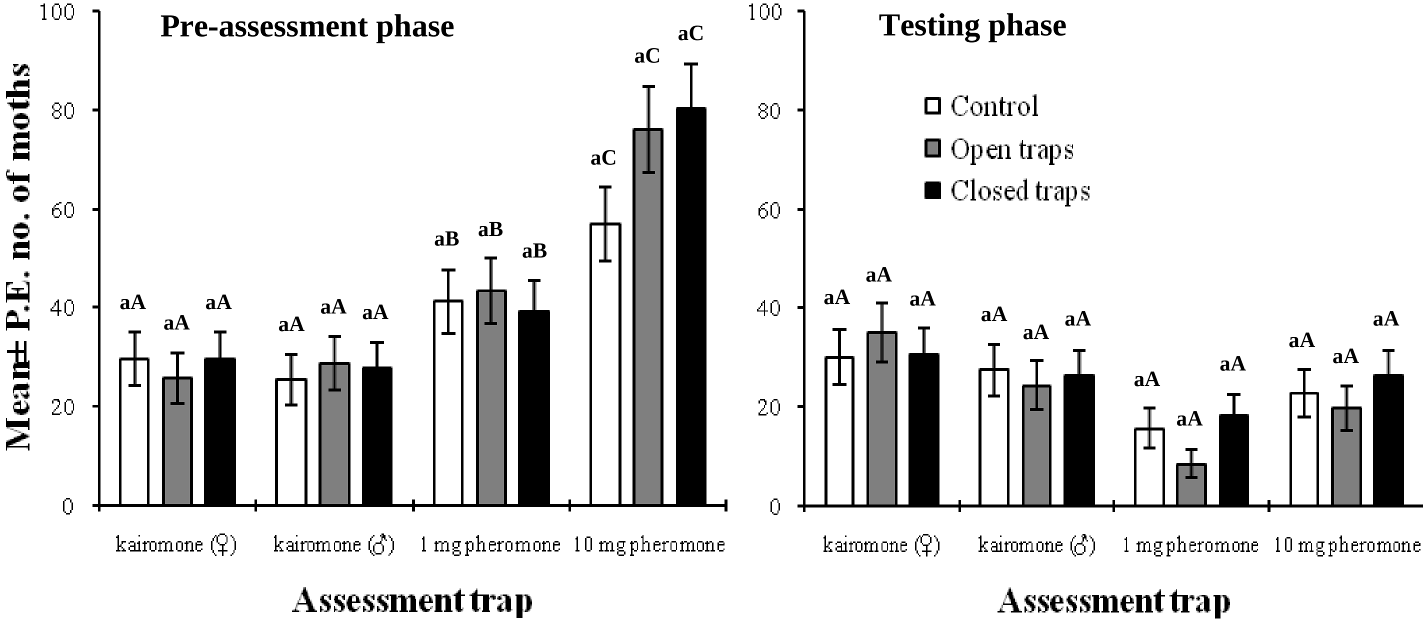
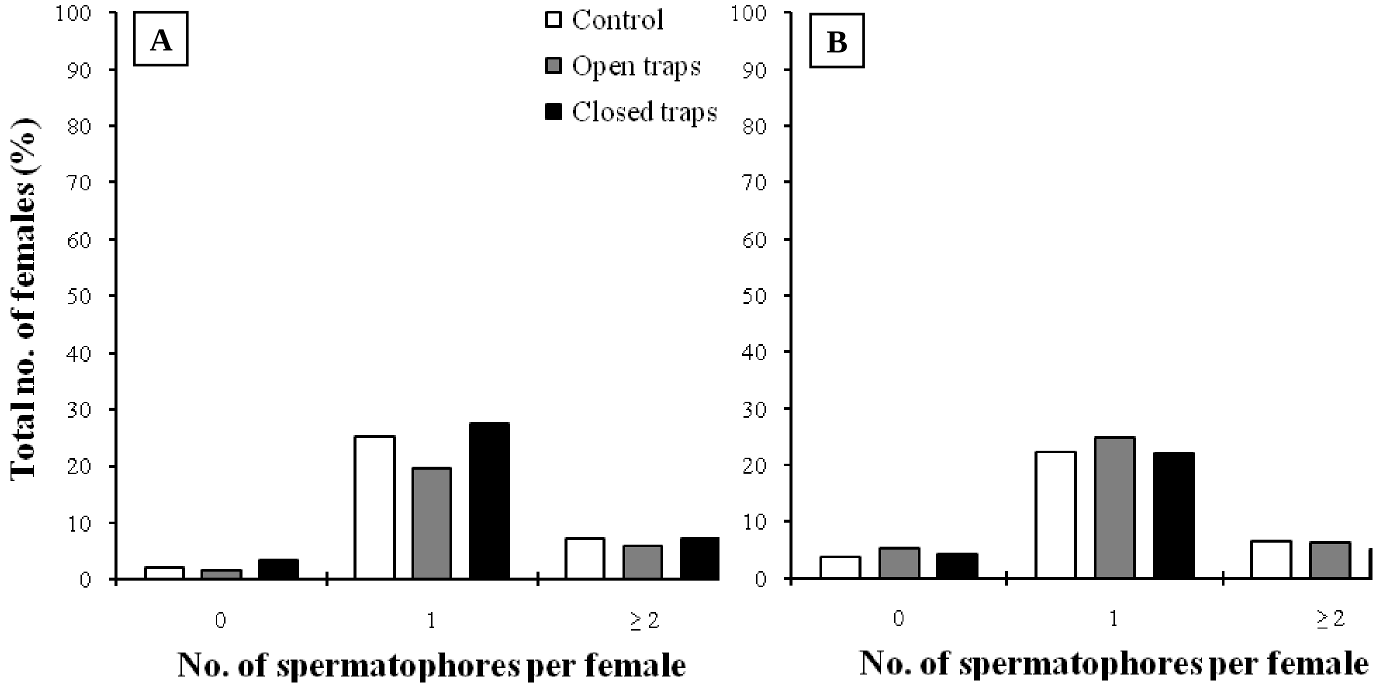

Pre-assessment phase
Testing phase
Mean± P.E. no. of moths
0
20
40
60
80
100
kairomone (♀)
kairomone (♂)
1 mg pheromone
10 mg pheromone
Assessment trap
□ Control
▨ Open traps
■ Closed traps
aA
aB
aC

Figure 3.6. The proportion of apple clearwing females with 0, 1 and 2 or more spermatophores captured in kairomone assessment traps in plots treated with (A) 1 mg pheromone or (B) 10 mg pheromone in open or closed traps.


A
B
Control
Open traps
Closed traps
Total no. of females (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
≥ 2
No. of spermatophores per female

Chapter 4. Diversity and abundance of arthropod by-catch in semiochemical-baited traps in conventionally- and organically-managed apple orchards

4.1. Introduction:

Intensive agricultural practices and global trade have contributed to an increase in the number and severity of insect pests worldwide (Krcmar-Nozic et al. 2000; Hulme 2009; Perrings et al. 2010) while concomitantly causing a decrease in biodiversity in managed and impacted natural ecosystems (Donald et al. 2006; Wilson et al. 2010).

The effect of insecticide use on biodiversity in agricultural landscapes has not been well studied to date. In European farms, 13 factors associated with intensive agriculture were studied to determine their impact on biodiversity levels in several selected taxa (Geiger et al. 2010). Pesticide use was consistently associated with decreased biodiversity of carabid beetles and birds sampled in the variously managed farms (Geiger et al. 2010). The presence of pesticides can negatively affect population densities of resident insectivorous bird species (Bouvier et al. 2011) most likely because of decreased prey abundance (Benton et al. 2002; Boatman et al. 2004) or because of direct neurotoxic effects (DeWitt 1956; Forsyth & Martin 1993; Fry 1995). Partitioning biodiversity measurements of plants, bees and ground-dwelling arthropods in organically and conventionally managed winter wheat fields showed an overall increased diversity of plants and bees both locally and regionally but decreased spider diversity regionally in organically managed systems (Clough et al. 2007). More locally, the extirpation of the Viceroy (*Limentis archippus* (Cramer)) from the Southern Interior of British Columbia (BC) can be attributed to the intensive use of pesticides (Guppy et al. 1994). Perennial cropping systems are generally believed to support greater levels of biodiversity than highly disturbed annual crops (Altieri 1999; Tscharntke & Kruess 1999). Several comparative studies conducted in various cropping systems showed higher local arthropod diversity in organic systems than in conventionally managed crops (Bengtsson et al. 2005; Schmid et al. 2011; Winqvist et al. 2011), although the effect varies with size of arable field and presence of adjacent wild habitats (Bengtsson et al. 2005; Gabriel et al. 2006).

Biodiversity estimates depend on both the scale (e.g. plot level vs. landscape level) and the group of organisms studied (Bengtsson et al. 2005), most likely due to an interplay of several factors such as organism size, sampling method, vegetative cover area and the presence of large patches of undisturbed habitat adjacent to crops. In general, small plots surrounded by large portions of wild habitat harbour a more diverse arthropod fauna than larger cultivated plots irrespective of how they are managed (Benton et al. 2003; Prasifka et al. 2005). Weed cover area has a significant effect on arthropod and bird diversity such that the greater and more diverse the weed cover the higher the overall diversity (Qureshi et al. 2010; Eyre & Leifert 2011). The presence of undisturbed wild habitats around the agricultural area increases local biodiversity in cultivated plots (Benton et al. 2003; Fuentes-Montemayor et al. 2011).

A suitable assessment method must be chosen to reflect the overall biodiversity levels of the target taxa in a cropping area. Biodiversity estimates are highly dependent on the type of trap, its placement (Miliczky et al. 2000) and colour. For example, the majority of diurnally-active arthropod groups sampled in either a salt marsh or a tropical forest showed a preference for yellow colour (Hoback et al. 1999; Abrahamczyk et al. 2010). Yellow traps have been demonstrated to be a good tool to monitor populations of beneficial insects and parasitoids of apple orchard pests (Neuenschwander 1982). Colour contrast against the surrounding crop foliage may also be important for some visually-orienting insects. Allan & Stoffolano (1986a, 1986b) directly tested this assumption and determined that the contrast between the spectral reflectance of a trap and its background is responsible for the attraction of gravid female greenhead horse fly (*Tabanus nigrovittatus* Macquart) to variously coloured sticky panel traps.

In an attempt to minimize environmental degradation and loss of biodiversity, there has been movement away from the use of synthetic pesticides and fertilizers and toward the adoption of more environmentally sustainable practices (Geiger et al. 2010). The major goal of organic agriculture is the long term sustainability of agroecosystems and minimization of detrimental environmental impact (Raynolds 2000; Pimentel et al. 2005). A lack of policy regulating organic food production and its enforcement in the European Union (Moschitz & Stolze 2007; Stolze & Lampkin 2009) and the United States has caused debate over what should be considered organic food (Harrison 2008;

Darnhofer et al. 2010). In addition, some organically approved pesticides are detrimental to many non-target arthropods. One such example is Entrust®, a spinosad-based, fungal-derived neural regulator, which causes involuntary muscle contractions, paralysis and death of arthropods (Kirst 2010). Spinosad has collateral effects on several non-pest arthropods including parasitoids (Elzen et al. 2000; Arthurs et al. 2007; Cossentine et al. 2010) and predatory arthopods such as hover fly (Diptera: Syrphidae) larvae, minute pirate bugs (Hemiptera: Anthocoridae) (Smith et al. 2008) and the crab spider *Philodromus cespitum* (Walckenaer) (Araneae: Philodromidae) (Řezáč et al. 2010). Its lethal effect is especially severe in *Doru taeniatum* (Dohrn) (Dermaptera: Forficulidae) earwigs (Cisneros et al. 2002). A second example of an organically-approved insecticide with non-target effects is *Bacillus thuringiensis* variety *kurstaki* (Berliner), a bacterial-derived insecticide used against the larval stage of many lepidopteran pests (Höfte & Whiteley 1989). Its non-target effects include all foliage feeding lepidopteran larvae (Johnson et al. 1995; Boulton & Otvos 2004; Boulton et al. 2007). Some direct lethal effects have been reported on Monarch butterflies (*Danaus plexippus* (Linnaeus)) fed pollen from the transgenic *B.t.* event 176 corn lines (Losey et al. 1999; Hansen-Jesse and Obrycki 2000), although those lines account for less than 2% of the total transgenic *B.t.* corn grown in the US and are being phased out (Gatehouse et al. 2002). *B.t.k.* also has indirect negative fitness effects on predatory insects, such as green lacewings, fed with diseased larvae (Hilbeck et al. 1998).

Individual studies examined the lethal or sublethal effect of pesticides on various groups of arthropods. However, examination of pesticide applications *in situ* on the overall abundance and diversity of arthropod communities continues to lag behind laboratory studies. The first goal of this study was to determine whether semiochemical-baited apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) traps capture a wide variety of non-target arthropod groups, which could potentially affect the local biodiversity. Several other experiments indicate that sex pheromone-baited yellow-coloured traps used in monitoring or control of various moth pests in orchard systems capture a wide variety of non-target arthropods such as various Hymenopteran groups (Meagher & Mitchell 1999; Clare et al. 2000; Meagher 2001). The second goal of this study was to document whether arthropod diversity (as measured to family level) is different between conventionally- and organically-managed apple orchards in the Southern Interior of BC, as measured by capture of non-target arthropods in semiochemical-baited traps already in use to manage the apple clearwing moth in apple orchards.

4.2. Materials and Methods:

Study sites

Arthropod diversity and abundance were sampled at 16 paired sites in Cawston, BC (49.15 N & -119.74 W) using semiochemical-baited traps targeting the apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) (Figure

2.1). Eight sites consisted of apple orchards organically certified by the British Columbia Certified Organic Program for more than 20 years (organic management). The other eight sites consisted of apple orchards treated with synthetic pesticides and fertilizers (conventional management) (Table 4.1). Of the eight orchards in each management type, four were superspindle-type plantings with an average density of 5235 apple trees / ha and the other four were standard plantings with an average planting density of 882 apple trees / ha (Table 4.1).

Semiochemical-baited traps

Arthropod biodiversity and abundance were sampled using two types of semiochemical-baited traps that are being tested as a potential management option against the apple clearwing moth in apple orchards in the Southern Interior of BC (Chapters 2 & 3). Sex pheromone-baited traps consisted of yellow non-saturating Unitraps (AgBio Inc., Westminster, CO, USA) baited with grey halobutyl rubber septa (West Co., Lyonville, PA, USA) impregnated with 1 mg of the sex pheromone of the apple clearwing moth, (*Z,Z*)-3,13 octadecadienyl acetate ((Z,Z) 18:Ac) (Pherobank, Wageningen, The Netherlands; > 95% isomeric purity) diluted in HPLC grade hexane (Aldrich Chemical Co., Milwaukee, WI, USA). Yellow kairomone traps (Figure 2.1B) were baited with 300 ml fresh Concord grape juice (SunRype^{TM}, Kelowna, BC), a known attractant for the apple clearwing moth (Judd 2008).

Experimental design

Arthropods were sampled from 12 June to 31 July 2009, which corresponded to most (> 60%) of the apple clearwing moth flight. At each site, two pheromone and two kairomone traps were deployed ~1.5 m above the ground, were separated from each other by at least 30 m and were positioned a minimum of 20 m from the edge of the orchard. At the site and grape juice in the kairomone traps was replaced weekly and pheromone lures were replaced every four weeks. Traps were checked weekly and all captured arthropods, including the apple clearwing moths, were placed into 14.8 ml vials (Fisher Scientific, Pittsburgh, PA, USA), transported to the laboratory and stored at 0°C until they could be processed for identification to family and/or genus.

Cleaning procedure

Arthropods captured in juice-baited kairomone traps required cleaning before they could be identified. Upon removal from the freezer, the catch from each individual vial was transferred into ~ 300 ml distilled water, kept at 40 – 50°C and stirred periodically. After five minutes in the water, specimens were transferred to a tray and allowed to dry for ~ 5 minutes before they were placed into 40 ml HPLC grade chloroform (Fisher Scientific, Pittsburgh, PA, USA) to be dehydrated for 24 hours. Small (< 5 mm) or soft-bodied arthropods (e.g. Neuroptera) were kept separately from the larger ones. Insects were removed from the chloroform, allowed to dry for another 24 hours and then stored in 15.8 ml vials for identification to family level and/or genus at a later time (Table 4.2).

Statistical analyses

Community-level analysis was conducted using non-metric multidimensional scaling (McCune et al. 2002) of the shortest ranked distances in multidimensional space of raw untransformed trap counts (specimens / arthropod family) in order to assess variation attributed to orchard management, planting type and trap type on family level diversity, as replicated every week over a seven-week period. P-values comparing family-level arthropod distributions between pheromone and kairomone traps, organically- and conventionally-managed orchards and standard (average of 882 trees / ha) and superspindle (average of 5235 trees / ha) tree plantings were extracted from ordination graphs using a multiple response permutation procedure (McCune et al. 2002) and were adjusted with a Bonferroni correction procedure (to reject null hypothesis p-value $\leq \frac{\alpha}{n}$, where $\alpha = 0.05$ and n = number of pairwise comparisons, 3 in this case). All community level analyses were done in PC-ORD (Windows version 5.10, MjM software™). The only arthropods captured that were not included in the analyses were the apple clearwing moths, worker ants (Hymenoptera: Formicidae) and thrips (Thysanoptera).

Representative species were extracted from the overall dataset and analyzed separately to determine if season-long pooled trap captures varied between the two management types. Orchard planting type was not significant in the community-level analysis and was not included as a variable in individual analyses. General Linear Mixed Effects Models with square root transformed season-long pooled trap catch specified as the dependent variable and orchard management type as fixed factor with individual orchard specified as a random variable were designed for each chosen taxon. Taxa were chosen for detailed analysis based on their importance to ecosystem function in this managed setting. Analyzed taxa included five predators (*Agulla* species complex (nr. *unicolor* Carpenter) (Raphidioptera: Raphidiidae), *Chrysopa* sp. *Chrysoperla* sp. (nr. *carnea sensu lato* (Stephens)) (Neuroptera: Chrysopidae), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and *Stethorus punctum* (LeConte) (Coleoptera: Coccinellidae)), which are potential biological control agents. Pestiferous species were also considered for individual analyses. The mullein bug, (*Campylomma verbasci* (Meyer) (Hemiptera: Miridae)), was frequently captured and is considered an important pest of tree fruits in this region (Smith & Borden 1990; McBrien et al. 1994a). Interestingly, the meal moth *Pyralis farinalis* (Linnaeus) (Lepidoptera: Pyralidae), a pest of stored products (Levinson & Buchelos 1981; Madrid & Sinha 1982), was captured in semiochemical-baited traps positioned in the orchard setting in high enough numbers to be analyzed separately. Other herbivores included in individual analyses were the sap beetle, *Glischrochilus quadrisignatus* (Say) (Coleoptera: Nitidulidae)) and *Telamona praealta* (Fowler) (Hemiptera: Membracidae). These two latter species are not apple orchard pests in the Southern Interior of BC but are considered minor pests elsewhere.

4.3. Results

A total of 7001 non-target arthropod specimens, excluding ant workers (Hymenoptera: Formicidae) and thrips (Thysanoptera) were collected in all semiochemical-baited traps over the course of the seven-week sample period in 2009. Trap type had a significant ($P < 0.001$) effect on the family-level community composition of arthropods captured (Figure 4.2A). Of the total number of sampled specimens, 91% were collected in juice-baited kairomone traps, while the rest were collected in pheromone-baited traps. Only 13 families were recovered from pheromone-baited traps while 25 families were recovered from kairomone-baited traps. Many groups (e.g. Noctuidae, Lygaeidae, Cicadellidae, Membracidae, Sciaridae, Phoridae, Hybotidae, Apidae, Halictidae, Coccinellidae, Chalcidoidea, Formicidae (winged reproductives) and Salticidae) were represented by at least 7 specimens in both trap types. The family-level community composition between organic and conventionally-managed apple orchards was significantly different ($P = 0.001$, Figure 4.2B). Fifty seven percent of the total number of specimens was captured in organic orchards while 43% was captured in conventional orchards. Planting type did not have a significant effect on the family-level community composition of the arthropod specimens captured ($P = 0.56$, Figure 4.2C), with specimens captured in pheromone and kairomone traps being evenly distributed between superspindle and standard plantings. More families were recovered from kairomone-baited traps than pheromone traps and from traps placed in organic orchards as opposed to traps placed in conventional orchards (Figure 4.3).

Individual analyses of chosen taxa illustrated a significant effect of orchard management type on the population densities of some of the sampled species (Figure 4.4). Significantly more specimens of *Harmonia axyridis* (P = 0.02), *Pyralis farinalis* (P = 0.03) and *Telamona praealta* (P = 0.01) were captured in organic orchards than in conventionally managed orchards. The difference in the number of *Chrysopa* sp. captured in organic and conventional orchards was minimally significant (P = 0.05). For the remainder of the studied groups, trap capture did not differ significantly (P > 0.1) between organic and conventional orchards (Figure 4.4).

Out of the 6360 specimens captured in juice-baited kairomone traps, the highest percentage of total catch was represented by true flies (Diptera) with 24% of all recovered specimens. Beetles (Coleoptera) represented19%, moths (Lepidoptera) 18.5%, lacewings (Neuroptera) 14% and bees, bumblebees and wasps (Hymenotpera) 11% of the specimens captured in juice-baited kairomone traps. The rarest groups in the kairomone traps were true spiders (Araneae) with 0.4%, barklice (Psocoptera) with 0.03% and damselflies (Odonata) with 0.02% of all recovered specimens (Figure 4.5). Thirty-six percent of the fly families known from BC were recovered in kairomone traps positioned in apple orchards in this study. Kairomone traps also attracted a high diversity of beetles and moths with

14 and 11% of families known from BC in these two orders, respectively. Four of the eight neuropteran families known from BC and one snakefly (Raphidioptera) family of the two present in BC were recovered from kairomone traps (Figure 4.6).

At least 60% of families were represented by only one to two morphospecies. Samples of other families, Coccinellidae, Nitidulidae, Dermestidae, Muscidae, and Apidae consisted of at least five morphospecies. The most diverse groups sampled were the Noctuidae and the Chalcidoidea each of which contained at least ten morphospecies.

4.4. Discussion

In the present study I examined the biodiversity of non-target arthropods recovered as by-catch in semiochemical-baited traps targeting the apple clearwing moth in apple orchards under organic or conventional management in high density and standard plantings. I also examined the relative abundance of selected taxa important to various ecosystem services in these managed agroecosystems. I finally illustrated the suitability of kairomone traps as a surveying tool for various arthropod groups of economic or ecological significance.

Over the course of the seven weeks, 7001 specimens were recovered from both the apple clearwing moth sex pheromone-baited yellow Unitraps® and the Concord grape juice-baited yellow bottle traps. A large variety of arthropod families were recovered from both trap types. Some such as Apidae, Halictidae, Megachilidae and Syrphidae were more prevalent in the pheromone-baited traps. Bumblebees (*Bombus* sp.) and honeybees (*Apis mellifera* Linnaeus) (Apidae) and other acualeate Hymenoptera are a common by-catch in pheromone trapping experiments using yellow-coloured funnel traps (Herman et al. 1994; Weber et al. 2005). Adults belonging to these groups are important pollinators of flowers so their mass removal may negatively impact the level of pollination.

Most (91%) of the arthropod specimens were captured in the Concord grape juice-baited traps. The chemical profile of Concord grape juice is both complex and dynamic, changing as the juice ferments (Massa et al. 2008). Complex chemical profiles are attractive not only to the target pest but also to other arthropods. For example, traps baited with methyl eugenol, a known kairomone of the Oriental fruit fly (*Bactrocera dorsalis* (Hendel)) or a mixture of methyl eugenol and decaying Oriental fruit flies attract a large number of non-target species including 187 species endemic to the study area (Leblanc et al. 2009). Similar to my study the most captures were represented by Diptera (94.9% of total specimens captured) (Leblanc et al. 2009). Kairomone-based mass trapping system targeting the Oriental fruit fly might negatively affect the abundance of non-target groups, including endemics, pollinators, parasitoids and predators (Leblanc et al. 2009). Therefore, any traps designed to mass trap the apple clearwing moth should be further optimized in order to minimize their impact on non-targets.

Implications of management practices on the overall arthropod diversity

My results support the growing body of evidence that arthropod communities are more complex in organically-managed than conventionally-managed crops. There was a significantly greater overall diversity of arthropod families in organically- as compared to conventionally-managed orchards in this study. Assessment of biodiversity of arthropods in paired orchard studies indicates more diverse communities exist in organically- than conventionally-managed farms (Bengtsson et al. 2005; Hole et al. 2005; Schmid et al. 2011). Epstein et al. (2000) conducted a similar survey of arthropod fauna in paired conventional and organic apple orchards in Washington and Oregon and determined that pesticides have an adverse effect on the overall community composition of ground-dwelling arthropods captured in pitfall traps and population densities of carabid beetles, European earwigs (*Forficula auricularia* Linnaeus) and hunting spiders *sensu lato*. I did not quantify the presence of European earwigs captured in the 2009 arthropod survey but casual observations seem to indicate a preponderance of earwigs in organic as opposed to conventional orchards. Management type may facilitate (or inhibit) arthropod movement into apple orchards from adjacent habitats (Altieri & Schmidt 1986) and this can have an impact on the entire agroecosystem. High insect diversity (Benton et al. 2002) and habitat heterogeneity (Freemark & Kirk 2001; Genghini et al. 2006) is also strongly correlated with high bird diversity in apple orchards. Similarly, bat species richness is higher in organic than conventionally-managed agricultural landscapes, mostly because of higher prey abundance and cleaner water (Wickramasinghe et al. 2003). In a few cases, toxicity of pesticides sprayed in orchards has been shown to be directly responsible for the rapid decrease in various bird species (Forsyth & Martin 1993; Fluetsch & Sparling 1994). A second important feature of organic management is the restriction of synthetic fertilizers and promotion of organic manure, which can further enhance the overall biodiversity levels. Synthetic nitrogen-based fertilizers decrease soil fertility (Mäder et al. 2002), overall biodiversity of soil microorganisms (Sarathchandra et al. 2001; Gu et al. 2009) and microarthropods (Doles et al. 2001) and can further exacerbate pest densities by decreasing plant resistance mechanisms (Yardim & Edwards 2003).

Interestingly, planting type (i.e. standard vs. superspindle) did not affect overall arthropod composition. There are many factors that may have differed between the two planting types but canopy cover and habitat heterogeneity are two components of the system that were likely greater in the standard compared to the superspindle plantings. Canopy cover of Bolivian tropical and subtropical forests has a significant effect on the number of hymenopteran parasitoids captured in pan traps (Abrahamczyk et al. 2010). In the present study, both pheromone and kairomone traps captured more carnivores than herbivores, irrespective of the type of bait used but this did not vary with orchard planting type. Habitat heterogeneity increases local butterfly biodiversity in cereal field headlands and margins by promoting habitat availability (Rundlöf & Smith 2006). It is likely that the surrounding habitat in my study had more of an effect on biodiversity measurements than the planting type itself as traps were designed and positioned to capture actively flying insects. My sampling area included a wide variety of orchard types (e.g. apple, pear, peach, cherries or mixtures), vineyards and wild habitats including riparian areas on the Similkameen river banks. The dominant plant communities around orchards were Great Basin sagebrush (*Artemisia tridentata* Nuttall) shrub-steppe plant communities with interspersed *Pinus ponderosa* Douglas ex C. Lawson pines. Dispersal of many arthropod groups into promixal agricultural systems is a regular occurrence (Duelli et al. 1990; Samu et al. 1999; Fuentes-Montemayor et al. 2011). The rate of dispersal into agricultural habitats is likely dependent on plot size; the orchards in the present study were between 0.3 and 15 ha in size (Table 4.1). Farm size plays an important role in shaping biodiversity in agroecosystems of a wide variety of organisms such as birds, bumblebees, butterflies and herbaceous plants (Belfrage et al. 2005). To my knowledge the present study is the first to examine the effect of planting type on the diversity of arthropod fauna.

The effect of management on several economically important groups

I selected several individual taxa based on the ecosystem service they provide in order to illustrate that juice-baited kairomone traps could be used as a monitoring tool for these taxa and also to determine if individual populations were affected by orchard management type. The predaceous species studied may be important natural biological control agents in orchards and it is commonly believed that carnivorous species are more susceptible to pesticides than herbivorous insects (Mullin 1985; Roush & Daly 1990). The herbivore species represent pest and non-pest species within the orchard system and a stored products pest that presumably dispersed into the area. Selection of species groups is not only based on their importance in agricultural systems but also because no reliable population assessment methods currently exist for many of them.

On average, *Chrysopa* sp. and *Chrysoperla* lacewing populations are higher in conventional than in organic orchards, although results are not statistically significant for *Chrysoperla* sp. and only minimally significant for *Chrysopa* sp. The sensitivity of lacewings to pesticides varies for each group, with some groups (e.g. *Chrysoperla carnea*) more resistant and others (e.g. *Chrysopa formosa* Brauer) more susceptible to pesticide poisoning (Bozsik 2009). When organophosphates and organochlorines were first introduced they were reported to kill both larvae and adult *Chrysoperla carnea* (Bartlett 1964). Recent field studies show *C. carnea* can develop resistance to organophosphates and pyrethroid insecticides without fitness associated costs (Pathan et al. 2008; Sayyed et al. 2010). *Chrysopa oculata* (Say) larvae and adults are killed by exposure to Guthion®, a broad-spectrum organophosphate (Pree & Hagley 1985). In my study only one grower included Guthion® as part of his yearly spray schedule (Table 4.1) against the apple clearwing moth. *Chyropa lacciperda* Kimmis, a predator of the lac scale insect (*Laccifer lacca* (Kerr)) showed high mortality rates when exposed to low doses of various broad spectrum insecticides, including spinosad and carbosulfan (Singh et al. 2010). A few growers in this study used either Entrust® or Success® (Table 4.1), both of which are spinosad-based insecticides.

A high number of *Chrysopa* captured in kairomone traps deployed in conventionally-managed orchards may be the result of increased prey density in those plots. *Chrysopa* adults show a strong preference for aphids over other soft bodied insects (Canard 2001). All of the conventional orchards examined were severely infested with woolly aphids, a situation similar to the one observed by Shaw & Wallis (2008). Higher numbers of *Chrysopa* were captured in traps baited with floral volatiles and aphid sex pheromones than floral volatiles alone (Koczor et al. 2010). High numbers of lacewings in conventional orchards might also indicate that natural enemies impact lacewing populations in organic orchards. Parasitism rates by several hymenopteran groups such as *Telenomus* sp. (Hymenoptera: Scelionidae) can easily depress *Chrysoperla carnea* (*sensu lato*) populations (Karut et al. 2003). I found a large number of parasitoids in samples from organically-managed orchards and many of them belonged to the family Scelionidae. These diminutive hymenopterans are egg endoparasitoids of a wide variety of arthropod groups, including Lepidoptera, Hemiptera, Diptera, Neuroptera and Araneae (Austin et al. 2005) and are important biological control agents for some pest species (Caltagirone 1981; Clarke 1990).

The effect of pesticides on beetles in the family Coccinellidae depends on the chemical type and the species studied (Obrycki & Kring 1998). Multi-spotted Asian lady beetle (*Harmonia axyridis* (Coleoptera: Coccinellidae)), a generalist predator with a preference for aphids, was captured less frequently in conventionally-managed compared to organically-managed apple orchards in this study, whereas densities of the spider mite destroyer (*Stethorus punctum* (LeConte) (Coleoptera: Coccinellidae)) were unaffected by management type. These two species are important biological control agents of aphids and spider mites, respectively (Obrycki & Kring 1998; Biddinger et al. 2009). Both coccinellids are sensitive to growth regulators (James 2004) when exposed at the first instar stage and exposure caused close to 100% mortality in all larval stages of Multi-spotted Asian lady beetle (James 2004). Spider mite destroyer adults are susceptible to several insecticides, including organophosphates and growth regulators (Biddinger & Hull 1995), but in general are able to tolerate broad spectrum insecticides better than the Multi-spotted Asian lady beetle (James 2003). *Stethorus bifidus* Kapur, a related spider mite destroyer species, can move into apple orchards in response to increasing spider mite densities (Shaw & Wallis 2008) and therefore beetle density may reflect prey density in the variously managed orchards.

Other predators that were examined in this study include *Agulla* spp. (Raphidioptera: Raphidiidae) which were captured more commonly in organic than in conventional orchards, although these results are not statistically significant. *Agulla* larvae and adults are active, generalist predators and feed on a wide variety of soft-bodied insect species, including their own larvae (Cannings & Scudder 2007). Although *Phydippus* species complex (Araneae: Salticidae) was not examined in detail due to low numbers of specimens captured, more than 80%

of recovered specimens were captured in organic orchards. Jumping spiders are very sensitive to broad spectrum pesticide treatments and can be entirely absent from heavily sprayed conventional apple orchards (Miliczky et al. 2000).

The taxa examined here are effective predators that can regulate aphid populations in apple orchards. Coccinellids and salticids can migrate from adjacent wild areas into orchards most likely as a response to increased prey density (Miliczky & Horton 2005). Snakeflies (Neuroptera: Raphidiidae) have been recovered in adjacent habitats but not inside apple orchards in previous studies (Miliczky & Horton 2005), but this may be an artefact of inadequate sampling. Personal observations indicate that snakeflies are common in hedge rows and Great Basin sage brush habitats adjacent to apple orchards throughout the Southern Interior of BC. Their presence within orchards indicates that they may serve as biological control agents alongside the other four groups studied and therefore, the effect of various pesticides on the population densities of snakeflies deserves further consideration.

Among the herbivore species examined, two are significantly more abundant in organic than conventional orchards whereas the population densities of the other two are independent of orchard management type. The two species that were found more commonly in organic orchards are the meal moth, *Pyralis farinalis* (Linnaeus) (Lepidoptera: Pyralidae) and the treehopper *Telamona praealta* (Fowler) (Hemiptera: Membracidae). The presence of the meal moth within apple orchards is unexpected since this exotic species is an important pest of stored grain (Levinson & Buchelos 1981; Madrid & Sinha 1982). Many organic growers in BC use poultry manure as fertilizer, a known food source for meal moth larvae (Rueda & Axtell 1997). Terefore, the higher meal moth populations in organic orchards could be attributed to higher food availability for larvae in the area and/or high dispersal rates of adults into orchards with suitable oviposition sites such as decaying organic matter found in organic fertilizers.

Treehopper groups such as *T. praealta* (Yothers & Allen 1941) and *Ceresa* sp. (Beirne 1961) can become minor occasional pests in orchards due to the rapid population build-up and repeated infestation (Loye 1982). Parasitoids are of minor importance in regulating population densities (Beirne 1961) of *T. praealta*. Recommended control measures are lacking but Dormant Oil appears effective at controlling *T. praealta* populations (Yothers & Allen 1941). The biology of most treehoppers remains poorly known, but sound communication between sexes and social aggregations appears to play a prominent role in their reproductive biology (Lin 2006). Seventy four percent of *T. praealta* specimens recovered in this study were captured in grape juice-baited kairomone traps. To my knowledge this is the first report of a treehopper captured with kairomones and indicates a potential new tool to monitor emergent treehopper pests.

The other herbivore species include a well known pest of tree fruits, the mullein bug (*Campylomma verbasci* (Hemiptera: Miridae)), and the sap beetle *Glischrochilis quadrisignatus* (Coleoptera: Nitidulidae). Interestingly, there is no significant difference in the total number of *C. verbasci* specimens captured in organic and conventional orchards. Populations may be regulated by parasitoids or predators in organic orchards (McBrien et al. 1994a) and pesticides are frequently used in conventional orchards (McMullen & Jong 1970). *Campylomma verbasci* is both a pest on developing apple fruits (Pourhadji 2001) and also an important predator of various mite and aphid species (Ismailov et al. 2004; Almatni & Khalil 2008) and this plasticity in life history may allow it to be equally successful in the variably managed orchards in this study. The kairomone-baited traps may be a new way to effectively monitor or control *C. verbasci* populations. Early sampling techniques relied on active searches (Thistlewood & McMullen 1989), female-baited traps (Smith & Borden 1990) and later, on monitoring of males with sex pheromone-baited traps (Smith et al. 1991; McBrien et al. 1994a, 1994b).

The last herbivore species examined was the sap beetle *Glischrochilis quadrisignatus* (Coleoptera: Nitidulidae). This species is an important pest on fruit and vegetables in Ontario, Canada (Luckmann 1963; Pree 1968) but has not been documented as a tree fruit pest in BC. This species is readily captured in traps baited with food-derived kairomones in various cropping systems (Alm et al. 1985; Lin & Phelan 1991; Dowd 2005). Research on the susceptibility of this species to broad spectrum insecticides is inconclusive (Pree 1968).

The use of juice-baited kairomone traps to detect and monitor arthropods in orchard ecosystems

A total of 7001 arthropod specimens belonging to 102 families were captured over the course of seven weeks, starting with 19 June 2009. Of these, 91% were captured in yellow kairomone traps baited weekly with fresh Concord grape juice. The remainder were captured in yellow pheromone traps baited with 1 mg apple clearwing moth sex pheromone. Since both trap types were yellow and placed at the same height and in the same area, it is likely that olfaction is the primary mechanism by which most arthropods are orienting to the kairomone trap. By far the most diverse groups in kairomone traps were true flies, representing 24% of total arthropods captured in kairomone traps. Similarly, Leblanc et al. (2009) showed that traps baited with either methyl eugenol or decaying fruit flies (*Bactrocera dorsalis* (Hendel)) captured a wide variety of arthropods, including other fly species, mirids, nitidulid beetles, honey bees and crambid moths. Response of flies to food-based volatiles is well known and has been the subject of extensive research. Vinegar flies (*Drosophila melanogaster* Meigen) show long range attraction to acetic acid (Becher et al. 2010), an important by-product of fermenting fruit (Becher et al. 2010). Caribbean fruit flies, *Anastrepha suspensa* (Loew) are attracted to both fruit- and host-derived kairomones (Nigg et al. 1994).

A total of 1138 Lepidoptera specimens were captured in kairomone traps. Noctuidae family was the best represented with 51.1% of catch, followed by Tortricidae with 27.5%, Pyralidae with 13.5% and Crambidae with 5.0%. The most specious were the noctuid moths with at least 10 species captured, although identification was exceedingly difficult due to the poor condition of specimens. Noctuid adults are readily attracted to various fruit-derived or floral-derived synthetic attractants (Landolt 2000; El-Sayed et al. 2005; Reddy et al. 2007) and this kairomonal response has been successfully exploited in the attract-and-kill of the alfalfa looper moth (*Autographa californica* (Speyer) (Camelo et al. 2007). Three tortricid species were present, the most common of which was codling moth (*Cydia pomonella* (Linnaeus)). The Similkameen valley, where this study was located, is undergoing an active sterile insect release program with male and female codling moths being released throughout apple orchards in the summer months (SIR 2011). Codling moth adults are known to respond to various food attractants, such as pear ester (Light et al. 2001; Landolt & Guédot 2008) or a combination of pear ester and acetic acid (Landolt et al. 2007). This kairomonal attraction can be further incorporated into monitoring of codling moths in apple orchards (Knight et al. 2005; Yang et al. 2005). Among the pyralids captured, two invasive species to North America were captured most frequently. The most abundant species in my samples was the meal moth, followed by the clover hay moth *Hypsopygia costalis* (Fabricius). Male and female meal moths show a dose dependent response to a mixture of acetic acid and 3-methyl-1-butanol (Landolt 2005).

Of a total of 1256 beetle specimens captured in juice-baited traps, 53 belonged to two species in the family Cerambycidae, the most common of which was *Centrodera spurca* (LeConté). This species is attracted to lights or infrequently, to sugar-based baits (Leech 1963).

Kairomone traps consistently captured neuropterid predatory groups, including antlions (*Brachynemurus* species complex, *Dendroleon speciosum* Banks and *Myrmeleon exitialis* Walker, Neuroptera: Myrmeleontidae) and snakeflies (*Agulla* sp., Raphidioptera: Raphidiidae). Both larvae and adults of antlions and snakeflies are predatory on soft-bodied insect groups. Raphidiid snakeflies also feed on pollen (Aspöck 2002). Some antlion species can be monitored with light traps (Szentkirályi et al. 2001), although wind speed, rainfall, air temperature and sedentary life styles affect their response to light sources (Szentkirályi et al. 2005). Currently, no reliable method exists to assess snakefly populations, although some ecological studies rely on foliage beating or sweep netting techniques to assess their populations (Miliczky & Horton 2005; Arthurs et al. 2007). By contrast, attraction of chrysopid lacewings to kairomones has been well researched (Zhu et al. 2005; Tóth et al. 2006; Jones et al. 2011). Two mantispid specimens (*Climaciella brunnea* (Say), Neuroptera: Mantispidae) were captured at the end of the sample period. Both specimens were observed to sit within traps and waited for prey to approach them. *Climaciella brunnea* adults are ambush-style predators of smaller arthropods, but can also feed on pollen and nectar (Batra 1972; Boyden 1983) and therefore, it is impossible to tell whether the captured mantispids responded to the bait itself or to prey within the trap. In 2010, I repeated the arthropod biodiversity study in the same orchards (data not shown) and captured a second mantispid species (*Leptomantispa pulchella* (Banks)) known to occur only in the southern portion of Okanagan valley of BC (Cannings & Cannings 2006). This study is the first report of *L. pulchella* captured in food-based kairomone traps.

A total of 74 spider specimens belonging to three different families were captured in this study, 33.8% of which were captured in kairomone traps. Salticid spiders in the genus *Phidippus* represented only 28% of the total spider catch in kairomone traps but were the dominant spider group overall and represented 50% of the total spider catch. Jumping spiders are very active predators and are usually trapped using pitfall traps (Abraham 1983; Brennan et al. 1999; Whitehouse et al. 2002), although trapping efficiency depends on the type of immobilizing liquid placed inside traps (Schmidt et al. 2006). The most common spiders in kairomone traps were crab spiders in the family Thomisidae, with 40% of the total kairomone trap catch. They have an ambush-style predatory behaviour (Foelix 2011) similar to mantispids and their presence in kairomone traps was most likely incidental since all but one species of jumping spider (Jackson 2009; Meehan et al. 2009) are strictly carnivorous. It is possible that jumping spiders associated the yellow colour of the pheromone and kairomone-baited traps with prey availability. For example, *Phyddipus* sp. jumping spiders can learn to associate a particular colour with location and availability of prey and also with nesting sites (Jakob et al. 2007). It is possible that brightly coloured traps presented themselves to approaching spiders as a beacon of shelter suitability and prey availability.

Visual orientation to yellow traps or a combination of visual and olfactory cues may have resulted in the capture of some of the sampled arthropod specimens in yellow-coloured pheromone and kairomone traps. For example, naive bumblebees show a strong innate attraction to the contrasting colour of flowers (Lunau 1990). Hover flies show a greater attraction to yellow than to green and yellow-green pan traps (Laubertie et al. 2006). In general, flies are most attracted to lower frequency wavelengths varying between 400 and 800 nm (Allan

& Stoffolano 1986a). Mexican fruit flies (*Anastrepha ludens* (Loew)) are also attracted to lower frequency (500 to 580 nm) wavelengths with and without the food bait, yeast hydrolysate (Robacker et al. 1990). The wavelength of yellow colour is around 570 nm, well within the attractive frequency range exhibited in flies.

In conclusion, semiochemical-baited traps used for mass trapping against the apple clearwing moth in apple orchards in the Southern Interior of BC capture a large number of non-taget arthropods. This indicates that a semiochemical-based mass trapping program might negatively affect local arthropod diversity. Nevertheless, both trap types can be used to sample and monitor actively moving arthropods. Coventional management practices such as the application of pesticide sprays and synthetic, nitrogen-based fertilizers (Table 4.1) may affect the overall arthropod community structure and population densities of key predator species that may act as biological control agents of various orchard pests. In addition, grape juice-baited kairomone traps can be a useful tool in the monitoring of several groups of economic importance for which no other reliable population assessment method currently exists and can be included in the tools available to measure arthropod biodiversity in apple agroecosystems. The response of several arthropod groups to kairomone traps is also recorded here for the first time.

4.5. Literature cited

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Table 4.1. The management and orchard characteristics of the apple orchards surveyed with apple clearwing moth pheromone- and kairomone-bated monitoring traps between 12 June and 31 July 2009.

Site no.	Orchard management			Orchard characteristics							Adjacent fields
	Type	Pesticides	Fertilizers	Age (years)	Size (ha)	Caliper size (m)	Density (trees / ha)	Canopy height (m)	Rootstock	Apple varieties	
1	Certified organic	Entrust®	None	11	1.64	0.015	5625	3.6	M26	Ambrosia	apples, grapes, shrub-steppe
2	Certified organic	Entrust®, Isomate-P®	Compost, Pacific Bio Organic Fish Fertilizer	02 – 15	11.51	0.082	6875	3.6	M9, M26	Ambrosia, Braeburn, Fuji, Gala, Granny Smith	apples, cherries, peaches, shrub-steppe
3	Certified organic	Dormant Oil, Entrust®	Compost, Groundskeeper's Organic Advantage	14 – 24	1.7	0.068	3073	2.5	M9, M26	Ambrosia, Gala	apples
4	Certified organic	Dormant Oil, DiPel®	PlantonicTM Fish Essence Bio-Organic fertilizer, Turkey manure	10	0.32	0.055	2320	3.6	M9	Granny Smith, Fuji, Wine sap	apples, fallow field, pears
5	Certified organic	Dormant Oil	None	30	1.33	0.164	750	6	Antonovka	Earligold, MacIntosh, Newton, Spartan, Yellow Transparent	apples, pears
6	Certified organic	Dormant Oil, DiPel®	Plantonic^{TM} Fish Essence Bio-Organic fertilizer, Turkey manure	25	0.9	0.176	691	3.6	M26	McIntosh	apples, curcurbits, fallow field, peaches, tomatoes
7	Certified organic	Dormant Oil, Entrust®	Compost, Turkey manure	45	1.54	0.198	571	4	Antonovka, M104	Spartan	apples, poplar thicket
8	Certified organic	Dormant Oil, DiPel®	PlantonicTM Fish Essence Bio-Organic fertilizer, Turkey manure	47	0.66	0.090	229	4.3	M7	Golden Delicious, Red Delicious, Spartan	apples, cucurbits, fallow field, walnuts
9	Conventional	Altacor®, Diazinon, DiPel®, Dormant Oil, Envidor®, Movento®	"orchard mix" ammonium sulphate (selected trees)	10 – 12	3.96	0.045	5438	2.7	M9	Ambrosia, Fuji, Golden Delicious, Pink Lady, Spartan	apples
10	Conventional	n/a	n/a	n/a	2.62	0.065	7597	2.6	n/a	Gala	apples, cherries, fallow field, shrub-steppe
11	Conventional	Dormant Oil, Guthion®, Intrepid®, Movento®, Rimon®, Sevin®	AgrowChem Pacific high yield fertilizer for apples and pears	6	0.65	0.088	5500	2.7	M9	Ambrosia	apples, peaches
12	Conventional	Altacor®, Success®	n/a	05 – 21	15.2	0.064	5450	3.0	M9	Ambrosia, Fuji, Gala, Granny Smith	apples, shrub-steppe
13	Conventional	Admire®, Movento®, Sevin®, Success®	Ammonium nitrate	14	1.06	0.084	1675	2.7	M9	Royal Gala	apples, vineyard
14	Conventional	Altacor®, Dormant Oil	Green Valley Agricultural Inc. Ammonium sulphate	31	0.98	0.169	837	3.6	Antonovka	Golden Delicious, Spartan	apples
15	Conventional	Dormant oil, Guthion®, Intrepid®, Movento®, Rimon®, Sevin®	AgrowChem Pacific high yield fertilizer for apples and pears	26	0.35	0.124	1100	3.6	Mark	Fuji	apples, pears
16	Conventional	Altacor®, Success®	n/a	26	6.2	0.139	1202	3.0	M4, M26	Gala, Granny Smith, Red Delicious	apples, scrubland, vineyard

Table 4.2. References used for family-level and/or genus-level identification of arthropod specimens captured in apple clearwing moth pheromone- and kairomone-baited monitoring traps.

	Order	**References**
1	Araneae	Ubick et al. 2005
2	Coleoptera	Parsons 1943; Gordon 1985; Scudder & Cannings 2005
3	Dermaptera	Engel 2003
4	Diptera	Scudder & Cannings 2006
5	Hemiptera	Beirne [1961]; Cannings & Scudder 2005
6	Hymenoptera	Stephen et al. 1969; Goulet & Huber 1993; O'Toole & Raw 1991; Michener et al. 1994
7	Lepidoptera	Scudder & Cannings 2007
8	Neuroptera	Scudder & Cannings 2007
9	Odonata	Cannings et al. 2000
	Opiliones	Pinto-Da-Rocha et al. 2007
10	Orthoptera	Capinera et al. 2004
11	Psocoptera	Mockford 1993
12	Raphidioptera	Aspöck et al. 1991, Scudder & Cannings 2007

Figure 4.1. Distribution of the experimental sites chosen for the 2009 non-target arthropod survey in Cawston, BC. Grey indicates orchards under conventional management and white indicates organic orchards. Rectangles with black bars indicate standard plantings of an average of 882 apple trees / ha and empty rectangles indicate superspindle type plantings of an average of 5235 trees / ha.

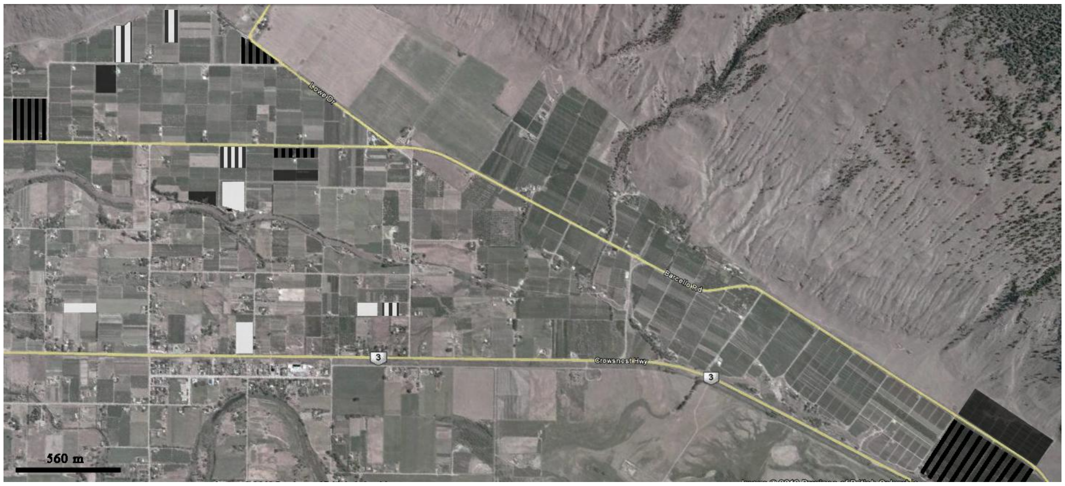

Lowe Dr
Barcello Rd
Crowsnest Hwy
3
3
560 m

Figure 4.2. Ordination output of the non-metric multidimensional scaling of the shortest ranked distances in multidimensional space of raw untransformed trap counts (specimens / arthropod family) in order to assess variation attributed to orchard management, planting type and trap type on family level diversity replicated every week over a seven-week period. Size of triangles indicates how close the datapoint is to the viewer (3D location of the datapoint. (A) Bait type, (B) management type and (C) planting type. There was a significant difference in the distribution of specimens / family captured in pheromone and kairomone traps ($P < 0.001$) and also between organic and conventionally managed orchards ($P = 0.001$). Planting type did not affect the distribution of arthropod specimens / family captured in pheromone and kairomone traps ($P = 0.56$).

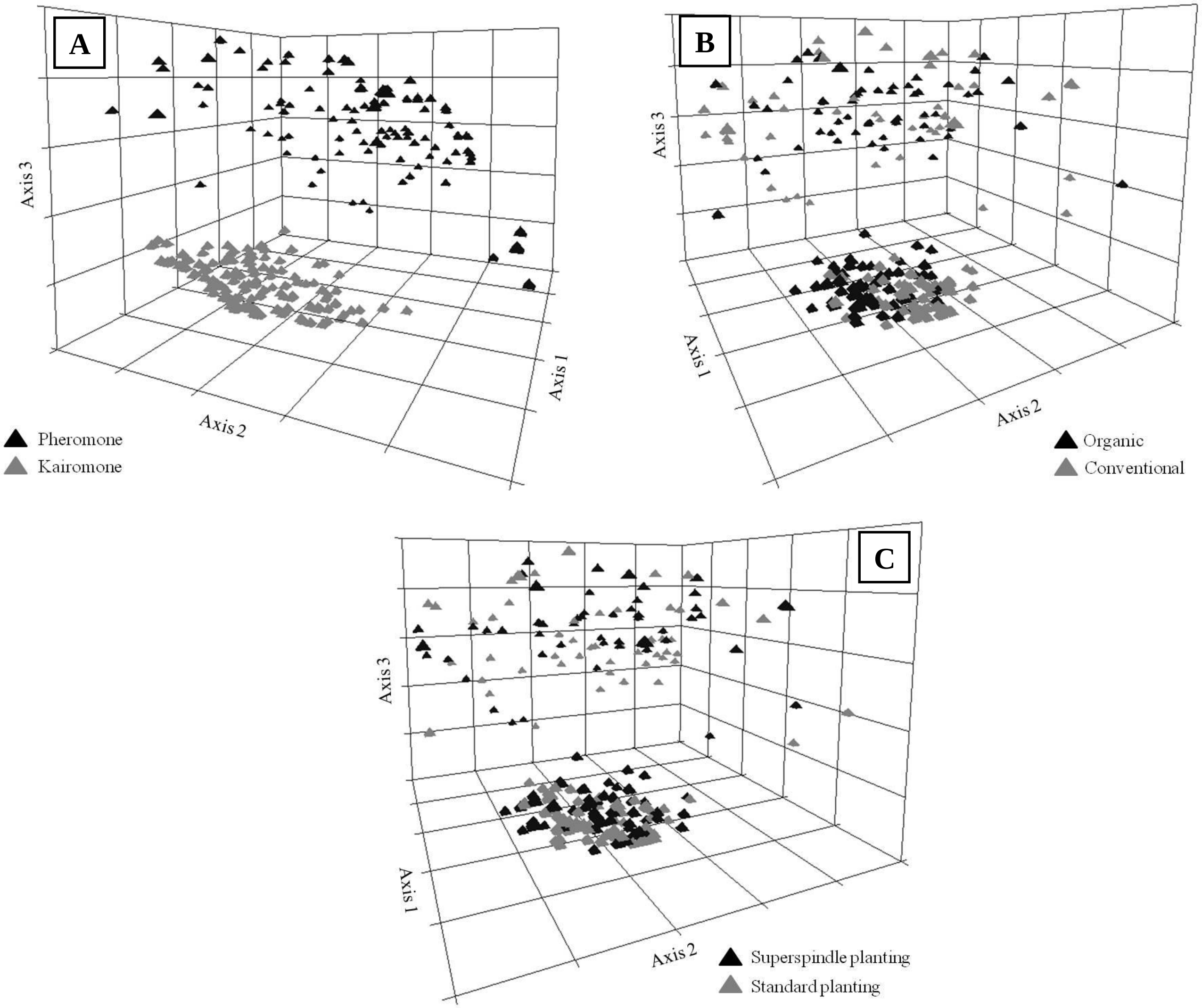

A
Axis 3
Axis 2
Axis 1
▲ Pheromone
▲ Kairomone
B
Axis 3
Axis 1
Axis 2
▲ Organic
▲ Conventional
C
Axis 3
Axis 1
Axis 2
▲ Superspindle planting
▲ Standard planting

Figure 4.3. The effect of management type on the mean-total number of adults captured ± Poisson Errors (P.E.) in various target taxa. A portion of the variability in catch of each group was explained by blocking for orchard.

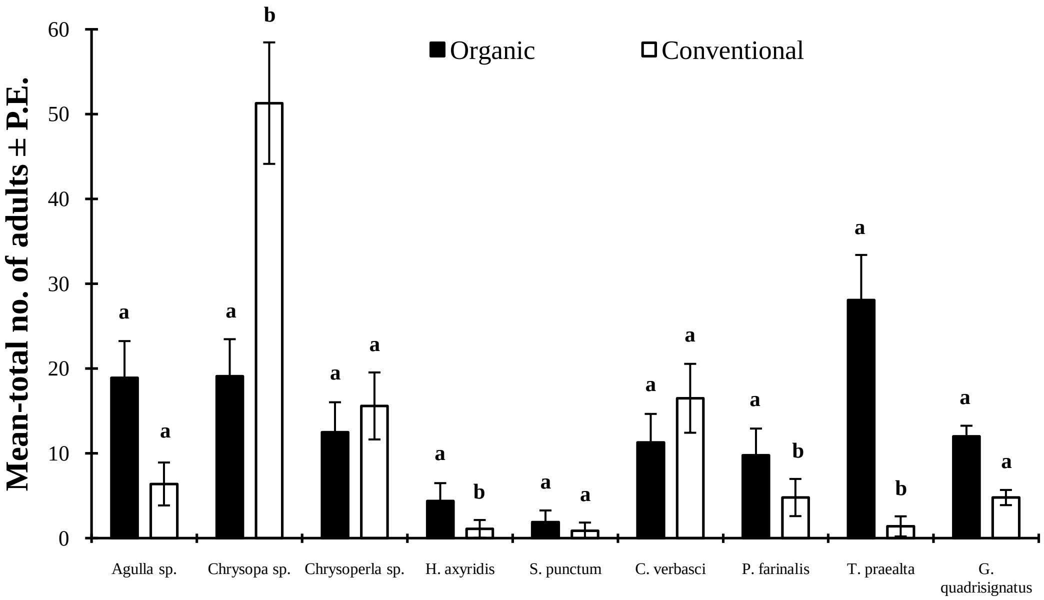

Organic
Conventional
Mean-total no. of adults ± P.E.
60
50
40
30
20
10
0
a
a
a
b
a
a
a
b
a
a
a
a
a
b
a
b
a
a
Agulla sp.
Chrysopa sp.
Chrysoperla sp.
H. axyridis
S. punctum
C. verbasci
P. farinalis
T. praealta
G. quadrisignatus

Figure 4.4. Total number of families / arthropod order captured in organically and conventionally managed apple orchards in (A) kairomone traps and (B) pheromone traps in 2009. The non-target arthropod survey started on 12 June and ended on 31 July 2009.

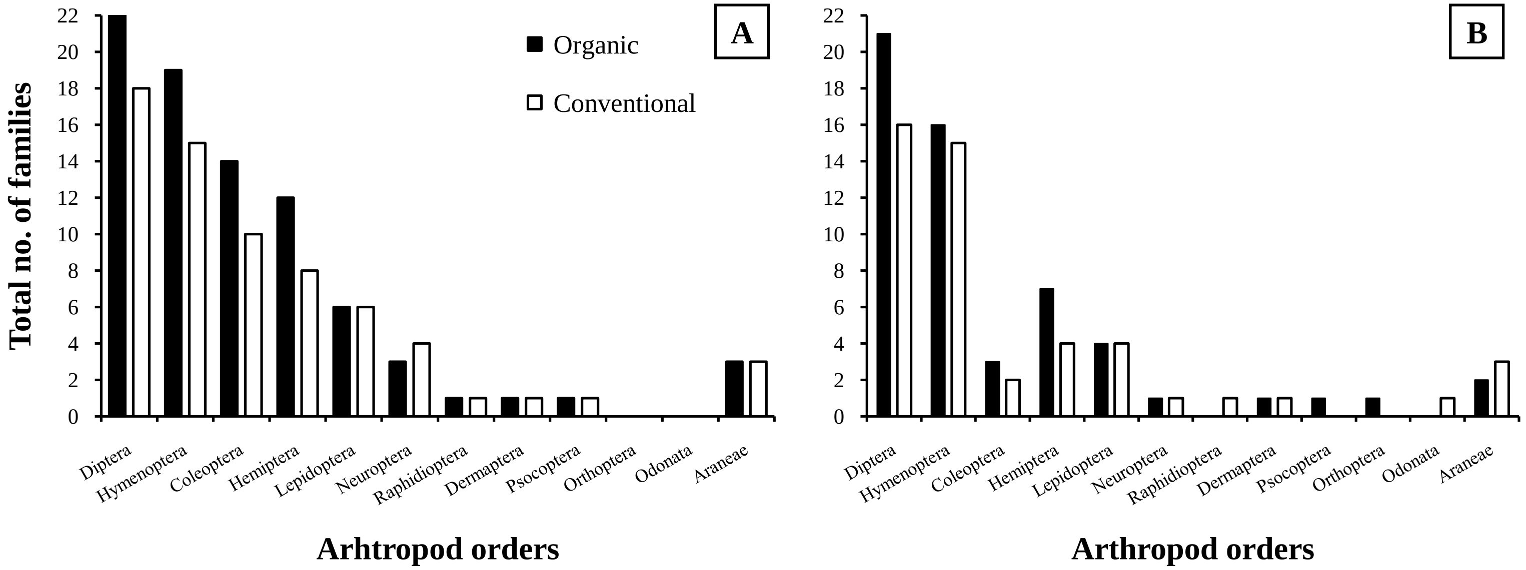
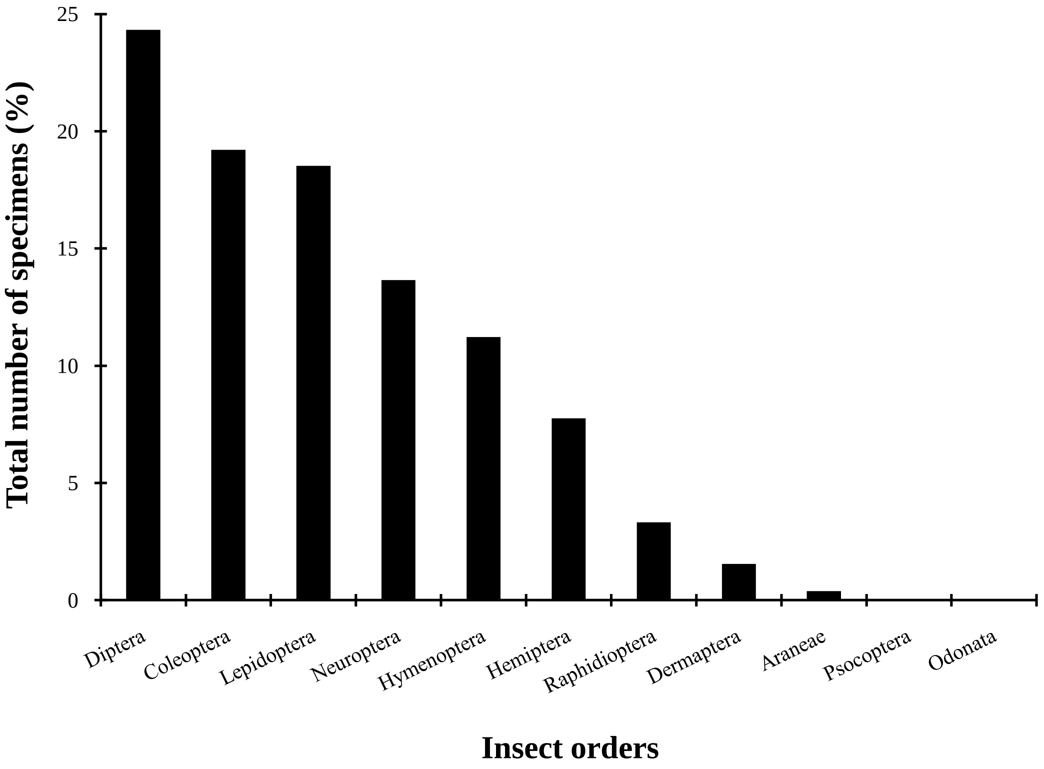
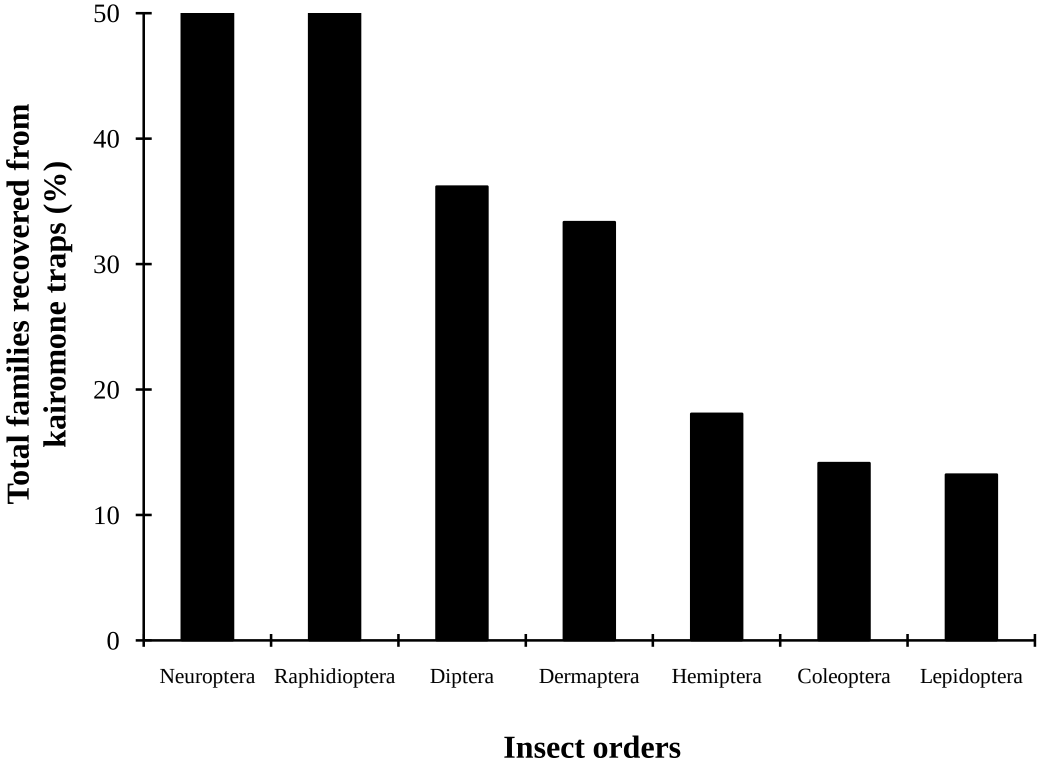

A
B
Organic
Conventional
Total no. of families
22
20
18
16
14
12
10
8
6
4
2
0
Diptera
Hymenoptera
Coleoptera
Hemiptera
Lepidoptera
Neuroptera
Raphidioptera
Dermaptera
Psocoptera
Orthoptera
Odonata
Araneae
Arhtropod orders
Arthropod orders

Figure 4.5. The total distribution of specimens / arthropod order captured in both pheromone and kairomone traps targeting the apple clearwing moth during the 2009 non-target arthropod survey in apple orchards in Cawston, BC.



25
20
15
10
5
0
Total number of specimens (%)
Diptera
Coleoptera
Lepidoptera
Neuroptera
Hymenoptera
Hemiptera
Raphidioptera
Dermaptera
Araneae
Psocoptera
Odonata
Insect orders

Figure 4.6. The total percentage of families known from British Columbia recovered from apple clearwing moth kairomone traps during the 2009 non-target arthropod survey in Cawston, BC.



Total families recovered from kairomone traps (%)
50
40
30
20
10
0
Neuroptera
Raphidioptera
Diptera
Dermaptera
Hemiptera
Coleoptera
Lepidoptera
Insect orders

Chapter 5. Concluding discussion

5.1. Research summary

The apple clearwing moth (*Synanthedon myopaeformis* (Borkhausen)) is difficult to manage with conventional insecticides in apple orchards (Ciglar & Masten 1977) because larvae are protected by bark. Feeding damage caused by high population densities can cause declines in tree vigour and fruit yield (Dickler 1976). Pheromone-based management such as mass trapping (Trematerra 1993; Önuçar & Ulu 1999) and mating disruption (Kyparissoudas & Tsourgianni 1993; Stüber & Dickler 1987) has potential for integrated pest management of this species. In this study, I compared pheromone and kairomone-baited mass traps at different densities (13 – 100 traps / ha) in an effort to develop a mass trapping tool for this invasive pest in the Southern Interior of British Columbia (BC). The very high moth densities present in BC require that up to 100 sex pheromone-baited traps / ha at peak flight and at least 100 traps / ha baited with Concord grape juice kairomones be deployed to effectively manage the apple clearwing moth. In practice, possible negative biodiversity implications caused by the attraction and removal of large numbers of non-target arthropods may demand further refinements to the two trap types before a mass trapping control tactic can be successfully implemented. The range of attraction of the yellow funnel traps baited with 10 mg of apple clearwing moth sex pheromone is around 20 m and the range of attraction of yellow traps baited with 300 ml fresh Concord grape juice is less than 5 m. Although not extensively studied, the range of attraction of semiochemical-baited traps is important for the design and implementation of mass trapping of various pests (Schlyter 1992; Byers 2007). Volatiles from traps separated by less than 2 attractive radii interact with one another and result in trapping interference (Dodds & Ross 2002) with subsequent disorientation of responding adults (Wall & Perry 1978) and aggregation around traps (Yamanaka et al. 2003).

Disruption of pheromone-based mate-finding behaviour as a result of pheromone-based mass trapping occurred when plots were treated with 1 mg pheromone-baited mass traps at 22 traps / ha. Male moths were equally disrupted by pheromone treatment in open and closed traps, indicating that male moth removal from the population does not enhance the “mating disruption” effect of pheromone alone. Similarly, the presence of insecticides in an attract-and-kill formulation targeting Oriental fruit moth (*Grapholita molesta* (Busk)) is not necessary to effectively control moth populations (Evenden & McLaughlin 2004). Nevertheless, the effect of actually removing males from the population suppresses some dense moth populations faster and for a longer time than pheromone-only tactics such as mating disruption (Suckling & Brockerhoff 1999).

Many moth species use an interaction of pheromonal and host volatile cues in a synergistic fashion (Light et al. 1993; Reddy & Guerrero 2004; Yang et al. 2004; Landolt et al. 2007). In this study, it appears that kairomone and pheromone plumes interact with one another to affect responsiveness of the apple clearwing moth. More males were captured in kairomone-baited traps when they were positioned in close proximity to pheromone traps than in isolated kairomone-baited traps. According to Knight et al. (2005) traps baited with kairomone and pheromone lures loaded individually captured fewer codling moth (*Cydia pomonella* (Linnaeus)) males than traps baited with a mixture of both pheromone and kairomones. It is also likely that the larger range of attraction of pheromone traps attracts moths from greater distances, which are then inadvertently caught in the efficient but low attraction radius kairomone traps. Trap catch of females was decreased in kairomone-baited traps when they were positioned in close proximity to pheromone traps as compared to catch in isolated kairomone-baited traps. The behavioural mechanism remains unknown.

Flight of male apple clearwing moth can be monitored effectively with both pheromone-baited and kairomone-baited traps. By comparison, Concord grape juice-baited kairomone traps provide a non-competitive lure that can monitor both males and females in pheromone-treated and untreated orchards and also permit assessment of mating status of females. A comparison of the male and female flight curves in early, mid and late season indicate that protandry may be an important characteristic of apple clearwing moth populations. The continued presence of mated females in orchards after males stopped responding to pheromone traps has direct implications for pest management (Reddy & Guerrero 2001) and as such, monitoring of female flight can provide useful information for the precise timing of control measures (Light et al. 2001). A large proportion of females (68.8 – 72.5%) captured in kairomone assessment traps in pheromone-treated and control plots were mated only once, with a small proportion mated twice or more. The small proportion (7.2 – 13.2%) of virgin females captured is not necessarily an indication of failure of pheromone-based management, since kairomone-baited traps can be more attractive to mated than to virgin females (Light et al. 2001; Knight & Light 2005). Mating status did not differ between treatments and control and therefore indicates that pheromone-based mass trapping may be ineffective at interfering with mating behaviour when moth populations are excessively high. Nevertheless, delayed mating may still cause a decline in fertility rates and hatching of larvae.

Some semiochemical-baited traps targeting the apple clearwing moth may also have an ecological function to monitor biodiversity levels in apple orchards under different planting densities and under different management options. Yellow pheromone and kairomone traps attract a wide variety of non-target arthropods, an indication that in their current state both trap types can be used to survey and monitor biodiversity but not to mass trap the apple clearwing moth. Based on captures in both pheromone and kairomone-baited traps, organically-managed orchards appear to support higher arthropod diversity levels than conventionally-managed orchards. Detailed examination of several economically and ecologically important taxa showed that most groups were more abundant in organic than in conventional orchards. The exception was green lacewings in the genus *Chrysopa* (Neuroptera: Chrysopidae). *Chrysopa* lacewings were more abundant in conventional orchards, although the statistical significance was minimal. *Chrysopa* lacewings show a preference for soft-bodied arthropods such as woolly aphids (Canard 2001), which occurred in highly dense populations in all of the conventional orchards examined.

The arthropod community in the superspindle apple plantings closely matched that recovered from standard apple plantings. The lack of a significant effect can be attributed to either a sampling bias, since traps were only attractive to actively moving insects or to a similar surrounding habitat.

5.2. Future directions

My thesis provides the basis for further research into two major areas. The first one concerns the control of the apple clearwing moth in apple orchards. Further research could examine the effect of large-plot mass trapping with yellow funnel traps baited with apple clearwing moth sex pheromones at a density of 25 traps / ha on the next generation of larval and pupal densities. In the current state, Concord grape juice may not be useful in area-wide mass trapping of the apple clearwing due to the large array of non-target arthropods captured and the labour-intensive nature of such work. Further refinements to kairomones are necessary to produce a commercially-available kairomone lure that can be incorporated into semiochemical-based management of the apple clearwing moth. These should include, but not be limited to quantification of the chemical profile of grape juice volatiles essential for triggering attraction and behavioural orientation of apple clearwing moths to traps, maximizing the range of attraction and increasing kairomone longevity and stability in the field. The ultimate goal is the development of a high capacity kairomone-baited trap since the current trap only captures adults on its surface (similar to a Delta trap) due to the surface tension of the juice and, therefore, it is vulnerable to rapid saturation with both apple clearwing and other insects. Research on kairomone-based attract-and-kill formulations has shown great promise since both sexes can be removed (Camelo et al. 2007) and deserves further consideration. Furthermore, since Concord grape juice volatiles are attractive to codling moths (*Cydia pomonella* (Linnaeus)), which is another major pest of apple orchards, research into the development of a dual lure could lead to significant advancements in the targeted control of the two species especially in organically-managed orchards.

Management of the high apple clearwing moth populations present in North America will most likely require a combination of several semiochemical-baited techniques. Inclusion of other control options such as biological control is dependent on a reduction of pesticide use, including spinosad-based neural regulators. The non-target effects of the spinosad-family of insecticides include parasitoids and earwings. Currently, research on whether earwigs are effective predators of apple clearwing moth larvae is lacking, but they have been shown to provide excellent control for other pests (Glen 1975; Phillips 1981; Mueller et al. 1988). Extensive apple clearwing moth larval and pupal collections did not reveal any parasitoids in 2006, whereas many of the pupae collected in subsequent years appeared to be parasitized. In June 2007, I discovered an ichneumonid parasitoid feeding externally on apple clearwing moth pupae in an organic apple orchard.

Therefore, species-level identification of natural enemies that may be potentially important in this system deserves further exploration.

The second research area my present research leads to is the incorporation of grape juice and related food-derived kairomones into arthropod surveys of other cropping areas as well as undisturbed habitats. I determined that Concord grape juice can be an effective biodiversity sampling tool of various arthropod taxa, including other apple orchard pests such as the mullein bug, *Campylomma verbasci* (Meyer), important predators such as coccinellids, snakeflies and green lacewings and a wide variety of parasitoids relative to other sampling tools such as pitfall or light trapping. Such broad attraction has been shown previously in other baits with complex chemical profiles (Leblanc et al. 2009) and can rival light traps. Therefore, grape juice-baited kairomone traps can be further developed to survey the arthropod fauna of other crop types or examine the arthropod communities of undisturbed areas.

In conclusion, my research documented the effect trap density and bait type required for mass trapping of apple clearwing moth populations in the Southern Interior of British Columbia. Information on phenology of both sexes and the presence of protandry will assist growers with integrated pest management of the apple clearwing moth. In addition, I have examined the mechanisms of action of pheromone-based mass trapping targeting apple clearwing moth adults and whether active removal of males is necessary to prevent mating. This present work also examined the effect of orchard management on the arthropod communities and abundance of several economically important taxa in apple orchards and documented the suitability of kairomone traps as biodiversity sampling tools. These results provide the underlying basis for future research into semiochemical-based control of the apple clearwing moth and development of kairomone traps as predictive tools of arthropod faunal composition in either managed or undisturbed landscapes.

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