ATTRACTIVENESS OF SEMIOCHEMICALS TO GREEN LACEWINGS FOR BIOLOGICAL CONTROL IN POME FRUIT

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Chair

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ATTRACTIVENESS OF SEMIOCHEMICALS TO GREEN LACEWINGS FOR BIOLOGICAL CONTROL IN POME FRUIT

Abstract

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Laboratory experiments to determine the attraction of green lacewings to pome fruit semiochemicals were conducted by Y-tube olfactometer and flight chamber assays. In Y-tube assays tested odors included pear psylla, pear psylla honeydew, washed pear psylla-damaged leaves, undamaged leaves, mechanically-damaged leaves, pear psylla honeydew + undamaged leaves, pear psylla + undamaged leaves, pear psylla-damaged leaves + pear psylla, and pear psylla + undamaged leaves + honeydew. Pear psylla-damaged pear leaves with pear psylla present were found to be significantly attractive to lacewings, but other tested odor and odor combinations were not attractive. In the flight chamber, no odors tested (pear branches infested with pear psylla, apple branches, pear psylla, wintergreen oil, methyl salicylate, and squalene) were found to be more attractive to lacewings than filtered air.

Field experiments to determine green lacewing attraction to methyl salicylate in pear, apple, and surrounding sagebrush steppe were conducted in Orondo, Entiat, and Wenatchee, WA. Three sites were established, each included 10ha apple, pear, and sagebrush plots. Each plot had five methyl salicylate-baited and five unbaited yellow sticky traps, which were monitored weekly for green lacewing capture. Methyl salicylate in the three habitat types was variable in its attraction to green lacewing species. Methyl salicylate was significantly attractive to *Chrysopa* *nigricornis* in pear, apple, and sagebrush, *Chrysoperla plorabunda* in pear, and *Chrysopa coloradensis* in sagebrush.

Field evaluations of the attractiveness of five lure types (control, methyl salicylate in glass vials, methyl salicylate in controlled release lures, squalene in glass vials, and a combination of squalene in glass and methyl salicylate in glass) to green lacewings was conducted in pear and sagebrush in Peshastin, WA. Three experimental sites each contained pear and sagebrush plots with five yellow sticky traps baited with each lure type per plot. *Chrysopa nigricornis* was found to be significantly more attracted to squalene than to methyl salicylate and control lures in both habitats, and combination treatments were more attractive than both lure types individually. In sagebrush, methyl salicylate was found to be significantly more attractive to *C. coloradensis* than other lures.

TABLE OF CONTENTS

CHAPTER

1. A FIELD EVALUATION OF METHYL SALICYLATE IN APPLE, PEAR, AND SAGEBRUSH

2. A NEW FIELD ATTRACTANT OF *Chrysopa nigricornis*

3. 3. Y-TUBE ASSAYS

4. FLIGHT CHAMBER ASSAYS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

Arthropod predator-prey interactions and host location by predators and parasitoids have been widely studied. Location of hosts is often by a combination of cues, including auditory, visual, and chemical. Predators must engage in a searching activity using multiple signals to pinpoint host location. Predators that do not rely on visual or auditory cues likely exploit chemicals that hosts use for mating, aggregation, dispersal, alarm, and host location. Specialist predators have evolved mechanisms to recognize host odors, and to use these kairomones in location activities; they may also use volatile chemicals emitted from host plants following herbivore damage. Generalist predators feed on a variety of prey or hosts, and would need to exploit specific host-produced kairomones or other cues to locate prey. Although generalists may use cues other than chemical, including visual and auditory, it is difficult to explain how many predators locate adequate prey without using semiochemicals. Generalists may use herbivore-induced plant-produced volatiles to locate prey and hosts (Reddy, 2002). Generalist predators may distinguish more plant-produced volatiles than prey-produced odors, as they must visit a variety of host plants and prey if they are to survive and reproduce.

Plants produce a variety of volatile compounds; conifers produce, among others, terpenes and pinenes, and many deciduous plants produce methyl salicylate and ethanol. These compounds can be produced at all times, at certain life stages, or following damage, such as herbivore damage. Plants respond to herbivore feeding damage and salivary compounds by producing mixtures of volatiles in a different quantity, ratio, or composition than if herbivore attack was absent, and they may be expressed systemically (Dicke and van Loon, 2000).

Plants release a mixture of volatile compounds following herbivore attack, although it is uncertain whether this release is evolutionarily linked with herbivore combat using predator and parasitoid 'bodyguard' attraction, or just a consequence of being attacked and damaged. The volatile blends that have thus far been detected from plants are commonly composed of as many as 200 unique compounds (Dicke et al., 1990). Evidence for the active recruitment of 'bodyguards' includes the active production of these compounds rather than a passive release, and systemic emission of volatiles rather than localized release (Dicke and van Loon, 2000). Thus, it is evident that arthropods occupy a world saturated with chemical information available for predators to utilize in prey location.

Pels and Sabelis (2000) showed that over short distances, the predatory mite *Phytoseiulus persimilis* arrests at a higher rate in areas of prey patches than in areas with only plant-produced volatiles, due not to plant-produced compounds but instead to prey-related physical cues. This experiment suggests that, at least in close proximity, these mites do not use plant-produced compounds to locate prey. However, Pels and Sabelis (2000) were not able to demonstrate any significant differences over long distances, further suggesting that these predatory mites do not use plant volatiles. Van Tilborg et al. (2004) also demonstrated that in a laminar flow situation, the same predatory mites appeared unresponsive to plant odors when either well-fed or starved, but appeared more responsive to presence or absence of a flow of air.

Parasitoids likely respond to specific chemical cues when locating hosts. For example, Hilker et al. (2000) showed that the parasitoid *Chrysononotomyia ruforum* Krausse was attracted to volatiles from *Pinus sylvestris* only after the deposition of eggs of the sawfly *Diprion pini* L., and not after mechanical damage. This suggested that there is some chemical change initiated after egg deposition that the parasitoid used to locate hosts. Mumm et al. (2005) demonstrated that these parasitoids must learn which plant volatiles to be attracted to, but that the range of attractive volatiles is relatively narrow. That these specialist parasitoids would learn a narrow range of plant-produced compounds is of interest because it may have evolutionary implications for the continuation and development of this tri-trophic relationship. Specialists should be attracted to only a small number of stimuli, whereas generalist arthropods must be able to exploit food sources that are unevenly distributed. Therefore, the question of food location strategies, such as what chemical cues do generalists use to locate food, must be explored.

One likely host location strategy of generalist arthropod predators is using herbivore- or damage-induced plant volatiles. However, each herbivore species may elicit a particular and unique response in host plants, resulting in a unique blend of volatiles released (Drukker et al., 2000). In a novel experiment where *Arabidopsis thaliana* L. was genetically transformed to constitutively release induced indirect defense volatiles in the absence of herbivore attack, it was shown that the predatory mite *Phytoseiulus persimilis*, in both open plantings and y-tube olfactometer assays, preferred the undamaged genetically modified plants to the herbivore, herbivore-damaged, and undamaged non-transformed plants (Kappers et al., 2005). This study demonstrated that there are indeed chemicals released by the plants that are utilized by arthropod predators in host location and orientation. Drukker et al. (2000) demonstrated a distinct difference in field-captured and first generation laboratory-reared anthocorid predators; there was a learned behavior and prey preference based on prior exposures and feedings, and this behavior was linked not just with the plant volatile, but also with the plant volatile in combination with a successful predation event. This study demonstrated that preference toward plant-produced infochemicals is a learned behavior. If predators must first successfully capture prey before associating and exploiting plant volatiles, many predators must be unsuccessful and starve before learning to recognize volatiles; an unlikely scenario. It is probable that arthropod predators do have some degree of learning but the results obtained by Drukker et al. (2000) may simply be an artifact of rearing insects in the laboratory on a food substrate that is uniform and unrealistic for arthropods in nature.

Oviposition by adult females on plants releasing herbivore-induced volatiles is possibly a means of determining predator attraction. In insects where the immature is the primary predatory stage, such as the neuropteran chrysopids, it would be advantageous for the adult to deposit eggs in areas of high potential prey densities, determined by presence or absence of plant volatiles. Evolutionarily, it would be beneficial to deposit progeny in areas where they will have the greatest chance of encountering prey. Reddy et al. (2004) explored oviposition of *Chrysoperla carnea* Stephens and potential relationships with damaged plants, and determined that oviposition was significantly higher on injured leaves than on undamaged leaves. This study suggested that there is possibly a chemical component mediating oviposition. *Chrysoperla carnea*, a generalist predator, may deposit eggs in areas where plant defense volatiles are being released in order to ensure survival of progeny and continuation of their genetic line, and continue to co-evolve with plant compounds to locate prey. Reddy (2002) also demonstrated that *C. carnea* is more attracted to both mechanically and mite damaged plants than to the prey

Tetranychus ludeni Zacher mites. Many studies in North America that refer to *C. carnea* may be *Chrysoperla plorabunda* Fitch, because *C. carnea* does not occur outside of Europe.

Generalists and specialists use plant-produced defensive volatiles in host location, though evidence suggests that they react differently to the same volatiles. There is still much research needed in the area of close-up prey location and predator arrestment. There are also gaps in determining whether different predators react to the same chemicals in a blend, or if specific chemicals in that blend are necessary for attraction. Much of this work could be further progressed with advances in synthesizing plant volatiles and combining them in life-like combinations. Other study areas would be in the evolution and proliferation of these mechanisms, to determine whether plants developed these as a means to attract ‘bodyguards’ or simply as an artifact of past attempts at herbivore defense.

Pear Psylla

Pear psylla, *Cacopsylla pyri* (L.) and *Cacopsylla pyricola* Foerster (Hemiptera: Psyllidae), are monophagous insect pests of pear in pear growing regions of Europe and North America; *C. pyricola* is present in N. America while both species are present in Europe (Bues et al., 2000; Horton and Lewis, 1996; Horton et al., 1992; Kapatos and Stratopoulou, 1999; Krysan, 1990). There are two distinct seasonal forms in western North America, a dark over-wintering form, and a light summer form which is responsible for most of the damage caused directly to pears (Horton and Lewis, 1996; Horton et al., 1992; van de Baan and Croft, 1991; Unruh and Krysan, 1994). In the fall of the year, pear psylla move to over-wintering sites; they begin to reenter orchards in the early spring as temperatures begin to warm (Horton et al., 1992). There are many predators and parasitoids of pear psylla that are important to biological control efforts, as well as a suite of pesticides available in chemical control programs. Pear psylla has developed resistance to many insecticides since pesticide use first began in pear orchards in the early 1900's.

Chemical control of pear psylla

Chemical control programs for pear psylla consist of two distinct phases, winter/spring treatments and summer treatments. Winter/spring treatments, also known as dormant sprays, are applied when adults begin to lay eggs in the spring (Burts, 1985). In the 1980's, pyrethroids (fenvalerate, permethrin, and flucythrinate) were the main chemical control tactic employed in North America for adults and nymphs, although many growers were advised to limit treatments to once or less per season (Burts, 1985; Pree et al., 1990). In western North America, pyrethroids were used for over-wintering adults, whereas in eastern North America growers used pyrethroids during the warmer midsummer period (Burts, 1985; Pree et al., 1990). Pyrethroids and organophosphates have also been used extensively in the pear growing regions of Europe (Bues et al., 2003). In Switzerland, insecticide resistance in pear psylla led to cessation of organophosphate use in the late 1970's, and organophosphates were replaced by pyrethroids until the late 1980's, when teflubenzuron use began (Schaub et al., 1996). In France, deltamethrin and the synergist piperonyl butoxide have been used in pear psylla control (Bues et al., 2003). There are several other pesticides that have been used, and can be used for pear psylla control.

While the use of chemical controls for pear psylla has been considered necessary for pest management, difficulties or failures of control have mostly been attributed to pesticide resistance (Pree et al., 1990). Pear psylla has developed resistance to almost every insecticide used against them, and the resistance conferred is often not restricted to one pesticide but is apportioned to classes of pesticides. As discussed previously, pear psylla in Switzerland have developed resistance to organophosphates and pyrethroids, and then developed resistance to teflubenzuron within six years, with two treatments per year (Schaub et al., 1996). Schaub et al. (1995) also determined that resistance was spread among regions by wind blown pear psylla from areas of high resistance to areas of low resistance, ascertained by presence of resistant individuals in organically managed orchards. Pear psylla populations in southern Ontario also began showing pyrethroid resistance following two pyrethroid applications per year for six years; the over-wintering stage was additionally shown to be more resistant than was the summer stage (Pree et al., 1990). In France, pear psylla developed resistance to the organophosphate azinphosmethyl due to repeated use against codling moth, and also exhibited cross resistance to several other insecticides (deltamethrin, fenvalerate, mevinphos, chlorpyrifos, mocrotophos, phosmet, methomyl, pirimicarb, amitraz) that were potentially useful against pear psylla (Bues et al., 2000). Pesticide resistance is often devastating to an agricultural industry, because of the costs associated with developing and implementing new pesticides.

Bues et al. (2003) showed that resistance can be delayed or slowed with the addition of synergists in pesticide applications. Van de Baan and Croft (1991) demonstrated that pear psylla in the Pacific northwest are more susceptible to pesticides when in the summer form, especially when insecticides are combined with synergists. This observation of winter forms being more resistant than summer forms is further substantiated by van de Baan et al. (1989), Unruh and Krysan (1994), and Bues et al. (1999). Van de Baan et al. (1989) also demonstrated that as the season progressed, susceptibility to many pesticides (fenvalerate, methiocarb, endosulfan, azinophos-methyl, perthane, and avermectin) increased, although resistance was demonstrated to some extent for all pesticides tested. This apparent seasonal susceptibility to pesticides may be used to determine proper timing of insecticides to produce the most mortality in pear psylla.

Predators are often adversely affected by the use of pesticides, and predator mortality amplifies the effects of the pest species. Hagley and Simpson (1983) demonstrated that applications of permethrin and azinphosmethyl reduced predator numbers in Ontario pear orchards, thereby reducing the potential for biological control of pear psylla. Most broad-spectrum insecticides can pose problems for biological control of pest species, and may allow for pest resurgences and secondary pest outbreaks.

Soft control

Because pear psylla is at risk to develop pesticide resistance, abandonment of traditional chemical control, at least in part, appears necessary. The adoption of soft control methods, such as the use of integrated pest management, biological control, insect growth regulators (IGR), non-persistent chemicals, and other methods to slow or halt the development of resistance may be the only remaining options in pear psylla control. There is also evidence to support the use of soft controls because natural enemies are allowed to survive and provide additional control.

One such soft control method is the use of insect growth regulators. Booth and Riedl (1996) demonstrated that in Oregon pear orchards treated with the IGR diflubenzuron, there were more natural enemies present than there were in conventionally treated orchards, but there was still a need for the use of some insecticides and acaricides to prevent fruit damage. The IGR fenoxycarb has been shown to cause many adverse effects in pear psylla, including disruption of diapause, reduced egg hatch, and increased nymphal mortality (Higbee et al. 1995, Krysan 1990). Higbee et al. also showed that a very short adult contact time (less than five seconds) resulted in reduced egg hatch for up to one week. Horton and Lewis (1996), however, showed that application of fenoxycarb in the fall may not be useful, since pear psylla was able to over-winter as mature adults and produce viable eggs in the spring. It has been shown that fenoxycarb can remain active for several weeks, and can reduce spring egg hatch when moisture in the form of overhead irrigation or rainfall is available (Horton and Broers, 1997). Although fenoxycarb is not currently registered for use, several others are available, and insect growth regulators are an attractive solution to pesticides because they are selective. However, like any other chemical used to combat pear psylla, resistance may eventually develop.

Pre-bloom oil sprays are another such soft control method for pear psylla. Westigard et al. (1986) showed that the application of an oil spray appeared to control pear psylla in Oregon, while allowing natural enemies to survive. Pre-bloom oils have also been shown to control pear psylla in Washington State, although with varying results (Burts, 1983). Puterka et al. (2000) investigated the effects of oils, and obtained high levels of early season control and also determined that the controls carried over to the following season. Over-wintering and summer pear psylla oviposition and feeding rates were also shown to be significantly reduced by Supreme oil (Weissling et al., 1997). Oils appear to demonstrate the best control abilities of a non-insecticidal compound, but alone will probably not be enough to prevent damage to pears.

Detergents have also been used in soft pear psylla control programs. Detergents act as hydrophilic compounds that serve to drown the insects in water by breaking the surface tension produced by cuticular waxes. Weissling et al. (1997) demonstrated that the use of detergents can significantly reduce winter-form pear psylla oviposition rates, but at a lower rate than oils. Detergents may also have application in winter sprays of orchards because they have been shown to raise pear psylla mortality due to freezing (Horton et al., 1996). Freeze-dried bacteria, *Pseudomonas syringae,* and detergents may also be used in concert to raise the freezing point of pear psylla, and raise winter-form mortality (Senft, 1996). Detergents may have some application, but will likely never become the only solution to the problem, and their use will probably be limited to combination treatments with other compounds including insecticides.

Botanical insecticides can also be used to control pear psylla, although like any other chemicals, resistance may develop over time. Nicotine compounds are among the most deadly natural compounds that can be used against insects. Puterka and Severson (1995) determined that a sugar ester from the leaf trichomes of wild tobacco at a concentration of 500 ppm can produce a mortality rate greater than 94% for pear psylla nymphs and adults. Botanicals can also be used as feeding deterrents, as Horton et al. (1995) demonstrated with the application of an extract from *Curcubita foetidissima* (the buffalo gourd) onto pear shoots; also, pear psylla honeydew was reduced. The use of botanically derived compounds in combination with pesticides may result in higher mortality and lower crop damage, but like any other chemical, pests may become resistant over time.

There are likely many pear psylla fungal pathogens; however their application and implementation may be difficult. Puterka et al. (1994) explored the virulence of several fungi (*Beauveria bassiana*, *Metarhizium anisopliae, M. flavoviride, Paecilomyces fumosoroseus*, and *Verticillium lecanii*), and found that all are pathogenic to pear psylla nymphs and adults, but to varying degrees, with the three non-*Metarhizium* species exhibiting greater than 90% mortality and *Metarhizium* spp. causing around 50% mortality. Fungal pathogens may prove to be useful biocontrol agents especially when insecticides are used in the same orchards. Fungal pathogens are living organisms and can evolve along with their host, in this case pear psylla, to slow the development of resistance.

The development of resistant pear lines may prove effective in the short term, but the ongoing co-evolution between plants and insects is proof that resistant lines will not always be effective. Several researchers have studied resistant pear strains (Butt et al., 1989; Bell and Stuart, 1990; Puterka, 1997; Bell and van der Zwet, 1999; Puterka et al., 2002), however, in a long-lived perennial crop such as pear it is doubtful that resistant strains will be useful tools in the long-term, since pear psylla will eventually develop resistance to plant resistance.

Semiochemicals are another possible control method for pear psylla. In pear as well as apple orchards, codling moth mating disruption with pheromones has been a highly successful control method; a similar technique may be useful for pear psylla. Other semiochemicals may be used to attract predators and parasitoids to orchards. Drukker et al. (1995) and Scutareanu et al. (1997) showed that volatiles from pear psylla-infested trees are attractive to predatory anthocorids, and may be useful in enhancing biological control. Predators in orchards will slow the development of resistance, and may also prevent pest resurgences and secondary pest outbreaks. Generalist predators in pear orchards have been well studied, and they have been shown to be beneficial (Solomon et al., 1989; Paulson and Akre, 1992; Bogya et al., 1999; Civolani and Pasqualini, 2003).

Green Lacewings

All agricultural systems have insect pests, though management strategies differ greatly depending on crop, grower, and government regulations. All insects, including agricultural pests, have predators, parasitoids, and pathogens that negatively affect their survival and fecundity. In agricultural systems it is desirable to eliminate pests, without eliminating natural enemies, but this has not been the primary management strategy of many growers. Many growers have relied on broad-spectrum insecticides to control insect pests, but these strategies have also eliminated natural enemies. In recent years, many of these broad-spectrum insecticides have been shown to have detrimental effects on the environment, causing a cascade of negative effects often leading directly to humans. In response to these negative environmental effects, there has been a push to either eliminate insecticide use, or move to more environmentally friendly, narrow-spectrum, non-persistent insecticides. So-called “selective insecticides” target a small range of insects, leaving natural enemies, and other non-target species largely unaffected. Natural enemies left undisturbed can continue to control pest outbreaks after application of selective pesticides, and can provide control of resistant strains of pests. One potential drawback of using selective insecticides is a secondary pest outbreak; a minor pest, unaffected by the insecticide and relieved of competition, can reach outbreak status quickly if not controlled. Survival of natural enemies may allow biological control of secondary pest outbreaks. There are many generalist predators commonly found in agricultural ecosystems, the most well known are ladybird beetles (Coleoptera: Coccinellidae) and green lacewings (Neuroptera: Chrysopidae). Both ladybird beetles and green lacewings have been extensively studied for application in biological control of pest insects.

Lacewing morphology and taxonomic placement:

Lacewing adults are soft-bodied, holometabolous insects with four membranous wings that are held roof-like over the body at rest. Although they have mandibulate mouthparts in both the adult and larva, in many species only the larva is predaceous with piercing sucking mouthparts, while the adult feeds on pollen or honeydew with chewing mouthparts (Borrer et al., 1992). Many species of the family *Chrysopa* are predators, while many species of *Chrysoperla* are pollen and honeydew feeders. Eggs of green lacewings are laid on stalks to prevent cannibalism, and larvae pupate in silk cocoons in the soil or bark cracks. The common green lacewings most often utilized in biological control efforts belong in the family Chrysopidae, which in North American contains 87 species (Borrer et al., 1992). Henry et al. (2002) suggest that there may be far more species within this family, based on characters other than morphological, including ‘mating songs’. Species of the *Chrysoperla carnea* complex (in Europe) produce substrate-borne songs by abdominal vibration to attract mates of the same species and to repel individuals of sibling species (Henry et al., 2002). The use of songs such as those identified by Henry et al. (2002) for species identification may be vital to the creation of an industry standard for insectaries.

Predators and parasitoids of lacewings:

While there are many predators of lacewings, most are within the same generalist predator guild. Cisneros and Rosenheim (1997) demonstrated that the generalist reduviid predator *Zelus renardii* Kolenati (Hemiptera: Reduviidae) would not only prey on aphids, but also on larval *C. carnea* (In North America may be *C. plorabunda*). It has also been shown that coccinellids will consume lacewing eggs if they are accessible (Phoofolo and Obrycki, 1998). Another arthropod shown to prey on chrysopids was female erigonid spiders, which increased mortality of lacewing larvae by as much as 44% (Dinter, 2002).

There are parasitoids that attack only lacewings, but it is of more importance to biological control to determine if parasitoids of pests affect beneficial insects. Silva and Stouthamer (1999) found five species of *Trichogramma* (Hymenoptera: Trichogrammitidae) that could parasitize both the bollworm, (*Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae), and *C. carnea* in

Portugal. The results from Silva and Stouthamer (1999) suggest competition as well as parasitism, both of which reduce biocontrol by lacewings.

Competitors:

There are many competitors of lacewings; most of these are also generalist predators. Kaplan and Eubanks (2002) have shown that red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), are able to protect cotton aphids (*Aphis gossypii* Glover (Hemiptera: Aphididae)) from predation by *C. carnea* (In North America may be *C. plorabunda*) and to act as competitors by preventing lacewings from feeding on their natural prey. Insects that compete more directly with lacewings by feeding on the same prey include: coccinellids, reduviids, mirids, anthocorids, nabids, staphylinids, carabids, dermapterans, and other lacewing families (Cisneros and Rosenheim, 1997; Dinter, 2002; Elliott et al., 2002; Horton et al., 2002; Phoofolo and Obrycki, 1998; Pilcher et al., 1997; Stewart et al., 2002). Spiders have also been shown to compete with lacewings (Dinter, 2002).

Environmental interactions:

Many non-insect factors influence the behavior of lacewings. Limburg and Rosenheim (2001) investigated the role of extra-floral nectar consumption by *C. plorabunda*, and found that it increased longevity at low prey densities. The architecture of plants has been shown to influence the efficiency of *C. plorabunda* larvae in finding aphid prey (Clark and Messina, 1997). Predation efficiency of *C. plorabunda* has also been shown to be influenced by epicuticular waxes produced by plants (Eigenbrode et al., 1999). Ballal and Singh (1999) reported that adult lacewings exhibit a preference when choosing plants on which to oviposit, and Schultz (1988) has shown that lacewing oviposition can be influenced by the composition of plant species in a field. Plant volatiles have also been reported to attract lacewings to varying degrees (Hooper et al., 2002; Reddy, 2002; Zhu et al., 1999). Several factors determine whether lacewings survive and locate prey, and, if the agricultural environment is manipulated in a favorable manner for lacewings, biological control can be more effective.

Lacewings in agricultural systems:

Lacewings can be found virtually everywhere that plants are found, in all of the growing regions of the world, provided that there are adequate prey for survival and reproduction (Borrer et al., 1992; Daly et al., 1998). Although species are found in specific, limited regions, due to biological control and augmentative release programs several are now found outside of their natural range. In pome fruit orchards of the Pacific Northwest, one native lacewing species is *C. nigriconis*, but it is common for other species, such as *C. carnea* (in North America may be *C. plorabunda*) and *Chrysoperla rufilabris* Burmeister to be released for biological control efforts, though lacewings marketed as *C. carnea* may actually be *C. plorabunda*.

Resistance and susceptibility to pesticides:

Within agricultural systems it is common for a combination of management strategies to be employed by growers, such as chemical insecticides, biological insecticides, and natural enemy releases. When natural enemies are released in combination with insecticides, it is important to select either enemies that are not susceptible to the insecticides used, or life stages of the natural enemy that are not highly susceptible to the insecticide.

In Ontario, Canada, *C. carnea* (In North America may be *C. plorabunda*) have been shown to be resistant to a wide range of broad-spectrum insecticides, including pyrethroids, organophosphates, and carbamates (Pree et al., 1989). An example of a broad-spectrum insecticide negatively influencing natural enemy populations was reported by Kleintjes (1997) in Wisconsin Christmas tree plantations. Kleintjes (1997) found that in blocks sprayed with

Phosphamidon-8, there were fewer lacewings than in unsprayed blocks, and concluded that a more timely application of the insecticide would have killed fewer predators. In this case lacewings were not resistant to Phosphamidon-8, and were killed.

Because synthetic, broad-spectrum insecticides can be extremely destructive to natural enemies, it is desirable to find alternative, selective biological insecticides and insect growth regulators. Although biological insecticides and IGRs may not kill natural enemies outright, it is still largely unknown how, if at all, they affect natural enemies. Liu and Chen (2000) and Chen and Liu (2001) studied the effects of IGRs on *C. rufilabris* and found that they adversely affect several life stages. Buprofezin, a chitin synthesis inhibitor, was found to not affect eggs, third-instar larvae, or pupae, but slowed the developmental process and increased mortality in first and second larval instars (Liu and Chen, 2000). This suggests that application be limited to times when susceptible instars are not present. Chen and Liu (2001) investigated the effects of pyriproxyfen, a juvenile hormone analog, on all immature stages of lacewings and found that only second instars were not susceptible, but that third instars were the most vulnerable. In contrast, regarding oviposition and egg fertility of treated females, Medina et al. (2003) found pyriproxyfen to be safe to adults and third instar larvae of *C. carnea* at the maximum recommended field rate. Another IGR commonly used in biological control is azadirachtin, a terpenoid from the neem tree, *Azadirachta indica* (Schmutterer, 1990). Azadirachtin has been shown to not increase mortality in newly emerged adults, but fecundity is reduced in a reversible way. The presence of the IGR delayed ovary maturation, but once the IGR was removed, the ovaries began to develop properly and fecundity was restored (Medina et al., 2004). Medina et al. (2003) found that azadirachtin was harmful at high doses to larvae and pupae, suggesting that these stages are the most vulnerable. In a study feeding neem-treated prey to larvae of the lacewing, *Mallada signatus* Schneider; the predators did not experience acute toxicity to the neem-fed prey, but experienced a delay in pupation, and a subsequent high mortality of pupae at high doses (Qi et al., 2001).

The toxic effects of spinosad, a compound derived from soil actinomycetes with insecticidal properties, has been shown to cause little mortality in *C. carnea* (In North America may be *C. plorabunda*) larvae exposed to a granular formulation (Cisneros et al., 2002), presumably because the larvae did not feed on the granules. The microsporidium *Nosema pyrausta* Paillot, which is often used to control European corn borer, *Ostrinia nubilalis* Hubner (Lepidoptera), is not infective to *C. carnea* (In North America may be *C. plorabunda*) that were fed infected hosts (Sajap and Lewis, 1989). Sajap and Lewis (1989) also demonstrated that lacewings may even play a role in dispersal of *N. pyrausta* because a significant portion of spores passed through the predators and were still infective to corn borers.

Hilbeck et al. (1998b), in experiments feeding *C. carnea* (In North America may be *C. plorabunda*) larvae an artificial diet containing the *Bacillus thuringiensis* (Bt) Cry1Ab toxin at a concentration of 100μg/ml, found that the toxin increased mortality, but developmental time of survivors was unchanged. Feeding transgenic Bt corn pollen to lacewings has, however, been found to have no effect on mortality in any larval stage (Pilcher et al., 1997). Lacewing larvae fed Bt corn-fed prey have been shown to have higher mortality and higher developmental times, although the observations may have been more a function of feeding on inferior prey, rather than the Bt toxin itself (Hilbeck et al., 1998a).

When using lacewings in biocontrol efforts, it is important to select pesticides that are not toxic to predators. It would be counterproductive to release, or attract lacewings to an area and then kill them with pesticides.

Use of lacewings in biological control:

Lacewings are commonly observed in agricultural ecosystems and can be important biocontrol agents. Lacewings also can be found in almost any cropping system. *Chrysoperla rufilabris* was found by Stewart et al. (2002) to be a highly efficient predator of azalea lace bugs, *Stephanitis pyrioides* Scott (Hemiptera: Tingidae), a common pest of nursery azalea landscape shrubs. In an orchard ecosystem, Chrysopidae and Hermerobiidae were found to be the most common over-wintering predators (Horton et al., 2002). Elliott et al. (2002) showed that *C. plorabunda* density increased with increasing aphid density, suggesting that they can quickly respond to pest outbreaks.

Gerling et al. (1997) demonstrated that *C. carnea* was not an efficient predator of whiteflies in Israeli cotton fields, possibly due to behavioral and nutritional preferences. Legaspi et al. (1994) reported that lacewings fed whiteflies required a longer development time, likely from inadequate nutrition. Longer development time may, however, increase residence time and therefore increase numbers of prey consumed.

Chrysoperla externa Hagen (formerly *C. lanata*) has been suggested by Albuquerque et al. (1994) for use as a biological control agent in field and row crops, and in citrus orchards in tropical and temperate regions of Central and South America. *Chrysopa oculata* Say and *C. carnea* (In North America may be *C. plorabunda*) often prey upon corn pests, resulting in increased pest mortality (Obrycki et al., 1989). *Chrysoperla carnea* (In North America may be *C. plorabunda*) has been released to control vineyard pests and has been shown to significantly reduce leafhopper densities (Daane et al., 1996).

Monitoring:

Many monitoring techniques can be used to follow lacewing population trends in agricultural ecosystems. Udayagiri et al. (1997) reported that *C. carnea* (In North America may be *C. plorabunda*) adults do not appear to be attracted to the same color (yellow) as aphids, suggesting that they do not use visual cues in habitat finding behaviors. Using colored sticky traps in lacewing monitoring would likely be an ineffective method of active trapping, but would capture some incidental lacewings.

Light trapping of lacewings has been reported by Nabli et al. (1999), suggesting the use of either black light (365, 430, 540 nm) or cool white light (440 and 580 nm) in light traps. Light trapping is an effective tool in monitoring nocturnal and crepuscular insects, but, if left unattended, insect scavengers can decimate samples. The phase of the moon is important to consider when light trapping, since it is brighter than any man-made light and will out-compete a light trap. Light trapping should be conducted in the new moon phase.

Lacewing populations can be monitored using more traditional entomological techniques than light traps, such as nets and beating sheets, provided that randomization is factored into the collection (Elliott et al., 2002). Corrugated cardboard bands placed on tree trunks can be used to monitor over-wintering and pupating lacewings when they are collected at regular intervals throughout the year. (Horton et al., 2002). Semiochemical traps may also be useful in population monitoring.

Production, rearing, and release:

Augmentative releases of lacewings into agricultural ecosystems are often used in biological control programs. All life stages can potentially be released, but the most commonly released stadia are eggs and adults. Larvae are reared on a number of substrates in the laboratory, including artificial diets (Cohen and Smith, 1998), aphids, and insect eggs, adults, and immatures. Lacewings received from insectaries are not necessarily uniform in species composition; contaminant species are often found in insectary populations due to the difficulty in identifying lacewings. Also, shipped lacewings will often cannibalize each other and not be in a useable condition when received (O'Neil et al., 1998).

An important factor in augmentative release of lacewings is prolonged, low cost, storage of various life stages. Tauber et al. (1997) determined a method for inducing short-term diapause in a Mexican population of *C. carnea* (In North America may be *C. plorabunda*) larvae based on variable, decreasing day length. Also, coupling short day length with low temperatures, Tauber et al. (1993) reported a moderate-term storage method for adult *C. carnea* (In North America may be *C. plorabunda*), in which greater than 90 percent of eggs laid after diapause were fertile. A long-term storage method, as described by Chang et al. (1995), was most efficient when pupae or young adults experienced a decrease in day length, a decrease in temperature, and a pre-storage high carbohydrate diet followed by a post-storage carbohydrate and protein diet.

The strategy employed in an augmentative release of lacewings often effects the survival and distribution of the insects. Daane and Yokota (1997) determined that larval releases were more effective than egg releases, primarily due to inefficient dispersion and cannibalism in egg releases. These results suggest that the distribution of green lacewings would not be uniform if eggs are not released in an efficient manner. Eggs sprayed in liquid solutions have been found to have approximately 36 percent survival regardless of carrier liquid (Wunderlich and Giles, 1999). Gardner and Giles (1996) explored the survival and uniformity of the discharge of lacewing eggs suspended in vermiculite, and found that the eggs had high survival, but there was variability in deposition.

Semiochemicals:

Although there have been few reports of lacewings using semiochemicals, research in this area can explain host-finding and mate-finding behaviors. The volatiles produced by different plant species may mediate host and habitat finding in lacewings (Reddy, 2002), as well as insect produced products. Zhu et al. (1999) found that male and female *C. carnea* (In North America may be *C. plorabunda*) adults will respond to several semiochemicals produced by corn, catnip extracts, and a prey species of aphid. Hooper et al. (2002) also demonstrated that the essential oil of catnip will elicit an orientation response, but that the enantiomerically pure diastereoisomers of the oil produced varying responses suggesting that lacewings respond more favorably to one of the two enantiomers of the catnip compound. Iridodial, an impurity from catnip oil, has also been shown to be attractive to green lacewings (Chauhan, 2004; Zhang et al., 2006). Also, in Washington state, methyl salicylate has been shown to be attractive to green lacewings in hops and grapes (James, 2003, 2005, 2006; James and Price, 2004). Monitoring lacewings in the field using semiochemical-baited traps has been demonstrated to be an effective tool (Hooper et al., 2002). Semiochemicals may also have many applications in biological control efforts. They can be used to attract native populations of predators to agricultural fields from surrounding habitats, and to increase biological control through augmentative release by increasing the arrestment rate of released individuals.

Future applications of lacewings in biological control:

There is still much unexplored potential for the use of lacewings in biological control efforts. More research is needed if successful biocontrol programs are to be established. Education of growers regarding use of lacewings for biological control is necessary for any program to succeed. Resistance to pesticides, both synthetic and natural, needs further research, as new pesticides are marketed every year. With new products, scientific research needs to determine effects on lacewings and other beneficials. Growers should also be informed about which beneficial insect life stages are safe and which are susceptible to insecticides, as well as which species are most ideally suited to their cropping system.

Intra-guild predation and competition need much more extensive research, specifically with in-field applications. Growers should have information regarding which species should be conserved, and which are expendable, if any. Each agricultural ecosystem is unique, so research regarding intra-guild predation and competition, as well as the efficiency of lacewings in each cropping system and on each pest needs exploration.

Industry standards need to be established as more research is conducted regarding species composition and range relative to areas of sale and release. Northern species, for example, should not be sold to growers in the south and vice versa. The *C. carnea* species complex needs to be resolved in order to determine which species are best suited for which areas. There is more need for taxonomists now than ever, since the biological control of invasive species is being highly regulated by the government.

Lacewings are likely one of the most valuable biocontrol agents in agricultural ecosystems. They are naturally resistant to many insecticides, and as generalist predators can control a wide spectrum of insect pests. Many species of lacewings are commercially available and easy to use in augmentative release strategies. In an effort to reduce pesticides use, lacewings and other insect predators are an attractive option. Many researchers have demonstrated reductions in the need for pesticides by increased biocontrol with lacewings in agricultural ecosystems.

Semiochemicals should be further and more extensively explored. These chemicals can be used to attract natural populations in the field, as well as to keep an augmented population where they are released. Host-finding chemicals, such as those produced by prey and prey host plants, are likely the most effective area to research for semiochemicals. Attracting female lacewings would be much more effective than attracting males, since they can lay hundreds of eggs in a lifetime on the plants in fields of interest. Though female insects often are not attracted to pheromones other than aggregation pheromones, pheromones may also prove valuable.

Biological control programs should be able to integrate all research and match ideal biocontrol agents with pests. As semiochemicals that are used by lacewings are discovered, they should be used to attract native, and to retain augmented and native lacewings in agricultural ecosystems. Properly identified lacewings released in augmentative control programs should be released into areas where they will be ideal control agents. Lacewings should not be released into areas or in conjunction with competitors, or predators and parasitoids that will reduce their efficiency. When employing chemical pesticide controls, growers should be aware of those that are safe to lacewings and which instars are vulnerable. Plants can be manipulated to provide favorable habitats to lacewings without sacrificing yield or marketability of the crop. Education of growers is necessary, since they are to be the ones to implement any monitoring programs.

INTRODUCTION TO RESEARCH

Following the work of David James (James, 2003, 2005, 2006; James and Price, 2004) with herbivore-induced plant-produced volatile compounds as field attractants of adult green lacewings in hops and grapes, laboratory studies were designed to test the level of attraction of pear and pear psylla volatiles compared to charcoal-filtered air to lacewings. The first study used a Y-tube olfactometer to test the attractiveness of pear-produced and pear psylla-produced volatiles to the three species of green lacewings that James (2003, 2005, 2006) and James and Price (2004) reported as attracted to the herbivore-induced plant-produced volatile, methyl salicylate. The results of the odor tests from the Y-tube study were largely not significantly different from charcoal-filtered air, suggesting that either adult green lacewings were not attracted to any of the odors tested, or that there is an inherent flaw in the Y-tube olfactometer as a test system for adult lacewings. Adult green lacewings, as a flying insect with migratory habit (Deulli, 1980) are severely restricted in a Y-tube and cannot fly, and as a result may not behave in the same way that they would in the field. Taking into account the restrictions on lacewing behavior, and the inconsistent results, we decided to abandon the Y-tube as a test system, and construct a flight chamber to allow for more normal behavior.

In the flight chamber we tested attraction of green lacewings to the same odors as the Y-tube, but added methyl salicylate, squalene, and apple branches. Although we were testing methyl salicylate, a documented field attractant of green lacewings (James, 2003), we still could not verify attraction in the laboratory. In the flight chamber, green lacewings were induced to fly, but normal flight was often interrupted by the walls and ceiling of the flight chamber. In addition to flight being restricted by the physical size of the chamber, nearly one out of every four lacewings tested did not respond within the three hour cutoff period. Most studies of this type have a much shorter stop time, but for lacewings in both the flight chamber and Y-tube, unless they were given three hours to respond, three out of four lacewings would not respond. As in the Y-tube assays, no tested odors were significantly more attractive to green lacewings than clean air, once again suggesting that either there is no latent attraction to the tested odors, or that the flight chamber we constructed is an inadequate test system for green lacewings. Due to inconsistent results in the flight chamber field plots were established to test methyl salicylate attraction in the field in apple, pear and sagebrush, and plots to compare the attraction of squalene and methyl salicylate in pear and sagebrush.

The methyl salicylate in pear, apple, and sagebrush study was designed to verify field attraction previously documented by James (2003, 2005, 2006) and James and Price (2004), since we could not verify those results in the laboratory. In the field we found methyl salicylate to be significantly attractive to *Chrysopa nigricornis* Burmeister, *C. plorabunda*, and *Chrysopa coloradensis* Banks, but not to *C. oculata*. However, we did not find that lacewings were attracted enough by methyl salicylate to move out of preferred habitats; the primarily sagebrush inhabiting species *C. coloradensis* only rarely moved from sagebrush to orchards, despite orchards and sagebrush being adjacent. Also, the primarily arboreal species, *C. nigricornis*, was found only rarely to be attracted to methyl salicylate in sagebrush, though orchards and sagebrush were adjoining. This study indicates that there is likely a low level of attraction or short distance of attraction in green lacewings to methyl salicylate, and possibly to other herbivore-induced plant-produced volatile compounds.

The squalene in pear and sagebrush study was designed to determine if lacewings are attracted to an essential steroid precursor that they must obtain in their diet, and to compare results with their attraction to methyl salicylate in two lure types. Combination treatments of traps baited with methyl salicylate and squalene together were also tested to determine if there was an additive effect of combining volatile compounds. In this study, for methyl salicylate, the same results as the previous study were found; *C. nigricornis* was attracted in pear, and *C. coloradensis* was attracted in sagebrush. Squalene, though was found to be twice as attractive as methyl salicylate to *C. nigricornis* in both pear and sagebrush, and the dual treatment was nearly four times as attractive. That *C. nigricornis* was significantly more attracted to squalene, and squalene combined with methyl salicylate, may have implications for lacewing lures in increasing biological control, and for improving monitoring techniques.

CHAPTER 1. A FIELD EVALUATION OF METHYL SALICYLATE IN APPLE, PEAR, AND SAGEBRUSH

INTRODUCTION

Prey location by insect predators is mediated by a combination of auditory, visual, and chemical cues. Chemical signals include those used by prey for mating, aggregation, dispersal, alarm, and host location. Predators may also detect plant-produced herbivore-induced volatile compounds. For example, oviposition of the green lacewing *Chrysoperla carnea* (In North America may be *C. plorabunda*) is higher on herbivore-injured leaves than on undamaged leaves, and its adults are more attracted to mechanically and mite-damaged plants than to the prey mite itself, *Tetranychus ludeni* Zacher (Reddy, 2002; Reddy et al., 2004). Also, *C. carnea* (In North America may be *C. plorabunda*) adults of both sexes will respond to corn, catnip extracts, and aphids (Zhu et al., 1999). Iridodial, an component of catnip oil, has been shown to be attractive to lacewings (Chauhan, 2004). In hops and grapes in Washington state, methyl salicylate, or wintergreen oil, has been shown to be attractive to several species of green lacewings, including *C. nigricornis* and *C. oculata* (James, 2003, 2005, 2006; James and Price, 2004). Semiochemicals such as methyl salicylate and irododial may be used to increase densities of resident populations of lacewings to increase biological control, or in augmentative biological control programs to increase the likelihood that lacewings remain in the targeted release area.

Lacewings are resistant to several insecticides (Pree et al., 1989), and provide biological control in many agricultural systems. However, lacewings can be highly dispersive, and have the capacity for migration of more than 40km per night (Deulli, 1980). A number of lacewing species are commercially available for use in augmentative release strategies, but lacewing migratory habit often hinders effectiveness as they disperse from release areas. In an effort to reduce pesticide use, augmentative release of lacewings can be an effective tactic (Daane et al., 1996). This study seeks to evaluate the effectiveness and applicability of methyl salicylate as an attractant to native green lacewings in pear and apple compared with surrounding native sagebrush steppe vegetation, and determine its influence on migration among habitats.

MATERIALS AND METHODS

An experiment to test methyl salicylate as a lacewing attractant in pear and apple orchards and surrounding native sagebrush steppe vegetation plots was conducted from 10 July through 12 September, 2007. Three experimental sites, each 2km^2, were established in Wenatchee, Entiat, and Orondo, Washington. Each site contained pear, apple, and sagebrush plots of at least 10 ha, in a randomized block design with sites as blocking factors, and lure types as the treatments. Lures were assigned randomly to trap locations within plots.

Traps and lures. Each plot had 10 4"x7" two-sided yellow sticky traps (Seabright Laboratories, Emeryville, CA, USA) positioned 25-30m apart. Five each of methyl salicylate and control lures were randomly assigned to trap positions. Methyl salicylate lures were 2ml amber glass vials baited with 1ml ReagentPlus^{tm} ≥99% methyl salicylate (Sigma-Aldrich Co., St. Louis, MO, USA; CAS # 119-36-8) and 1cm long cotton dental wicking plugs, while control lures were empty 2ml amber glass vials with cotton plugs (after James, 2006). Lures were placed horizontally into holes in the sticky trap. Lure release rate was determined by first loading 10 lures with 1ml of methyl salicylate, then measuring the initial weight of the entire baited lure with a digital balance. Lures were then placed in the field in the manner that they were on traps, and re-collected and weighed every day for eight days. At the end of the eight days, the daily average release from all 10 lures was calculated.

Traps were hung at approximately 2m height in fruit trees using twist ties, and approximately 2m high in sagebrush using 3m x 2cm-diameter electrical conduit poles, placed 30cm into the soil, with a 45cm-long bend in the top. Traps and lures were replaced weekly without re-randomization to prevent false positives and a carry-over effect the week following a baited lure in a trap location, and to allow for season-long analysis of trap data (as in Fleming (1951) replacement without re-randomization for season-long analysis, and Fleming et al. (1940) for Japanese beetle trapping). Using plant produced compounds as lure bait may have also influenced the surrounding vegetation and produced a ghost-like carry-over effect in capture. If lures were not replaced weekly they would not have lasted for then entire 10 week trapping period, and if traps were not replaced weekly, trap captures may have deteriorated or been damaged by scavengers and impeded identification of species. Captured lacewings from weekly samples were tallied and identified using the keys of Penny et al. (2000) and Garland (1984).

Monitoring. To determine the presence of lacewing populations and species composition, lacewings were monitored three times in the orchards and sagebrush using beating tray, leaf inspection, and light trap sampling techniques. In weeks one, three, and nine, 10 beating tray samples and 50 leaves were taken from each of the five trees surrounding each trap.

Two beating tray samples, one on the side proximal to the trap and one on the distal side, were taken from each tree with a trap and the four adjacent trees at the cardinal directions. Lacewing larvae and adults were tallied when captured on beating tray samples.

Ten leaves were collected randomly from each of the five trees sampled by beating tray, and were used to monitor egg deposition. The number of leaves with eggs were recorded as a measure of oviposition events. Each week, 750 leaves were inspected from each lure type in each crop.

In week five, a new moon phase week, a gasoline-powered 175W mercury vapor lamp was suspended against a white canvas sheet in each habitat of each site. All neuropterans attracted to the mercury vapor lamp were collected from dusk to 1:00 am. Lacewings were tallied and identified using Penny et al. (2000) and Garland (1984) keys.

Statistical Analyses. Total seasonal trap data for major captured species were ln(x+1) transformed, and then analyzed for crop, treatment, and site effects using an analysis of variance (SAS Institute, version 9); means were compared using Bonferroni's mean comparison test (α=0.05).

RESULTS

Lures. Methyl salicylate lures released approximately 0.12ml of methyl salicylate per day (Table 1) and would have been completely empty after 1-2 weeks in the field.

Table 1. Release rate of methyl salicylate lures

	Average daily loss in ml from 5 lures
day 1	0.069
day 2	0.094
day 3	0.104
day 4	0.157
day 5	0.16
day 6	0.11
day 7	0.09
day 8	0.19
Average loss	0.12175

Pear. Throughout the 10wk trapping period, 345 green lacewings and 51 brown lacewings were captured in pear. There were significantly more *C. nigricornis* captured on methyl salicylate-baited traps than on control traps (P=0.024) (Table 3), and significantly more were captured at the Orondo (P<0.001) and Wenatchee (P<0.001) plots than Entiat (Table 4). *Chrysoperla plorabunda* was found to be significantly more attracted to methyl salicylate than to control traps (P<0.001) (Table 3), and no differences were found among blocks (Table 5). Both *C. coloradensis* and hemerobiids were not significantly different among treatments.

Beating tray samples in pear demonstrated that larvae and adults were present in low densities throughout the study period. In week one, beating tray samples at control traps captured two adult green lacewings, and at methyl salicylate traps captured one adult brown lacewing. Week three control treatment beating tray samples captured two adult green lacewings, and those from methyl salicylate captured three adult green lacewings. In week nine, samples in the control treatment captured three adult green lacewings and two larvae, while in the methyl salicylate treatment three adult green lacewings, two adult brown lacewings, and four larvae were found.

Leaf samples confirmed that eggs were deposited in pear throughout the experiment, but in low density. In weeks one and three, leaf samples from control and methyl salicylate treatments each recovered one leaf with lacewing eggs. In week nine, leaf samples from control trees recovered eight leaves with lacewing eggs, and those trees surrounding methyl salicylate traps had two leaves with eggs.

There were 19 *C. nigricornis*, two *C. plorabunda*, six *C. coloradensis,* two *C. oculata*, 11 *Eremochrysa* spp., two hemerobiids, and 1 myrmeleontid captured at mercury vapor lamps placed in pear blocks.

Apple. In apple there were 38 brown lacewings and 80 green lacewings captured through 10wks. *Chrysopa nigricornis* was captured significantly more often on methyl salicylate traps than control traps (P=0.023) (Table 3), and more were captured at Wenatchee than Entiat (P<0.001) and Orondo (P=0.030) (Table 4). The numbers of *C. coloradensis*, C. *plorabunda*, and Hemerobiids were not significantly different between those caught in control traps and those on methyl salicylate baited traps.

Lacewing adults were verified as present by beating tray. In week one, beating tray samples from both control and methyl salicylate treatments each captured one adult green lacewing. Beating tray samples from week three captured no and one adult green lacewing from control and methyl salicylate treatments, respectively. In week nine, no lacewings were captured from control and methyl salicylate beating tray samples in apple.

Lacewing eggs were collected throughout the experiment on apple leaves. Eight leaves from those sampled in week one from control treatment trees had lacewing eggs, while methyl salicylate had three leaves with eggs. In week three, leaf samples found that control trees had 16 leaves with lacewing eggs, and methyl salicylate trees had 15 leaves with eggs. In week nine, leaf samples from control and methyl salicylate treatments had 20 and eight leaves with lacewing eggs, respectively.

Mercury vapor lamps placed in the apple blocks captured nine *C. nigricornis,* three *C. oculata*, six *Eremochrysa* spp., and three hemerobiids.

Sagebrush. Traps in sagebrush plots over 10wks captured seven brown lacewings and 247 green lacewings. There were significantly more *C. coloradensis* (P<0.001), and *C. nigricornis* (P=0.005) captured on methyl salicylate baited traps than control traps, but there was no treatment effect for either *C. plorabunda* or brown lacewings (Table 3). The Orondo block had significantly more *C. coloradensis* than the other blocks (P<0.001 for each) (Table 6).

No adult lacewings were captured in native vegetation by beating tray sample in week one from control treatments, while one adult green lacewing was captured from beating tray samples at methyl salicylate baited treatments. In week three, beating tray samples from control and methyl salicylate treatments captured no lacewings. Week nine beating tray samples captured one lacewing larva at control treatments, and none from methyl salicylate treatments.

Eggs were only found on leaves in leaf samples in weeks one and three in sagebrush. Week one control samples had two leaves with eggs, and methyl salicylate treated samples had four leaves with eggs. Week three leaf samples had zero and two leaves with eggs from control and methyl salicylate treatments, respectively. In Week nine, control and methyl salicylate treatments each had no leaves with eggs.

Sixteen *C. coloradensis*, four *C. nigricornis*, three *C. oculata*, one *C. plorabunda*, 55 *Eremochrysa* spp., seven hemerobiids, 11 myrmeleontids, two mantispids, one berothid, and two unknown green lacewings were captured at the mercury vapor lamps in native vegetation.

Table 2. Total capture of lacewings in three habitat types 10 Jul-12 Sept, 2007, from 15 traps of each lure type in each habitat and analysis of variance table

	PEAR		**APPLE**		**SAGEBRUSH**	
	Methyl Salicylate	Control	Methyl Salicylate	Control	Methyl Salicylate	Control
C. nigricornis	273*	10	45*	5	28*	0
C. coloradensis	16	0	9	1	218*	0
C. Plorabunda	39*	7	12	8	1	0
Hemerobiidae	23	28	28	10	6	1

within habitats * indicates significant difference between methyl salicylate and control lures at α=0.05

Chrysopa nigricornis

```
                            Sum of
     Source         DF      Squares          Mean Square      F Value    Pr > F
     Model          17      16.38697139      0.96393949       16.98      <.0001
     Error          72      4.08649160       0.05675683
     Corr Total     89      20.47346299

     Source         DF      Type I SS        Mean Square      F Value    Pr > F
     TMT            1       5.09272534       5.09272534       89.73      <.0001
     Site           2       2.64212776       1.32106388       23.28      <.0001
     Crop           2       2.33061776       1.16530888       20.53      <.0001
     Site*Crop      4       2.12042138       0.53010534       9.34       <.0001
     TMT*Site       2       1.85411496       0.92705748       16.33      <.0001
     TMT*Crop       2       1.29706536       0.64853268       11.43      <.0001
     TMT*Site*Crop  4       1.04989884       0.26247471       4.62       0.0022
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Chrysopa coloradensis

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                            Sum of
     Source         DF      Squares          Mean Square      F Value    Pr > F
     Model          17      12.58975352      0.74057374       28.56      <.0001
     Error          72      1.86685120       0.02592849
     Corr Total     89      14.45660472

     Source         DF      Type I SS        Mean Square      F Value    Pr > F
     TMT            1       2.42818988       2.42818988       93.65      <.0001
     Site           2       2.34785182       1.17392591       45.28      <.0001
     Crop           2       1.49772242       0.74886121       28.88      <.0001
     Site*Crop      4       1.24887298       0.31221824       12.04      <.0001
     TMT*Crop       2       1.62313909       0.81156954       31.30      <.0001
     TMT*Site       2       2.08104542       1.04052271       40.13      <.0001
     TMT*Site*Crop  4       1.36293191       0.34073298       13.14      <.0001
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Chrysoperla plorabunda

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                            Sum of
     Source         DF      Squares          Mean Square      F Value    Pr > F
     Model          17      2.60452392       0.15320729       3.60       <.0001
     Error          72      3.06168840       0.04252345
     Corr Total     89      5.66621232

     Source         DF      Type I SS        Mean Square      F Value    Pr > F
     TMT            1       0.56786890       0.56786890       13.35      0.0005
     Site           2       0.00794462       0.00397231       0.09       0.9109
     Crop           2       1.04763882       0.52381941       12.32      <.0001
     Site*Crop      4       0.36608478       0.09152119       2.15       0.0831
     TMT*Crop       2       0.35536047       0.17768023       4.18       0.0192
     TMT*Site       2       0.10831280       0.05415640       1.27       0.2861
     TMT*Site*Crop  4       0.15131353       0.03782838       0.89       0.4747
```

Table 3. Average weekly capture (se) of *C. nigricornis* from five traps of each lure type at three experimental sites each with three plot types 10 July - 12 September, 2007, and the total capture of each lure type from each plot

SITE	PLOT	LURE TYPE	AVERAGE NUMBER OF ADULT LACEWINGS CAPTURED PER WEEK (SE)										
			Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Total capture
Wenatchee	Pear	Methyl salicylate	0.2 (0.2)	1.4 (0.93)	1.6 (0.68)	3.2 (1.39)	3.6 (1.03)	1.8 (0.73)	0.6 (0.4)	0.8 (0.58)	0.4 (0.4)	0	68
		Control	0	0	0	0.2 (0.2)	0.2 (0.2)	0	0	0	0	0	2
	Apple	Methyl salicylate	0.4 (0.4)	0.6 (0.6)	0	0.8 (0.37)	2.8 (0.73)	1.6 (0.51)	1.2 (0.58)	0.6 (0.4)	0	0	40
		Control	0	0	0	0	0.4 (0.45)	0	0	0	0	0	5
	Sagebrush	Methyl salicylate	0	0.2 (0.2)	0.6 (0.4)	0	0.2 (0.2)	0	0.2 (0.4)	0.2 (0.2)	0	0	7
		Control	0	0	0	0	0	0	0	0	0	0	0
Orondo	Pear	Methyl salicylate	0	0	1 (0.77)	0.4 (0.4)	4.2 (2.13)	13 (3.36)	15.6 (2.62)	5 (2.12)	1 (0.55)	0.2 (0.2)	202
		Control	0	0	0	0	0.2 (0.2)	0.4 (0.4)	0.8 (0.8)	0	0	0	7
	Apple	Methyl salicylate	0	0	0	0	0	0.4 (0.4)	0	0.4 (0.24)	0	0.2 (0.2)	5
		Control	0	0	0	0	0	0	0	0	0	0	0
	Sagebrush	Methyl salicylate	0	0.6 (0.24)	0.6 (0.40)	0	0.4 (0.24)	0.8 (0.37)	1.2 (0.73)	0	0.2 (0.2)	0	19
		Control	0	0	0	0	0	0	0	0	0	0	0
Entiat	Pear	Methyl salicylate	0	0	0	0	0.2 (0.2)	0	0	0.2 (0.2)	0.2 (0.2)	0	3
		Control	0	0	0	0	0	0	0.2 (0.2)	0	0	0	1
	Apple	Methyl salicylate	0	0	0	0	0	0	0	0	0	0	0
		Control	0	0	0	0	0	0	0	0	0	0	0
	Sagebrush	Methyl salicylate	0	0	0	0	0	0	0.2 (0.2)	0.2 (0.2)	0	0	2
		Control	0	0	0	0	0	0	0	0	0	0	0

Table 4. Average weekly capture (se) of *C. plorabunda* from 5 traps of each lure type at three experimental sites each with three plot types 10 July - 12 September, 2007, and the total capture of each lure type from each plot

SITE	PLOT	LURE TYPE	AVERAGE NUMBER OF ADULT LACEWINGS CAPTURED PER WEEK (SE)										
			Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Total capture
Wenatchee	Pear	Methyl salicylate	0	0.4 (0.24)	0	1 (0.45)	2 (0.55)	0.2 (0.2)	0	0	0	0	18
		Control	0	0	0	0	0.2 (0.2)	0	0	0	0	0	1
	Apple	Methyl salicylate	0	0	0	0.2 (0.2)	0.2 (0.2)	0	0	0	0.2 (0.2)	0	3
		Control	0	0	0	0	0	0	0	0	0	0	0
	Sagebrush	Methyl salicylate	0	0	0	0	0	0	0.2 (0.2)	0	0	0	1
		Control	0	0	0	0	0	0	0	0	0	0	0
Orondo	Pear	Methyl salicylate	0	0	0.2 (0.2)	0	0	0.2 (0.2)	0.6 (0.2)	0.2 (0.2)	0	0	6
		Control	0	0	0	0	0	0	0	0	0	0	0
	Apple	Methyl salicylate	0.4 (0.24)	0	0	0.2 (0.2)	0.2 (0.2)	0	0	0	0.2 (0.2)	0	5
		Control	0.4 (0.4)	0.2 (0.2)	0	0	0.6 (0.6)	0	0.2 (0.2)	0.2 (0.2)	0	0	8
	Sagebrush	Methyl salicylate	0	0	0	0	0	0	0	0	0	0	0
		Control	0	0	0	0	0	0	0	0	0	0	0
Entiat	Pear	Methyl salicylate	1 (0.63)	0	0	1.2 (0.58)	0	0.6 (0.6)	0	0.2 (0.2)	0	0	15
		Control	0.2 (0.2)	0	0	0.2 (0.2)	0.6 (0.24)	0.2 (0.2)	0	0	0	0	6
	Apple	Methyl salicylate	0.2 (0.2)	0	0.2 (0.2)	0.2 (0.2)	0	0	0.2 (0.2)	0	0	0	4
		Control	0	0	0	0	0	0	0	0	0	0	0
	Sagebrush	Methyl salicylate	0	0	0	0	0	0	0	0	0	0	0
		Control	0	0	0	0	0	0	0	0	0	0	0

Table 5. Average weekly capture (se) of *C. coloradensis* from 5 traps of each lure type at three experimental sites each with three plot types 10 July - 12 September , 2007, and the total capture of each lure type from each plot

SITE	PLOT	LURE TYPE	AVERAGE NUMBER OF ADULT LACEWINGS CAPTURED PER WEEK (SE)										
			Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Total capture
Wenatchee	Pear	Methyl salicylate	0	0.4 (0.24)	0	0	0.2 (0.2)	0	0	0	0	0	3
		Control	0	0	0	0	0	0	0	0	0	0	0
	Apple	Methyl salicylate	0	0	0	0	0	0	0	0	0	0	0
		Control	0	0	0	0	0	0	0	0	0	0	0
	Sagebrush	Methyl salicylate	0	0	0.2 (0.2)	0.2 (0.2)	0	0.6 (0.6)	0	0	0	0	5
		Control	0	0	0	0	0	0	0	0	0	0	0
Orondo	Pear	Methyl salicylate	0	0.2 (0.2)	0.2 (0.2)	2.2 (1.71)	0	0	0	0	0	0	13
		Control	0	0	0	0	0	0	0	0	0	0	0
	Apple	Methyl salicylate	0.2 (0.2)	0.4 (0.4)	0	1 (0.55)	0.2 (0.2)	0	0	0	0	0	9
		Control	0	0	0.2 (0.2)	0	0	0	0	0	0	0	1
	Sagebrush	Methyl salicylate	0.4 (0.24)	3.4 (0.93)	16 (2.12)	10.4 (2.04)	7.8 (1.28)	0.6 (0.24)	1.8 (0.66)	0.6 (0.24)	0.4 (0.24)	0.2 (0.2)	207
		Control	0	0	0	0	0	0	0	0	0	0	0
Entiat	Pear	Methyl salicylate	0	0	0	0	0	0	0	0	0	0	0
		Control	0	0	0	0	0	0	0	0	0	0	0
	Apple	Methyl salicylate	0	0	0	0	0	0	0	0	0	0	0
		Control	0	0	0	0	0	0	0	0	0	0	0
	Sagebrush	Methyl salicylate	0	0	0.2 (0.2)	0.4 (0.24)	0.2 (0.2)	0	0	0.2 (0.2)	0.2 (0.2)	0	6
		Control	0	0	0	0	0	0	0	0	0	0	0

DISCUSSION

This field study was initiated to determine the applicability of methyl salicylate as a lacewing attractant in pear and apple, and to compare species capture in pear, apple, and surrounding native sagebrush steppe vegetation. Lacewing species were found to be variably attracted to methyl salicylate in all three habitat types. Methyl salicylate was significantly attractive to *C. nigricornis* in all three habitat types, but significantly more were captured in pear than apple and sagebrush. *Chrysoperla plorabunda* was attracted to methyl salicylate only in pear and not in the other habitat types. Also, *C. coloradensis* was significantly attracted to methyl salicylate only in sagebrush. Population estimates by beating tray, leaf, and light sampling techniques were much lower than densities estimated by baited traps, suggesting that standard field sampling methods may not be adequate for lacewings.

Of the three main species captured, *C. nigricornis* is primarily found in deciduous trees, and *C. coloradensis* and *C. plorabunda* are more widespread and common in all habitats (Johnson, 1995). It is generally believed that insects move freely among habitat types, with areas of native vegetation as a source and agricultural areas as a sink, and that conservation of native refuges will increase biological control in managed areas (Horton et al., 2002). However, in this study, despite the presence of chemical attractants, lacewings only rarely moved from preferred habitats into adjacent habitats of a different composition. *Chrysopa nigricornis* was found primarily in pome fruit plantings, and *C. coloradensis* was more common in sagebrush. At each experimental site, the three habitat types were within a $2km^2$ area, far under the potential lacewing migration distance of 40km per night, so movement from habitat to habitat could have been easily achieved. Methyl salicylate does not appear to draw lacewings into an area from surrounding habitats, as evidenced by the clear differences in species captured in each habitat. It is therefore probable that methyl salicylate is a short distance attractant. Alternatively, if methyl salicylate were a long distance attractant, it would be expected that *C. coloradensis* would have moved into orchards from the sagebrush, and *C. nigricornis* would move into sagebrush as the duration of the experiment increased; neither result was found with this study.

The results of this study are similar to those obtained by James (2003, 2005) and James and Price (2004) in hops and grapes, but are the first descriptions of methyl salicylate as a lacewing attractant in pome fruit and native sagebrush steppe vegetation, and first to explore its effects on movement among habitats. Despite the short distance relative to potential lacewing migration between plots within blocks, there were clear differences in capture among crops and blocks. These differences were likely influenced by differing pest complexes and management strategies, as well as habitat preferences. Pear psylla is found at measurable levels regardless of management strategy, due to insecticide resistance (Pree et al. 1989, van de Baan et al. 1989). For lacewings, pear psylla is an abundant prey species which can facilitate populations to increase throughout the season. In apple, the pest complex available to lacewings is dominated by aphids and mites, both sufficient prey for lacewings. However, management strategies in apple are more effective at reducing pest populations, lowering availability of prey. The sagebrush habitat is the most diverse in terms of lacewing prey, but also the most patchy with a seasonal succession of short lived desert plants available for insect herbivores. Based on prey availability, preferred habitat, and methyl salicylate acting as a short distance attractant, differences in habitat capture explain higher *C. nigricornis* populations in pear than apple, and higher populations of *C. coloradensis* in sagebrush than pear and apple.

Although native lacewings are present in agricultural systems, they are also available for augmentative release from commercially-produced sources. Augmentative releases of insect predators can be used to increase biological control of agricultural pests. Methyl salicylate and other chemical attractants may be used to arrest augmented lacewings to increase biological control, as well as attract and arrest native populations. Though significantly attractive to lacewings, further studies to determine attraction of methyl salicylate over distance should be undertaken to better optimize augmentative release and conservation biological control strategies for green lacewings.

CHAPTER 2. A NEW FIELD ATTRACTANT OF *Chrysopa nigricornis*

INTRODUCTION

Green lacewings are predatory on a variety of soft-bodied insect pests, including aphids and pear psylla. The species of green lacewings most often providing biological control can migrate 40km or more per night (Deulli, 1980). Plant-produced volatiles may mediate host and habitat finding in green lacewings (Reddy, 2002). For example, Zhu et al. (1999) found that *Chrysoperla carnea* (In North America may be *C. plorabunda*) will respond to semiochemicals produced by corn, catnip, and aphids. Iridodial, an impurity from catnip oil, has also been shown to be attractive to green lacewings (Chauhan, 2004; Zhang et al. 2006). Also, in Washington state, methyl salicylate has been shown to be attractive to green lacewings in hops and grapes (James, 2003, 2005, 2006; James and Price, 2004), and pear, apple, and sagebrush steppe vegetation (RTC and JED unpublished data). Semiochemical attractants may attract green lacewings, and ensure that augmentitively released lacewings remain in the release area to provide increased biological control.

Squalene, an essential steroid precursor, is a chemical attractant of ticks (Yoder et al. 1993), a sex pheromone of garter snakes (Mason et al., 1989), produced in sharks livers (Pietsch and Jaeger, 2007), by such plants as apple (Dutton et al. 2002), olive (Grigoriadou et al. 2007), and palm (Lau et al., 2007), and in mammalian skin (Ramasastry et al., 1970). Neuropterans cannot produce squalene, and must obtain it in their diet for normal steroid production (Behmer and Nes, 2003). This study seeks to determine if green lacewings are attracted to squalene, as compared to methyl salicylate, a documented herbivore-induced plant-produced lacewing attractant, in pear plantings and sagebrush steppe vegetation.

MATERIALS AND METHODS

An experiment was designed to compare field attraction of squalene, methyl salicylate, methyl salicylate in Suterra™ controlled-release experimental biolure F173, and a dual treatment of squalene and methyl salicylate, to unbaited control lures and each other. Three sites in Peshastin, WA were selected as study sites. Study sites consisted of adjacent pear and sagebrush steppe plots each 5ha, large enough to contain 25 baited yellow sticky traps (Seabright Laboratories™) 25-30m apart with a 30m border. The five treatments were assigned randomly to trap locations within plots, and sites were blocking factors in a randomized block designed study.

Traps. Within each plot there were five traps each of control, methyl salicylate, F173, squalene, and dual baited traps. Lures (excluding F173) were constructed of 2ml amber glass vials with 1cm cotton dental wicking stoppers. Control lures were left empty, while baited lures had 1.5ml of either methyl salicylate or squalene. F173 lures were provided pre-loaded by Suterra™ and used for the duration of the experiment; release rate was not measured for F173 lures. Control, methyl salicylate, F173, and squalene traps were composed of one lure affixed to a yellow sticky card, whereas dual baited traps were composed of one methyl salicylate baited and one squalene baited lure affixed to one yellow sticky card. Baited traps were assigned randomly to trap locations within each plot. Excluding control and F173, lures were replaced weekly from 3 Aug – 21 Sept 2007. Traps and lures were replaced weekly without re-randomization to prevent false positive responses and carry-over effects due to stimulation of surrounding plants to release damage-induced volatiles the week following a baited lure in a trap location, and to allow for season-long analysis of trap data (as in Fleming (1951) replacement without re-randomization for season-long analysis, and Fleming et al. (1940) for beetle trapping). If lures were not replaced weekly they would not have lasted for then entire 10 week trapping period, and if traps were not replaced weekly, trap captures may have deteriorated or been damaged by scavengers and prevented identification of species. Also, all yellow sticky cards were replaced weekly and neuropterans were tallied and identified using the keys of Penny et al. (2000) and Garland (1985). In pear plots, traps were hung with aluminum twist ties from trees at 2m height; in sagebrush plots, traps were suspended from 3m x 2cm diameter electrical conduit poles, placed 30cm into the soil, with a 45cm long bend in the top, approximately 2m height.

Lure release rate was determined by first loading 10 lures with 1ml of either methyl salicylate or squalene, then measuring the initial weight of the entire baited lure with a digital balance. Lures were then placed in the field in the manner that they were on traps, and re-collected and weighed every day for eight days. At the end of the eight days, the daily average release from all 10 lures was calculated.

Monitoring. Lacewing populations were monitored at the beginning and end of the experiment using beating tray, leaf inspection, and light sampling techniques to determine the density of lacewing populations and species composition. In weeks two and seven, 10 beating tray samples and 50 leaves were taken from each of the five trees surrounding each trap.

Two beating tray samples, one on the side proximal to the trap and one on the distal side were taken from each tree with a trap and the four adjacent trees at the cardinal directions.

Lacewing larvae and adults were tallied when captured on beating tray samples. Ten leaves were collected randomly from each of the five trees sampled by beating tray, and were used to monitor egg deposition. The number of leaves with eggs were recorded as a measure of oviposition events. Additionally, a gas-powered 175W mercury vapor lamp was placed in each crop in week six (a new moon phase week), and all neuropterans attracted from dusk to 1:00 am were tallied, collected, and identified.

Statistical Analyses. Data for season trap catches were ln(x+1) transformed to normalize data and then analyzed for relevant species captured for treatment, plot, and site effects by an analysis of variance (SAS institute, version 9), and means were compared by Bonferroni's pre-planned comparisons test (α=0.05).

RESULTS

Methyl salicylate lures in the field released approximately 0.12ml/day at 75°F (Table 1), while squalene lures in the field released approximately 0.043ml/day at 75°F (Table 5). Methyl salicylate lures required regular replacement due to high daily release, and for standardization of lures, all lures were replaced weekly.

Table 6. Release rate of squalene lures

	Average daily loss in ml from 5 lures
day 1	0.041
day 2	0.038
day 3	0.045
day 4	0.049
day 5	0.037
day 6	0.043
day 7	0.039
day 8	0.048
Total loss	0.34
Average loss	0.043

Of 5504 green lacewings captured over 8 weeks in pear, there were 4681 *C. nigricornis*, 74 *C. plorabunda*, 1 *C. coloradensis*, and 748 indistinguishable due to wasp damage; there also were 97 brown lacewings and 22 snakeflies captured (Table 7). There were 1648 green lacewings captured in sagebrush, 1095 *C. nigricornis*, 16 *C. plorabunda*, 433 *C. coloradensis*, and 70 were indistinguishable due to wasp damage; also captured were 23 brown lacewings, 2 snakeflies, and 13 *Nothochrysa californica* (Table 7). There were significantly more *C. nigricornis* captured in pear than sagebrush (P<0.001), and there were significant block effects, with blocks one (P<0.001) and two (P<0.001) capturing more than block three (Table 8). Also, there were significantly more *C. coloradensis* captured in sagebrush than pear (P<0.001), and there were significantly more captured in blocks one (P<0.001) and two (P<0.001) than block three (Table 9). Significantly more *C. plorabunda* were captured in pear than sagebrush (P<0.001). Hemerobiids were captured significantly more often in block one than two (P<0.001) and three (P<0.001), and more often in pear than sagebrush (P<0.001). Wasp damaged insects were those that were not identifiable beyond family level, and were treated as a category of data (in this case species) and not included in species analyses. However, there was significantly higher wasp damage on both squalene and dual-baited traps than control (P<0.001 for both) and methyl salicylate (P<0.01 for both) and F173 traps (P<0.01 for both), significantly higher damage in pear than sagebrush (P<0.001), and significantly higher damage in block one than two (P<0.001) and three (P<0.001). That there was more damage on squalene traps may have been due to higher number of captured lacewings and higher availability of this food source.

Chrysopa nigricornis. Methyl salicylate (P<0.001), F173 (P<0.001), squalene (P<0.001) and dual (P<0.001) baited traps captured significantly more *C. nigricornis* than control traps.

Methyl salicylate (P=0.022), squalene (P<0.001), and dual (P<0.001) baited traps captured significantly more *C. nigricornis* than F173 baited traps. Squalene (P<0.001) and dual (P<0.001) baited traps captured significantly more *C. nigricornis* than methyl salicylate baited traps (Figures 3, 4).

Chrysopa coloradensis. Methyl salicylate (P<0.001), and F173 (P<0.001) baited traps captured significantly more *C. coloradensis* than control traps. Methyl salicylate traps also captured significantly more *C. coloradensis* than squalene (P<0.001) and dual (P<0.010) baited traps. F173 (P<0.001) baited traps, and dual (P<0.001) baited traps also captured significantly more *C. coloradensis* than squalene baited traps (Figure 5).

Hemerobiidae. No treatments were significantly different from control traps for Hemerobiid capture, but methyl salicylate baited traps captured significantly more than squalene (P=0.002) baited traps.

Monitoring. Beating tray samples demonstrated that larvae and adults were present in pear throughout the experiment (Table 10), however few adults were captured for identification. Beating tray samples were inconclusive in sagebrush (Table 10), but did demonstrate the presence of adults and larvae.

Leaf samples from pear blocks confirmed that there were eggs present at the beginning and the end of trials. In pear in the second week, control leaves had eight leaves with eggs, methyl salicylate leaves had four, F173 had five, squalene had six, dual had five leaves with eggs, out of 750 leaves per lure type sampled. In week seven, control leaves had eight, methyl salicylate had three, F173 had four, squalene had five, and dual had six leaves with eggs, out of 750 leaves per lure type sampled. Leaf samples from sagebrush showed that few eggs were present in week two, and the final week had no eggs recovered. In week two, control leaves had two leaves with eggs, methyl salicylate leaves had two, F173, squalene, and dual had zero leaves with eggs, out of 750 leaves per lure type sampled. In week seven, control leaves, methyl salicylate, F173, squalene, and dual each had zero leaves with eggs, out of 750 leaves per lure type sampled.

The mercury vapor lamp captured two *C. plorabunda*, 16 *C. nigricornis*, and one hemerobiid in pear in week six. In week six there were 43 *C. coloradensis*, 18 *C. oculata*, 21 *C. nigricornis*, seven *C. plorabunda*, eight *Eremochrysa* spp., eight hemerobiids, one berothid, and two myrmeleontids captured at the mercury vapor lamp.

Table 7. Total season capture by family and species per lure type in pear and sagebrush steppe in Peshastin, WA 3 August – 21 September, 2007 and analysis of variance table

	Control	Methyl Salicylate	F173	Squalene	Methyl salicylate + Squalene
			Pear		
Chrysopidae	*206*^{a}	*715*^{c}	*387*^{b}	*1695*^{d}	*2501*^{d}
C. nigricornis	*181*^{a}	*634*^{c}	*348*^{b}	*1388*^{d}	*2130*^{d}
C. coloradensis	-	*1*	-	-	-
C. oculata	-	-	-	-	-
C. plorabunda	*11*	*34*	*9*	*5*	*15*
Hemerobiidae	*16*	*32*	*23*	*7*	*19*
Raphidioptera	*1*	*6*	*2*	*3*	*10*
N. californica	-	-	-	-	-
Wasp damaged	*14*^{a}	*46*^{a}	*30*^{a}	*302*^{b}	*356*^{b}
			Sagebrush Steppe		
Chrysopidae	*50*^{a}	*418*^{c}	*253*^{b}	*329*^{d}	*598*^{d}
C. nigricornis	*22*^{a}	*182*^{c}	*80*^{b}	*306*^{d}	*505*^{d}
C. coloradensis	*21*^{a}	*205*^{c}	*146*^{c}	*8*^{a}	*53*^{b}
C. oculata	-	*9*	*13*	-	*12*
C. plorabunda	*2*	*4*	*2*	*2*	*6*
Hemerobiidae	*4*	*8*	*3*	*3*	*5*
Raphidioptera	*1*	*1*	-	-	-
N. californica	-	-	*5*	*3*	*5*
Wasp damaged	*5*	*18*	*12*	*13*	*22*

Different letters within species rows indicate significant difference among treatments at α=0.05

Chrysopa nigricornis

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                                 Sum of
Source               DF          Squares          Mean Square        F Value        Pr > F
Model                29          54.70952989      1.88653551         18.20          <.0001
Error                120         12.43781960      0.10364850
Corrected Total      149         67.14734949

Source               DF          Type I SS        Mean Square        F Value        Pr > F
Tmt                  4           29.46402449      7.36600612         71.07          <.0001
Site                 2           3.76541969       1.88270985         18.16          <.0001
Crop                 1           11.18808771      11.18808771        107.94         <.0001
Tmt*Crop             4           0.25967676       0.06491919         0.63           0.6446
Tmt*Site             8           1.01614431       0.12701804         1.23           0.2900
Site*Crop            2           8.02693441       4.01346721         38.72          <.0001
Tmt*Site*Crop        8           0.98924252       0.12365531         1.19           0.3089
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Chrysopa coloradensis

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                                 Sum of
Source               DF          Squares          Mean Square        F Value        Pr > F
Model                29          21.30869443      0.73478257         13.33          <.0001
Error                120         6.61490240       0.05512419
Corrected Total      149         27.92359683

Source               DF          Type I SS        Mean Square        F Value        Pr > F
Tmt                  4           3.29666553       0.82416638         14.95          <.0001
Site                 2           2.05547029       1.02773515         18.64          <.0001
Crop                 1           9.58769286       9.58769286         173.93         <.0001
Tmt*Crop             4           3.07476031       0.76869008         13.94          <.0001
Tmt*Site             8           0.48011511       0.06001439         1.09           0.3757
Site*Crop            2           2.25724464       1.12862232         20.47          <.0001
Tmt*Site*Crop        8           0.55674569       0.06959321         1.26           0.2695
```

Table 8. Average weekly capture (se) of *C. nigricornis* in Peshastin, WA 3 August - 23 September 2007 in two crop types at three sites from five lure types, and season capture from each lure type at each plot

			Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
A	PEAR	Control	1.8 (1.07)	1 (0.45)	1.8 (0.66)	9.2 (1.28)	6.8 (2.40)	4.2 (1.07)	0.8 (0.58)	0.2 (0.2)	129
		Methyl salicylate	10.6 (1.12)	7.2 (1.59)	16 (3.36)	12.6 (3.39)	11.4 (2.46)	4.4 (2.50)	0.6 (0.60)	0	314
		F173	3.8 (1.43)	7.4 (1.29)	10.8 (2.42)	15.2 (2.56)	7.2 (3.07)	2.2 (0.73)	0.4 (0.4)	0.2 (0.2)	236
		Squalene	18.2 (3.43)	13.4 (3.28)	31 (8)	25 (2.07)	26.8 (5.21)	11.4 (3.01)	2 (0.71)	1.6 (0.2)	647
		Methyl salicylate + Squalene	29.2 (6.70)	15.8 (4.45)	46.6 (6.1)	38.6 (6.48)	31.2 (9.91)	18 (4.82)	1 (0.4)	0.2 (0.2)	903
	SAGEBRUSH	Control	0	0	0	0	0.6 (0.6)	0.2 (0.2)	0	0	4
		Methyl salicylate	0	0.2 (0.2)	1.2 (0.8)	0.2 (0.2)	1.8 (1.2)	0.2 (0.2)	0	0	18
		F173	0	0.6 (0.4)	1 (0.63)	1.4 (1.17)	2 (0.63)	0	0	0	25
		Squalene	0	0.2 (0.2)	2.4 (0.6)	1.2 (0.97)	8.8 (2.71)	1.4 (0.68)	0	0	70
		Methyl salicylate + Squalene	0	2.2 (1.07)	9.4 (2.36)	6.6 (1.44)	17.8 (2.67)	3.6 (1.5)	0	0	198
B	PEAR	Control	0	0.2 (0.2)	0.8 (0.22)	2.8 (1.57)	4.4 (2.32)	0.8 (0.63)	0.4 (0.45)	0	47
		Methyl salicylate	2.8 (0.89)	4.6 (2.66)	14.4 (5.98)	14.6 (3.13)	13.4 (3.19)	4 (1.03)	0.4 (0.4)	0	271
		F173	2.2 (0.81)	1.6 (1.07)	3 (2.88)	7 (3.11)	5.6 (3.03)	0.8 (2.31)	0	0	101
		Squalene	12 (3.33)	15.2 (3.22)	26.4 (4.56)	30.8 (6.61)	25 (4.93)	6.2 (3.47)	1 (0.4)	0.4 (0.2)	585
		Methyl salicylate + Squalene	25.8 (5.69)	26.8 (4.72)	50.2 (10.52)	47.6 (10.03)	47.2 (9.02)	13 (5.23)	2.4 (2.08)	0.6 (0.58)	1068
	SAGEBRUSH	Control	0	0	0	1 (1)	0.4 (0.4)	0	0	0	7
		Methyl salicylate	0	0	2.8 (1.36)	4 (1.92)	7.4 (3.44)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	74
		F173	0	0	1 (0.32)	0.8 (0.20)	2.2 (1.11)	0.2 (0.2)	0	0	21
		Squalene	0	0.6 (0.4)	3 (2.53)	5.2 (1.59)	7.6 (0.98)	2.4 (0.75)	0.2 (0.2)	1.2 (0.58)	101
		Methyl salicylate + Squalene	0	0.8 (0.58)	5.2 (1.32)	5 (1.52)	11 (2.28)	1 (0.32)	0	0.4 (0.24)	117
C	PEAR	Control	0	0	0	0.40 (0.24)	0.60 (0.4)	0	0	0	5
		Methyl salicylate	0	0.2 (0.2)	1.8 (0.58)	5.6 (1.91)	1.4 (0.75)	0.2(0.2)	0.6 (0.4)	0	49
		F173	0.2 (0.2)	0	0	0.4 (0.24)	1 (0.77)	0.2(0.2)	0.4 (0.4)	0	11
		Squalene	3 (0.55)	2.8 (0.97)	7.8 (2.78)	7.8 (0.97)	7.4 (1.33)	1.8(0.97)	0.4 (0.24)	0.2 (0.2)	156
		Methyl salicylate + Squalene	3.8 (0.8)	2.4 (0.81)	9.4 (2.2)	9.6 (2.14)	4.8 (0.58)	1.4(0.75)	0.2 (0.2)	0.2 (0.2)	159
	SAGEBRUSH	Control	0	0	0.4 (0.24)	0.8 (0.58)	0.4 (0.24)	0.6(0.4)	0	0	11
		Methyl salicylate	0	2.8 (1.62)	7.4 (5.51)	3.4 (1.75)	3.2 (1.16)	0.2(0.2)	0.8 (0.8)	0.2 (0.2)	90
		F173	0	1.2 (0.49)	2.4 (0.81)	2 (1.1)	1 (0.32)	0.2(0.2)	0	0	34
		Squalene	0	2.6 (0.93)	11 (3.58)	5.4 (1.69)	6.2 (0.97)	1.2(0.37)	0.2 (0.2)	0.4 (0.4)	135
		Methyl salicylate + Squalene	0	7 (3.21)	13 (3.79)	6 (3.16)	8.8 (2.08)	1.6(0.75)	1.4 (0.98)	0.2 (0.2)	190

Table 9. Average weekly capture (se) of *C. coloradensis* in Peshastin, WA 3 August - 23 September 2007 in two crop types at three sites from five lure types, and season capture from each lure type at each plot

			Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
A	PEAR	Control	0	0	0	0	0	0	0	0	0
		Methyl salicylate	0	0	0	0	0	0	0	0	0
		F173	0	0	0	0	0	0	0	0	0
		Squalene	0	0	0	0	0	0	0	0	0
		Methyl salicylate + Squalene	0	0	0	0	0	0	0	0	0
	SAGEBRUSH	Control	0	0.2 (0.2)	0.4 (0.24)	0.2 (0.2)	1 (0.77)	0.6 (0.4)	0	0.4 (0.4)	14
		Methyl salicylate	0	4.4 (4.15)	7 (2.97)	4 (2.21)	3.8 (1.59)	1.8 (1.11)	0.2 (0.2)	1.6 (1.36)	114
		F173	0	2.2 (1.32)	7.6 (4.95)	1 (0.32)	2.6 (1.29)	1.6 (0.51)	0	0.2 (0.2)	76
		Squalene	0	0.2 (0.2)	0	0.2 (0.2)	0	0	0	0.6 (0.4)	5
		Methyl salicylate + Squalene	0	1.2 (0.49)	3.6 (1.12)	1.2 (0.97)	0.6 (0.24)	0	0	0	33
B	PEAR	Control	0	0	0	0	0	0	0	0	0
		Methyl salicylate	0	0	0	0	0	0	0	0	0
		F173	0	0	0	0	0	0	0	0	0
		Squalene	0	0	0	0	0	0	0	0	0
		Methyl salicylate + Squalene	0	0	0	0	0	0	0	0	0
	SAGEBRUSH	Control	0	0.2 (0.2)	0.6 (0.4)	0	0	0.4 (0.24)	0	0	6
		Methyl salicylate	0	4.4 (1.94)	5.4 (1.69)	0.6 (0.4)	4.6 (1.91)	0.6 (0.4)	0.2 (0.4)	0.4 (0.4)	81
		F173	0	2.6 (1.17)	6.2 (3.01)	1.4 (0.93)	1.8 (1.11)	0.6 (0.4)	0	0	63
		Squalene	0	0	0	0	0.6 (0.4)	0	0	0	3
		Methyl salicylate + Squalene	0	0.8 (0.8)	0.6 (0.4)	0.8 (0.2)	0.8 (0.58)	0.4 (0.24)	0.2 (0.2)	0	18
C	PEAR	Control	0	0	0	0	0	0	0	0	0
		Methyl salicylate	0	0.2 (0.2)	0	0	0	0	0	0	1
		F173	0	0	0	0	0	0	0	0	0
		Squalene	0	0	0	0	0	0	0	0	0
		Methyl salicylate + Squalene	0	0	0	0	0	0	0	0	0
	SAGEBRUSH	Control	0	0	0.2 (0.2)	0	0	0	0	0	1
		Methyl salicylate	0	1 (0.63)	0.8 (0.37)	0	0.2 (0.2)	0	0	0	10
		F173	0	0.8 (0.49)	0.4 (0.24)	0	0.2 (0.20)	0	0	0	7
		Squalene	0	0	0	0	0	0	0	0	0
		Methyl salicylate + Squalene	0	0	0.4 (0.4)	0	0	0	0	0	2

Figure 1. Season capture of *C. nigricornis* at three pear sites in Peshastin, WA, 3 August - 23 September 2007, on traps baited with methyl salicylate in glass lures, methyl salicylate in a suterra lure (f173), squalene in glass lures, and a combination treatment of squalene and methyl salicylate lures

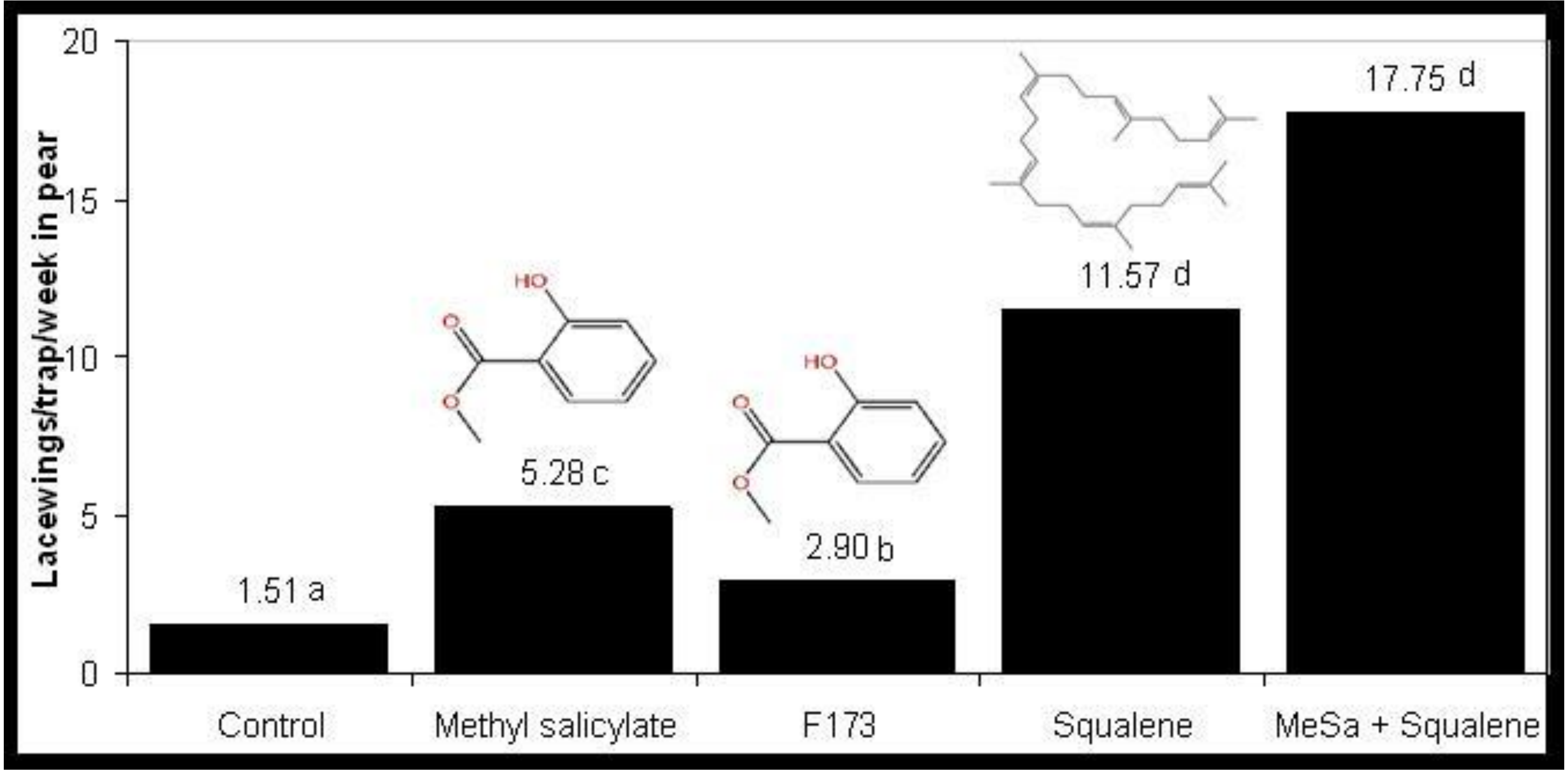


Figure 2. Season capture of *C. nigricornis* at three sagebrush sites in Peshastin, WA, 3 August - 23 September 2007, on traps baited with methyl salicylate in glass lures, methyl salicylate in suterra lures (f173), squalene in glass lures, and a combination treatment of squalene and methyl salicylate lures

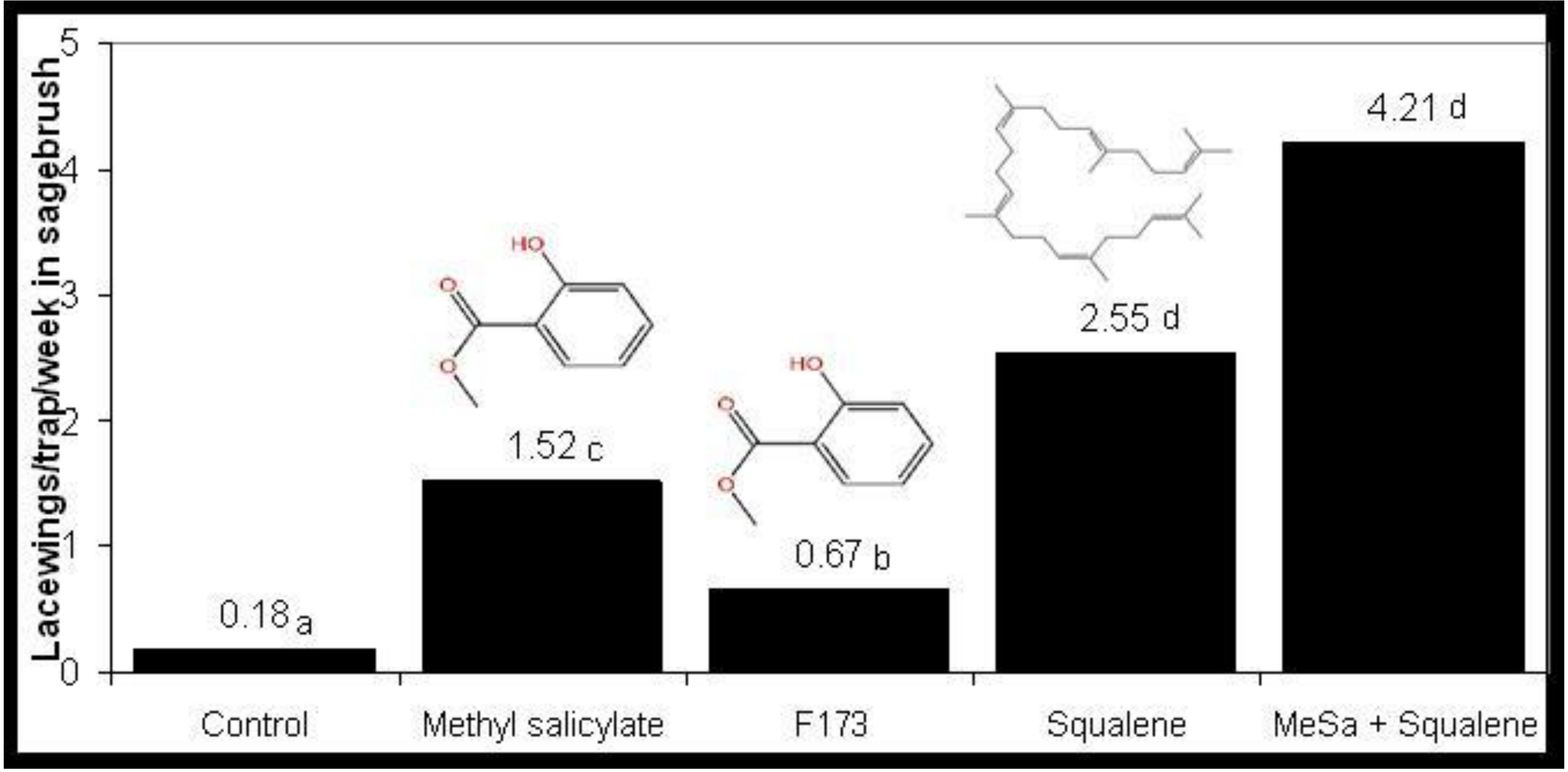


Figure 3. Season capture of *C. coloradensis* at three sagebrush sites in Peshastin, WA, 3 August - 23 September 2007, on traps baited with methyl salicylate in glass lures, methyl salicylate in a suterra lure (f173), squalene in glass lures, and a combination treatment of squalene and methyl salicylate lures

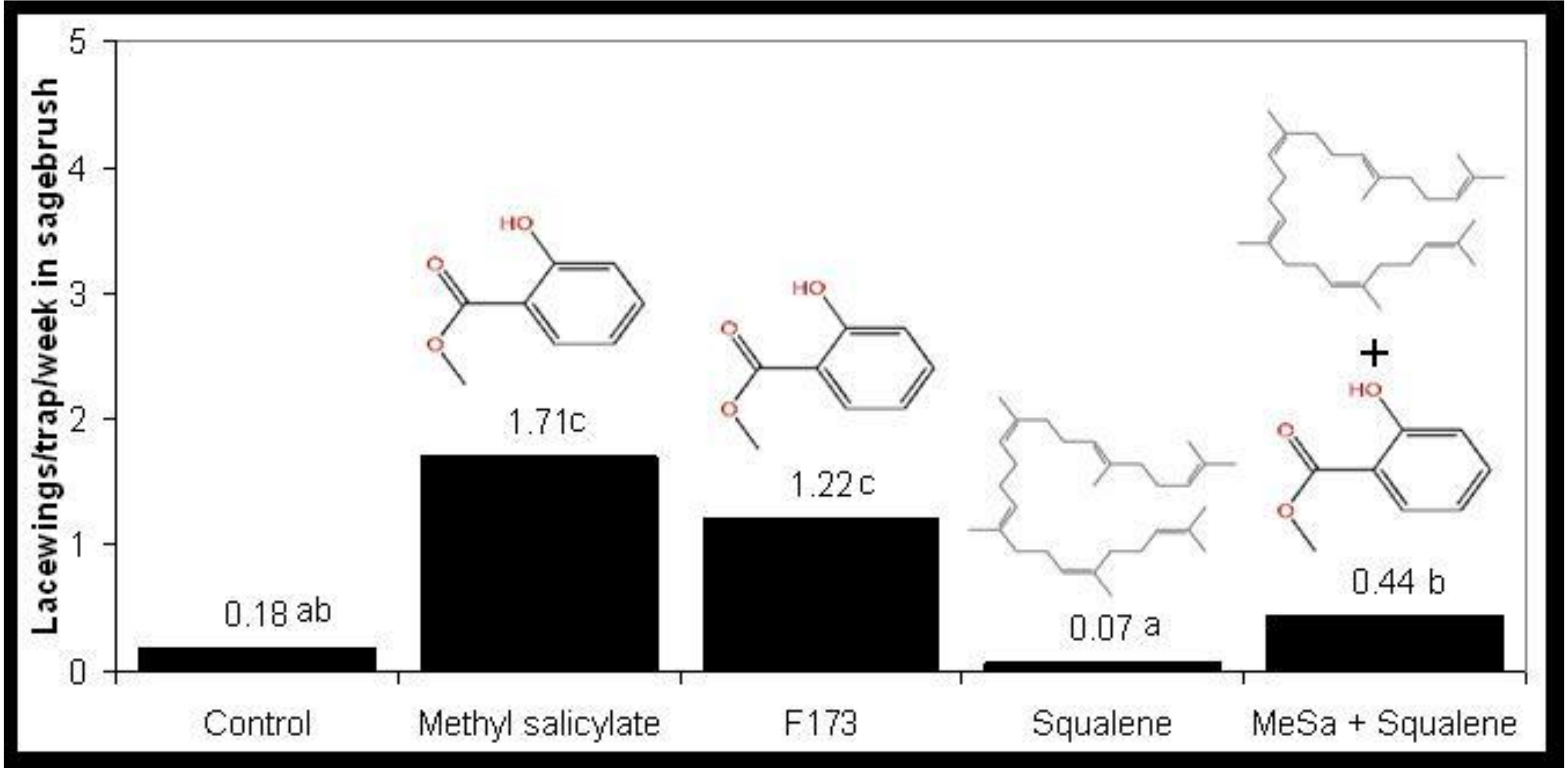


Table 10. Beating tray samples in pear and sagebrush plots in Peshastin, WA 10 August and 14 September 2007; total counts from 10 trays per trap, from 15 traps of each lure in each habitat

	Control		MeSa		F173		Squalene		Dual	
	Wk 2	Wk 7	Wk 2	Wk 7	Wk 2	Wk 7	Wk 2	Wk 7	Wk 2	Wk 7
					PEAR					
LWA	*1*	*9*	*1*	*10*	*1*	*2*	*5*	-	*4*	*11*
C. plorabunda	-	*5*	-	*3*	-	*1*	-	-	-	*1*
C. nigricornis	-	*2*	-	*1*	-	-	-	-	-	*3*
C. coloradensis	-	-	-	-	-	*1*	-	-	-	-
Brown LWA	-	*2*	-	*4*	-	-	-	-	-	*1*
LWL	*2*	*35*	*3*	*28*	*7*	*1*	-	*4*	*3*	*30*
					SAGEBRUSH STEPPE					
LWA	-	-	-	-	*1*	-	-	-	-	-
C. plorabunda	-	-	-	-	-	-	-	-	-	-
C. nigricornis	-	-	-	-	-	-	-	-	-	-
C. coloradensis	-	-	-	-	-	-	-	-	-	-
Brown LWA	-	-	-	-	-	-	-	-	-	-
LWL	-	-	-	-	-	*1*	-	-	*1*	-

LWA means lacewing adult
LWL means lacewing larvae

DISCUSSION

This study indicates that squalene is a potent attractant of *C. nigricornis,* and that the combination of methyl salicylate appears to have an additive effect. It is somewhat unexpected that *C. nigricornis* would be attracted to a compound as ubiquitous as squalene. Interestingly squalene appears to be specifically attractive to *C. nigricornis* and not to other species of lacewings, and in the case of *C. coloradensis* there may even be a repellency since the combination treatment has lower capture than methyl salicylate alone. Squalene may have potential use as a field attractant in crop systems that are dominated by *C. nigricornis*, or as an arrestant when augmentative releases are used. There are two possible explanations for squalene being more attractive to *C. nigricornis* than methyl salicylate, it is either more attractive over the same area, or it is attractive over a larger area. However, that there appears to be an additive effect in the dual lure suggests that squalene draws lacewings from a larger area, and the combination treatment is more attractive over short distance than either one alone.

Squalene has, to the best of our knowledge, never before been shown to be an attractive chemical for a predatory insect. The influence on behavior is unexpected since it is never limited where prey is not limited. Alone, squalene was more than twice as attractive as methyl salicylate, which has been shown to be an effective lacewing attractant, and in combination with methyl salicylate, squalene was almost four times as attractive as methyl salicylate alone. Combination lures could be used to increase biological control in areas of high pest density, especially if *C. nigricornis* is released augmentatively. Studies need to be undertaken to identify: distance over which squalene is effective, appropriate dose/acre, effectiveness as an arrestant on released *C. nigricornis*, and short term temporal trap efficiency compared to methyl salicylate.

CHAPTER 3. Y-TUBE ASSAYS

INTRODUCTION

Pear psylla is the key insect pest of pears in Europe and North America (Bues et al., 2000; Kapatos and Stratopoulou, 1999; Krysan, 1990). There are many predators and parasitoids of pear psylla as well as a suite of pesticides available for control efforts, but pear psylla has developed resistance to many insecticides since use began in the early 1900’s. Green lacewings are predatory on a variety of soft bodied insect pests, including pear psylla, however, the species of green lacewings most often used in biological control efforts can migrate 40km or more per night (Deulli, 1980). Plant-produced volatiles may mediate host and habitat finding in lacewings (Reddy, 2002). For example, Zhu et al. (1999) found that *Chrysoperla carnea* (In North America may be *C. plorabunda*) will respond to semiochemicals produced by corn, catnip, and aphids. This study was initiated to test whether lacewings are attracted to pear plant-produced compounds, pear psylla and controls, using a laboratory-controlled y-tube olfactometer (Barrows, 1907; McIndoo 1926).

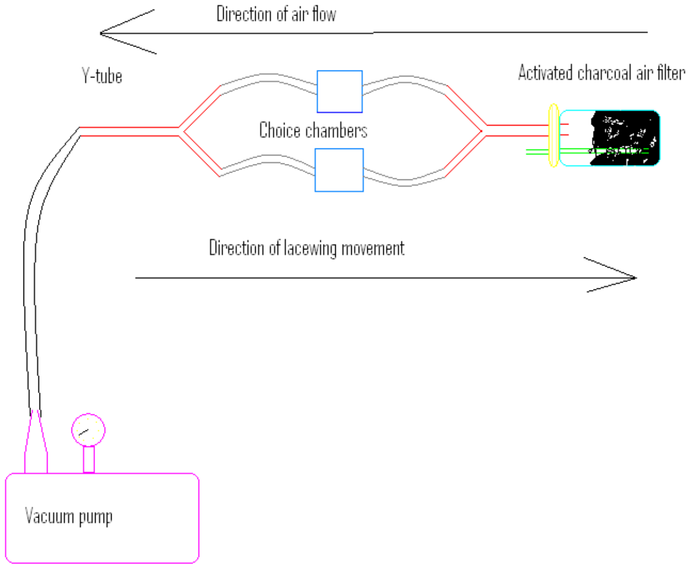
MATERIALS AND METHODS

Insects and plant materials. Green lacewings (*C. plorabunda*, *C. nigricornis, C. oculata*) were field collected at a 400W mercury vapor (MV) light at Washington State University Tree Fruit Research and Extension Center - Columbia View Plots (CV) in East Wenatchee WA, and in pear plots with a beat sheet and net at the Washington State University Tree Fruit Research and Extension Center in Wenatchee, WA (TFREC). Lacewings were held in a ~20°C 16:8hr L:D laboratory, and starved 24hr before use in trials. Pear psylla were field collected using a beating sheet and an aspirator from pear trees at TFREC, and used in trials the same day they were collected. Pear psylla honeydew was collected from infested pear leaves and used immediately after collection. Uninfested pear leaves were taken from pear saplings grown in a greenhouse at 20-25°C with natural day length at TFREC, while infested and pear psylla damaged pear leaves were taken randomly from trees grown in field plots at TFREC, and used within 2hr.

Y-tube. Lacewing responses to plant and insect material were tested in a glass Y-tube olfactometer which consisted of two screened odor source chambers (2.5x7.5x7.5cm, ¾in plexiglass), a regulated air delivery system (a Gilmont Instruments™ air flow regulator and a General Electric™ vacuum pump), an activated carbon filter, and a screened insect inlet adapter to prevent lacewings from entering the vacuum pump (figure 1). The olfactometer was kept under negative pressure to provide consistent air flow at 1.0 l/min. The Y-tube was 1 cm uniform internal diameter, with 8 cm arm length, and 10 cm main stem length. All experiments were conducted in dim red light to simulate crepuscular conditions by eliminating visual cues, and observed using Bushnell™ night vision goggles.

For all Y-tube experiments, lacewings were presented with an odor source on one side and clean air on the other side. A choice was recorded when the lacewing reached ¾ the distance of arm with odor source, negative choices were either no response, or response to the arm with charcoal-filtered air. Trials were initiated by inserting the lacewing into the screened insect inlet adapter and turning on the vacuum pump to begin air flow. In order to prevent side bias, the Y-tube was cleaned after every trial with soapy water and acetone, odor sources were switched every 3rd run, and the Y-tube was flipped every 2nd. Initially, 50 lacewings were tested with both sides blank (clean air). Fifty lacewings for each odor were offered 15 pear psylla adults and nymphs mixed in the odor source, 1 ml of honeydew, one clean undamaged leaf still attached to the sapling, one mechanically damaged leaf, and washed pear psylla damaged leaves. Choice combinations, including pear psylla honeydew + undamaged leaf (45 lacewings), pear psylla + undamaged leaf (25 lacewings), pear psylla + undamaged leaf + honeydew (25 lacewings), and pear psylla + washed psylla damaged leaf (25 lacewings), were also tested. Experiments were conducted from May-August, 2005.

Figure 4. Schematic of Y-tube



Statistical analyses. Data from Y-tube assays were analyzed using a g-test of goodness of fit (α=0.05). Although many studies of this type use a chi square analysis, the g-test of goodness of fit is more appropriate for binomial data generated by Y-tube assays.

RESULTS

Testing intrinsic bias with no odor on either side with 50 lacewings, 21 went to the left side 29 to the right (P=0.256), not significantly different. Odors which were also not significantly more than clean air were: fifteen pear psylla, pear psylla honeydew, washed pear psylla damaged leaves, undamaged leaves, mechanically damaged leaves, pear psylla honeydew + undamaged leaves, pear psylla + undamaged leaves, and pear psylla + undamaged leaves + honeydew (Table 1). The odor pear psylla + pear psylla damaged leaves was significantly more attractive than clean air, with 18 positive and 7 negative (P=0.025).

Table 11. Lacewing Y-tube olfactometer choice test responses

Odor	Positive	Negative	*P*
AIR	*21*	*29*	*0.256*
15 pear psylla	*27*	*23*	*0.571*
pear psylla honeydew	*21*	*29*	*0.256*
Washed pear psylla damaged leaves	*21*	*29*	*0.256*
undamaged leaves	*29*	*21*	*0.256*
Mechanically damaged leaves	*28*	*22*	*0.359*
pear psylla honeydew + undamaged leaves	*17*	*28*	*0.099*
pear psylla + undamaged leaves	*14*	*11*	*0.548*
pear psylla + undamaged leaves + honeydew	*10*	*15*	*0.315*
psylla + pear psylla damaged leaves	*18*	*7*	*0.025**

DISCUSSION

There are no known laboratory studies with adult lacewings that attempt to determine latent attraction to pear infochemicals by directly observing behavior in a Y-tube in the laboratory. With lacewings being one of the major biological control agents in tree fruit, these experiments were initiated to determine baseline attraction to tree fruit (with particular reference to pear) for increasing natural biological control, and increased retention of augmentatively released individuals.

Lacewings were not significantly attracted to either side of the Y-tube apparatus when only clean air was being passed through, suggesting that there is no intrinsic bias in the set-up. However, they were also not found to be significantly attracted to any tested odors other than pear psylla + pear psylla damaged leaves. Y-tube experiments were abandoned because it was determined that consistent results were unlikely since adult lacewings fly to find hosts. In a Y-tube lacewings cannot fly, so results may be skewed toward evenness due to an escape response rather than a host locating response. It was thought that a larger scale apparatus was needed to more accurately observe behavior (as in Brown et al. (1951) for mosquitoes), thus a flight chamber was constructed to allow for flight while retaining the basic design of a Y-tube.

CHAPTER 4. FLIGHT CHAMBER ASSAYS

INTRODUCTION

Prey location by insect predators is often mediated by chemical cues, including those used by prey for mating, aggregation, dispersal, alarm, and host location. Predatory insects may also detect plant-produced herbivore-induced volatile compounds. For example, oviposition and attraction of the green lacewing *Chrysoperla carnea* (In North America may be *C. plorabunda*) is higher on herbivore-injured leaves than on undamaged leaves (Reddy, 2002; Reddy et al., 2004). In hops and grapes in Washington state, methyl salicylate has been shown to be attractive to several species of green lacewings (James, 2003, 2005, 2006; James and Price, 2004). Semiochemicals such as methyl salicylate may be used to increase densities of resident populations of lacewings to increase biological control, or in augmentative biological control programs to increase the likelihood that lacewings remain in the targeted release area.

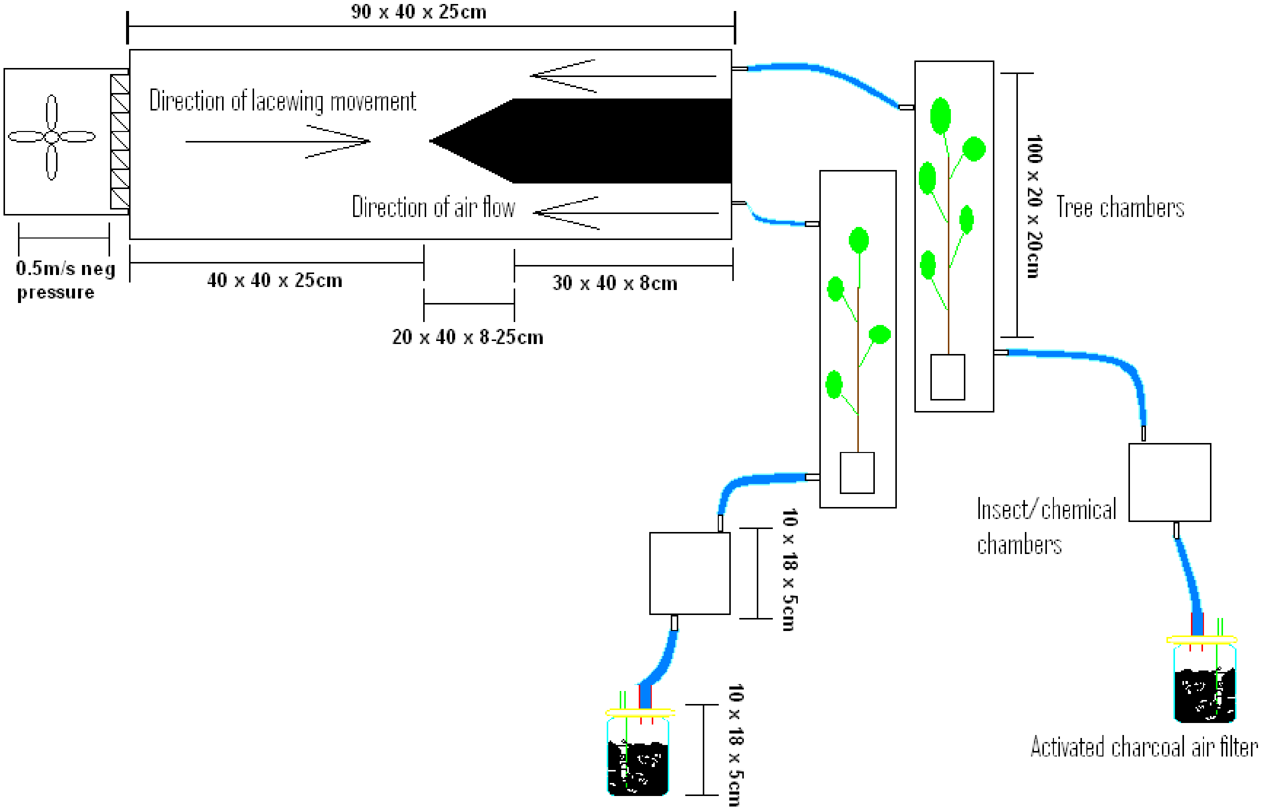
Although lacewings are resistant to several insecticides (Pree et al., 1989), and provide biological control in many agricultural systems, they can be highly dispersive (Deulli, 1980). A number of lacewing species are commercially available for use in augmentative release strategies, but lacewing migratory habit often hinders their effectiveness. In an effort to reduce pesticide use, augmentative release of lacewings can be an effective tactic (Daane et al., 1996). This study seeks to evaluate the attraction of apple and pear leaf volatiles, pear psylla, squalene and methyl salicylate to field collected green lacewings in a laboratory-controlled flight chamber.

MATERIALS AND METHODS

Green lacewings (*C. plorabunda*, *C. nigricornis*, *C. oculata*) were field collected at a 400W mercury vapor (MV) light at Washington State University Tree Fruit Research and Extension Center - Columbia View Plots (CV) in East Wenatchee WA, and in pear plots with a beat sheet and net at Washington State University Tree Fruit Research and Extension Center in Wenatchee, WA (TFREC). Lacewings were held in a ~20°C 16:8 (L:D) hr laboratory, and starved 24hr before use in trials. Pear psylla were field collected using a beat sheet and an aspirator from pear trees at TFREC, and used in trials the same day they were collected. Pear psylla infested pear branches and apple branches, 0.75-1m long, were cut randomly from trees at TFREC, and used within 2hr.

A sealed flight chamber with removable lid was constructed from 1cm plexiglass and hot glue. The flight chamber dimensions were 90x40x25cm, and was built with two air flow columns separated by a ‘V’ (figure 2). The air flow columns were 30x40x8cm before the ‘V’ expansion, which was 20x40x8-30cm, and the flight area was 40x40x25in. Laminar air flow was achieved by using a squirrel cage fan to maintain negative pressure and have equal air input and output at a rate of 0.5m/s, verified by hot wire anemometer. The flow characteristic was visually observed to be approximately laminar using titanium tetrachloride smoke puffers from E. Vernon Hill Inc. Two tree chambers were clamped to the flight chamber by 6x33cm screened openings. A 10x18x5cm insect/chemical chamber was clamped to the top of the tree chamber with a screened

Figure 5. Flight chamber schematic



opening, and clamped to the top was a 10x18x5cm carbon filter. Components were sealed using closed cell weather stripping foam and C-clamps.

Experiments were conducted in an 18-22° C laboratory with a 16:8h light cycle in the dark, with an 250W infrared heat lamp approximately five feet above the flight chamber as a light source, and observed using Bushnell™ night vision goggles. Trials were recorded on VHS using an array of 4 infrared cameras.

Pear psylla infested branches, apple branches, and other odors were used in one of the two tree chambers, or insect/chemical chambers in 50% of all trials respectively to remove side bias. Tree and flight chambers were cleaned and rinsed after every trial. All lights except infrared were turned off, and the squirrel cage fan was turned on for 20 min prior to inserting lacewings. After 20 min of acclimatization, one lacewing was placed in the flight chamber, and the response (positive or negative), and time to response (maximum of 3hr) was recorded. A response was recorded when the lacewing reached a line drawn 8cm from the end of the flight chamber. Reported are data for 30 lacewings that responded for pear branches and 20 for apple branches. Against clean air, 10 lacewings were offered to 50-100 pear psylla, 20 were offered 4µl of wintergreen oil, 10 were offered 4µl of methyl salicylate , and 5 were offered 4µl of squalene.

Statistical analysis. Data from flight chamber assays were analyzed using a g-test of goodness of fit (α=0.05). Often studies of this type use a chi square analysis, however, for data (binomial data) generated by flight chamber assays, the g-test of goodness of fit is more appropriate.

RESULTS

Lacewings were not significantly more attracted to tested odors (pear branches infested with pear psylla, cut apple branches, pear psylla, wintergreen oil, methyl salicylate, and squalene) than to filtered air in the flight chamber (Table 2). Data reported are only for lacewings that responded within three hours, but nearly ¼ flown in the flight chamber never responded.

Table 12. Lacewing flight chamber responses

Odor	Positive	Negative	*P*	No Response
PP Pear Branches	*18*	*12*	*0.271*	7
Apple Branches	*11*	*9*	*0.654*	4
Wintergreen Oil	*9*	*11*	*0.654*	5
Methyl salicylate	*3*	*7*	*0.119*	2
Squalene	*2*	*3*	*0.653*	4
Pear Psylla	*2*	*8*	*0.049**	4

DISCUSSION

Though lacewings were induced to fly in the flight chamber, responses to all tested odors were not significantly more than filtered air, suggesting that either there is no latent attraction to the odors tested, or the constructed flight chamber is an insufficient test system for lacewings. Further evidence that the flight chamber is an inadequate system for lacewings is that clean air was significantly more attractive than pear psylla to green lacewings. Also, despite the expected similarity of results from wintergreen oil and methyl salicylate, there were clear differences in lacewing responses to each. Results from flight chamber assays suggest that responses are more random chance than actual attraction. Herbivore-induced volatiles, based on field studies, are thought to be attractive to lacewings (James, 2003), but in both the Y-tube and flight chamber, herbivore-damaged plant materials and synthetic versions of volatiles were not attractive. Lacewings, a searching predator, may require a more complex system to allow for normal behavior, as well as a larger scale apparatus to allow more ordinary flight (as in Brown et al. (1951) for mosquitoes); nearly every lacewing tested flew into the wall or ceiling of the flight chamber, likely affecting their response from prey location to escape. It is also possible that lacewing chemoreceptors became saturated with chemicals and activity ceased, or that in such close proximity it appeared that they had already reached the host and searching was not necessary.

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