FLORAL LURES FOR ATTRACT AND KILL AND FOR SEASONAL MONITORING OF ALFALFA LOOPER, CORN EARWORM AND CABBAGE LOOPER MOTHS

by

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of LEONARDO DE AZEVEDO CAMELO find it satisfactory and recommend it to be accepted.

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Chair

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FLORAL LURES FOR ATTRACT AND KILL AND FOR SEASONAL MONITORING OF ALFALFA LOOPER, CORN EARWORM AND CABBAGE LOOPER MOTHS

Abstract

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Federal and State regulatory legislation, growing environmental issues, and worker safety related concerns have served to initiate research into the development of alternative approaches to conventional pesticides for managing pestiferous insects. Chemical lures derived from "moth-visited" flowers, which lure both sexes of alfalfa looper, corn earworm and cabbage looper moths, have been developed. These feeding attractants are dispensed from polypropylene vials that provide controlled release of the attractant. A killing station was tested in the field for use in combination with these lures as an attract and kill system. Alfalfa looper female moth activity was significantly reduced in field plots due to deployment of killing stations, evidenced by reduction of moths captured in monitoring traps. Two feeding attractant based killing stations designs were field tested against corn earworm moths. The shuttlecock based killing station significantly reduced the number of corn earworms captured in monitoring traps. In trials inside a screened building, oviposition on potted plants by female alfalfa looper moths was significantly reduced by the use of 2 killing stations. Both sexes of alfalfa looper and cabbage looper moths demonstrated high attraction rates to the killing station in wind tunnel assays, and exhibited high mortality rates when they contacted the killing station. Moths were less likely to be attracted to lures when fed sugar during the adult stage and fed alfalfa looper moths were less affected by killing stations in screen building trials, compared to unfed moths. Seasonal activity of corn earworm, cabbage looper and alfalfa looper moths were monitored in south-central Washington State during 2002, 2003 and 2004. Moths were monitored with the use of female sex pheromone and feeding attractant baited traps. A series of experiments were conducted in order to assess feeding attractants ideal release rates, different release systems, and lure composition for all three species. This attract and kill system has potential to be adopted for alfalfa looper and cabbage looper control in vegetables, and other high input field crops. Relevant advances in this technology include environmental benefits, attraction of female moths, and efficacy in reducing oviposition by female moths.

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Dedication

Without love and passion life would be shallow, regardless of any Science. Thank you Amber, I will love you forever.

Without my family's support this monumental, sometimes unkind task would be impossible. Thank you Mom, Dad, Marcelo, Elizangela, Mary and Joe you are the best family anyone can wish for.

Friends make it all worthwhile, my friends made everything that much more meaningful while providing a great deal of inspiration. Thank you Paulo, Igino, Mariana, Manoel, John, Tim, Adam and all members of the República Mata-Burro.

Thank you.

INTRODUCTION

Increasing environmental concerns, human safety hazards, and pesticide contamination in food, have led federal regulatory agencies to reduce the number of conventional pesticides available for pest management in the United States and Europe. A large number of formally reliable and widely used highly-toxic products are being replaced with safer, environmentally friendly tools. The use of insect attractants as a mechanism to aid pest management and monitor insect population activity has long been studied (Dethier, 1947). These attractants can be adapted for effective management of agricultural and urban pests, without causing the harms of toxic chemical cover applications. The use of sex pheromones for trapping adult male moths for example is commonly adopted in agricultural settings. Currently female sex pheromones are available for most Lepidopteran pests. However, those attractants have drawbacks including attraction of males only. Field sampling of the female moth population would provide information on the gender responsible for laying eggs that will ultimately cause damage to crops in the larval stage. Other than the use of blacklight traps, there are few reliable methods available for adult female moth trapping, commercial sampling, or for use as a pest management tool.

The use of attractants as a management strategy has a long history (Dethier 1947, Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, Charmillot et al. 2000). A method that relies on insect attractants to manage pests is attract and kill, where insects are attracted to a source where they contact a lethal dose of a killing agent. The killing agent of attract and kill may include sticky materials, insecticides or pathogenic microorganisms (Pedigo, 2002). Other management systems that use insect attractants include mass trapping, poisoned baits, and mating disruption, with much research effort on mating disruption and the use of poisoned baits (Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, and Charmillot et al. 2000). Poisoned baits have been defined as a formulation that combines an edible substance with a pesticide (Pedigo 2002). Mass trapping and attract and kill, for example, have been previously proved to be an effective method against the boll weevil (Lloyd et al. 1981, Villavaso et al. 1998, respectively), bait sprays effective against tropical fruit flies (Steiner et al., 1961 and Vargas et al., 2001), and attract and kill effective against the codling moth (Charmillot et al. 2000). However, success against other Lepidopteran pests has been somewhat limited. Generally, these methods have been successful in tree fruit, field, and forest crops as well as with stored products and household pests (Lloyd et al. 1981, Roelofs 1981).

The corn earworm *Helicoverpa zea* (Boddie), cabbage looper *Trichoplusia ni* (Hübner), and alfalfa looper *Autographa californica* (Speyer) are moths in the family Noctuidae that are agricultural pests throughout the south-central region of Washington State. This region has extensive areas devoted to vegetable, fruit, and hay production. The corn earworm is distributed from southern Canada southward into Argentina and Chile and has been introduced into Hawaii (Crumb, 1956, and Hardwick, 1965, 1996). Foliar insecticide applications are the most common method of managing corn earworm in field crops. The corn earworm can cause damage to the region's tomatoes, sweet and field corn, feeding on the fruits and corn ears, respectively. In the western part of the United States it overwinters as a pupa in the soil as far north as southern Washington State (Eichmann 1940, and Klostermeyer 1968). This insect is also migratory, undoubtedly contributing to regional pest pressures (Hardwick, 1965).

The cabbage looper is a pest of a variety of vegetable crops of several distinct taxonomic groups (Sutherland and Greene, 1984) including crucifers, Solanaceae, Curcubitaceae, Unberlliferae, Amaranthaceae, and others. Both cabbage looper and alfalfa looper can feed on cole crops and lettuce (McKinney, 1944) damaging local crops between May through late fall. Overwintering by the cabbage looper occurs in southern parts of North America in the pupae stage, attached to host materials (Metcalf and Flint 1962), and moths can migrate into the northern States and into southern Canada.

The alfalfa looper is a widely distributed and polyphagous foliage feeder in western North America. Even though, it is considered to be a less serious pest than both the cabbage looper (Vail et al., 1989), and the corn earworm. Damage caused by the larvae includes the destruction of the heads of cole crops, and defoliation of potatoes, peas, sugarbeets, alfalfa, beans, mint, spinach and other minor crops (Brewer, 1995). Once the variable economic threshold is reached foliar insecticide applications that target the early instars of these species are the most common management technique used to prevent damage (Godfrey et al., 2005; Baird and Homan, 1996).

Here, we discuss field implementation of an attract and kill system using novel floral-based feeding attractants, and provide experimental results that demonstrate a significant reduction in numbers of female alfalfa looper moths, and a reduction of eggs laid by moths when killing stations were deployed. Efficacy of the attractants and killing stations was assessed for both sexes of corn earworm, alfalfa looper, and cabbage looper moths in wind tunnel studies. Also, corn earworm, alfalfa looper, and cabbage looper moth attraction to the feeding attractants in a wind tunnel was compared for both sexes when adults were fed or not fed sugar. Population levels of adults of corn earworm, alfalfa looper, and cabbage looper moths in the south-central region of Washington State were monitored throughout the growing season during 2002, 2003, and 2004. Populations were monitored with sex pheromone and with feeding attractant baited traps. The primary objective of this part of the study was to provide a seasonal picture of the male and female activity of these three species in the agricultural area of south-central Washington State. Lastly, we conducted studies designed to improve field efficiency of previously field tested and novel floral derived feeding attractants, in order to capture higher numbers of female and male corn earworm and alfalfa looper moths in monitoring traps.

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Chapter 1

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A kairomone based attract-and-kill system effective against female alfalfa looper *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) reproduction.

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CHAPTER I

A kairomone based attract-and-kill system effective against female alfalfa looper *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) reproduction.

ABSTRACT

Federal and State regulatory legislation, growing environmental issues, and worker safety related concerns have served to initiate research into the development of alternative approaches to conventional pesticides for managing pestiferous insects. Chemical lures derived from "moth-visited" flowers, which lure both sexes of alfalfa loopers, have been developed. This feeding attractant is dispensed from polypropylene vials that provide controlled release of the attractant for extended periods of time. A killing station was tested in the field for use in combination with these lures as an "attract and kill" system. Female moths captured in monitoring traps were reduced by 92.7% in 2003 and by 80.0% during 2004. In screen building trials using the same killing-stations, oviposition on potted plants by female alfalfa loopers was significantly reduced by the use of 2 killing stations. Moths laid an average of 4.33 eggs compared to 287.67 in control. Alfalfa looper moths demonstrated a high attraction rate to the killing station in wind tunnel assays and exhibited a 90.9% mortality rate when female insects contacted the killing station. Moths were less likely to be attracted to lures when fed sugar during the adult stage and fed moths were less affected by killing stations in screen building trials, compared to unfed moths. This

attract and kill system has potential to be adopted for alfalfa looper control in vegetable and other field crops.

INTRODUCTION

The alfalfa looper *Autographa californica* (Speyer) is a widely distributed, destructive foliage feeder in North America (Berry, 1998). Damage caused by the larvae includes the destruction of the heads of cole crops to be used as processed foods, and defoliation of potatoes, peas, sugarbeets, alfalfa, beans, mint, spinach and other minor crops (Brewer, 1995). Alfalfa looper distribution includes the entire United States and parts of Canada (Metcalf and Flint, 1962). In some locations populations do not reach economic injury level because viral diseases, predators, and parasites lower its numbers (Berry, 1998). Natural enemies, however, may not be sufficient to reduce heavy infestations, particularly in agro-ecosystems. Economic injury thresholds for alfalfa looper vary among cropping systems. Once the economic threshold is reached, foliar insecticide applications that target the early instars are the most common management technique used to prevent damage (Godfrey et al., 2005; Baird and Homan, 1996). Disadvantages of regular insecticide applications as a method of controlling insect pests often include human health hazards, impacts on natural enemies, elevated costs, environmental contamination and residues on food crops.

The use of attractants as a management strategy has a long history (Dethier 1947, Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, Charmillot et al. 2000). A method that relies on insect attractants to manage pests is attract-and-kill, where insects are attracted to a source where they contact a lethal dose of a killing agent. The killing agent of attract-and-kill, also known as lure-and-kill, may include sticky materials, insecticides or pathogenic microorganisms (Pedigo 2002). Other management systems that use insect attractants include mass trapping, poisoned baits, and mating disruption, with much research effort on mating disruption and the use of poisoned baits (Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, and Charmillot et al. 2000). Poisoned baits have been defined as a formulation that combines an edible substance with a pesticide (Pedigo 2002). Mass trapping and lure and kill, for example, have been previously proved to be an effective method against the boll weevil (Lloyd et al. 1981, Villavaso et al. 1998, respectively), bait sprays effective against tropical fruit flies (Vargas et al., 2001), and lure and kill effective against the codling moth (Charmillot et al. 2000) although, success against other Lepidopteran pests has been somewhat limited. Generally, these methods have been successful in tree fruit, field, and forest crops as well as with stored products and household pests (Lloyd et al. 1981, Roelofs 1981).

Several problems have been observed in attract and kill systems for moths that utilize sex pheromone as an attractant, including a delayed efficacy in reducing larvae numbers in the field, caused by the male only attraction to female sex pheromones (Minks, 1997). Charmillot et al. (2000) demonstrated that in some cases applications should be made at least one week before the flight season, and that the application of sex pheromone based attract and kill systems should occur only in areas where the initial pest population is low and there is sufficient isolation from external sources of infestations. The attraction and management of adult female moths would directly impact the reproductive potential of a pest species, reducing the number off eggs laid and consequently reducing the number of larvae infesting the field.

As larvae are the primary source of damage (Shorey, 1976) attract and kill of females would be a significant advancement in the management of pest Lepidoptera. Grant (1971), Cantelo and Jacobson (1979), Haynes et al. (1991), Heath et al. (1992), Pair and Horvat (1997), Lopez et al. (2000), and Landolt and Smithhisler (2003) have described floral odors that are attractants to both sexes of some Lepidoptera pest species. Phenylacetaldehyde is one of the volatile compounds discussed in these studies and is attractive to several noctuid moths including economical insects such as the alfalfa looper (*Autographa californica* (Speyer)), soybean looper (*Pseudoplusia includens* (Walker)), celery looper (*Anagrapha falcifera* (Kirby)), and the cabbage looper (*Trichoplusia ni* (Hübner)), among others. Landolt and Smithhisler (2003) found that the odorants of flowers of Oregongrape shrubs, which are visited by insect pollinators, are attractive to alfalfa looper moths. Landolt et al. (2006) demonstrated that the combination of phenylacetaldehyde plus beta-myrcene, both floral volatiles emitted by Oregongrape, was particularly strong in attracting both sexes of adult alfalfa loopers. Landolt et al. (2001) using a double component floral lure captured a ratio of one female to two male alfalfa loopers. Strong female attraction would be a great asset for the development of an effective attract and kill technique. The annihilation of female moths should directly reduce the number of eggs laid and consequently, decrease the numbers of larvae in the subsequent generation. Haynes et al. (1991), Landolt et al. (1991), Hitchcox (2000), and Landolt and Higbee (2002) found no indication that the responsiveness of female cabbage looper moths to floral derived feeding attractants, and other chemical lures was limited to early or late in life. For other noctuid species unmated females and mated females with eggs readily respond to the feeding attractant lures in laboratory and field studies (Hitchcox, 2000; Landolt and Higbee, 2002). Smith et al. (1943) observed that female *Autographa* spp. captured with floral attractants were both unmated and/or mated with eggs in all stages of development.

We hypothesized that female alfalfa looper numbers can be reduced by the deployment of killing stations that combine a floral chemical attractant (phenylacetaldehyde and beta-myrcene) and a killing agent (Permethrin®). This should reduce the number of larvae in the subsequent generation which will reduce damage to crops. We also discovered and demonstrated that the attraction of female moths to the feeding attractant is stronger when moths have not recently fed.

Here we discuss field implementation of killing stations using floral based feeding attractants as well as experimental results that demonstrate a reduction in numbers of female alfalfa looper moths. The reduction of eggs laid by moths when killing stations were deployed was assessed on a smaller scale using screened buildings. Efficacy of the attractant and killing stations was assessed for both sexes of alfalfa loopers in wind tunnel studies. Also, alfalfa looper attraction to the feeding attractants in a wind tunnel was compared for both sexes when adults were allowed to feed on sugar versus starved moths.

MATERIAL AND METHODS

Killing stations: Killing stations were adapted from Everett Mitchell (USDA-ARS Gainesville, FL, deceased) and Landolt (2002), they consisted of: (1) a cone shaped target for the moth to contact, (2) a chemical lure at the base of the cone, and (3) a killing agent applied to the cone target, all integrated into a single unit (Figure 1). The cone is a white plastic badminton birdie (shuttlecock) measuring 7.6 cm tall with a 6.5 cm wide opening. The soft red rubber cork at the 2.5 cm base was replaced with an 8 ml polypropylene vial (Nalgene 2006-9025, Fisher Scientific, Pittsburg, PA) as the dispenser for the attractant. Each vial was loaded with 5 ml of the feeding attractant lure, a combination of phenylacetaldehyde (2.19 mg) and beta-myrcene (2.81 mg) (Sigma-Aldrich Chemicals, Milwaukee, WI). The components were added to cotton balls placed inside the vials, with a 10 ml plastic graded syringe. A single hole with a 3.0 mm diameter was drilled into each vial lid to provide the desired release rate of the volatilized chemicals, 43.0 μg per hour (Landolt et al. 2001). The Nalgene vial was then attached to the base of the shuttlecock in place of the red shuttlecock cork, with the lid of the vial facing into the cone. The internal and external surfaces of the cone were coated with Teflon® Hitch Ball Lube grease (Reese Products Inc., Elkart, IN) mixed with 7% by weight technical grade Permethrin® (FMC Corp., Princeton, NJ). Killing stations were placed in the field using 8 gauge wires that suspended the killing stations approximately 12cm above the alfalfa crop canopy.

Insect rearing: Alfalfa looper colonies were started with eggs obtained from females trapped in black light traps during mid-March. Eggs were surface sterilized with a 1.5% bleach/distilled water solution and set-up for hatching under room conditions (22° C. ±, 60% H.R.). After eclosion larvae were placed into individual 30.0 ml plastic cups (Solo cup company, Highland, IL) containing a small piece of artificial diet (Southland Products, Lake Village, AR). Larvae were reared throughout the pupa stage. When a pupa was completely developed, it was removed from the cup, separated by gender and placed into a screen rearing cage (20.0 cm x 20.0 cm x 20.0 cm). Water and a sugar-water mixture were made available to emerging moths. After adult emergence, three male moths were placed in a plastic cup with 1 female (water and sugar-water provided) so that mating could occur. After female moths laid eggs, they were surface sterilized and the rearing process was repeated so that sufficient numbers of adults were obtained for experiments. Moths were reared on a 14:10 scotophase with lights on from 7:00 pm until 9:00 am.

Large scale field trials: Large scale experiments were conducted in commercially grown alfalfa-hay fields in Yakima and Benton Counties of Washington State's south-central valley during the 2003 and 2004 growing seasons. Field plots of 2.02 hectares were monitored for alfalfa loopers with a single feeding attractant trap beginning in mid-May 2003 and again in 2004. Trials started when several alfalfa looper moths were captured per trap per week signaling the start of the alfalfa looper flight in the Yakima Valley.

Plot monitoring and data collection: Alfalfa looper adult monitoring during trials was accomplished with two sex pheromone baited traps and two feeding attractant baited traps located in each experimental plot. Males were monitored with Universal moth traps (Unitraps™ Agrisense, Fresno, CA) with green lids, yellow cones and white buckets (Figure 2). For killing of trapped specimens, a 6.5 cm^2 piece of Vaportape® (Hercon Environmental, Emigsville, PA) was placed inside of each bucket part of the trap. The Vaportape® was stapled into the internal wall of the bucket. Tissue paper was added to the bucket to absorb water entering the trap. For monitoring of male alfalfa looper moths, Unitraps™ traps were baited with alfalfa looper female sex pheromone made in our laboratory; 1.0 mg of Z7-12:Ac plus 10% (0.1 mg) of Z7-12:OH, and applied to a red rubber septum (The West Company, Lititz, PA). For the monitoring of female moths, Universal moth traps were loaded with the same feeding attractant used in the killing stations, a combination of phenylacetaldehyde (2.19 grams) and beta-myrcene (2.81 grams) (Sigma-Aldrich Chemicals, Milwaukee, WI). Vials were attached to the internal wall of the bucket trap with a thin wire that held the lure in the upright position. To facilitate counting and preservation of specimens captured, traps were emptied daily during the 2003 trials. During the 2004 season, traps were emptied every other day. Trap contents were placed into Ziplock® plastic bags (S.C. Johnson and sons, Racine, WI) and transported to the laboratory where they were stored in a freezer. Insects were later sorted, identified, and separated.

Monitoring traps positioning in each plot: Each 2.02 hectare plot was divided into 4 different quadrants with a monitoring trap placed in the center of each quadrant (Figure 3). Each plot had 2 sex pheromone traps and 2 feeding attractant monitoring traps placed in alternating positions. Pheromone traps were placed in the northwest and southeast quadrants with feeding attractant traps in the northeast and southwest quadrants. Each trap was 73.0 meters away from the adjacent trap.

2003 field experiments: Tests were conducted for 14 days. During the first seven days, all plots were monitored with the two pheromone and two feeding attractant traps without the deployment of killing stations. On the eighth day, killing stations were deployed in plots designated to receive the killing station treatment. Control plots were left without killing stations. Plots were monitored daily throughout the 14 days. Treatments than consisted of: (T1) 0 killing stations per hectare and (T2) 125 killing stations per hectare. Killing stations were positioned at a 1.0 meter height which was 12.0cm (± 3.0cm) above the canopy of the crop. Killing stations were distributed in a grid format with bait stations positioned uniformly throughout the 2.02 hectares (Figure 3). Four replicates were conducted from late May through mid August of the 2003 growing season. Experiment was set up as a Complete Randomized Block Design with repeated measures. Data analysis was done using a pre-test/post-test with repeated measures analysis (Brogan and Kutner 1980), to compare numbers of female and male alfalfa looper moths captured before and after deployment of killing stations. This was done in order to determine if numbers of moths captured in traps were significantly reduced after killing station deployment. Numbers of female and male alfalfa looper moths captured before and after killing stations were deployed were also compared between treatments using an Analysis of Variance to determine if there were significant reductions in numbers of moths captured in plots treated with 125 killing stations per hectare compared to 0 killing stations per hectare (control) during the same period.

2004 field experiments: Experiments conducted during the 2004 growing season were designed to assess efficacy of killing stations for reducing numbers of moths and larvae in plots, as well as to evaluate the long term durability of killing stations. Plots were monitored with both feeding attractants and pheromone traps for 20 days with killing stations deployed on the beginning of the experiment. Following the methodology in 2003, 2.02 hectare plots were monitored with a feeding attractant trap, beginning in mid May 2004 to determine suitable timing to start the experiments. Experiments were replicated four times between mid May to mid August 2004. Treatments consisted of (T1) 0 killing stations per hectare and (T2) 125 killing stations per hectare. Trials lasted 20 day and monitoring traps were emptied every other day. Larva numbers were monitored with sweep net samples executed at 4, 8, 12, 16 and 20 days after the start of each replication. Samples were conducted by dividing each plot in quadrants and executing 200 sweeps in random locations within each quadrant. A standard 54.0 cm diameter sweep net (Bioquip Products, Rancho Dominguez, CA, 90220) was used for sampling. Experiment was set up as a complete randomized block design with repeated measures. Data analysis for the 2004 experiments was done by using an Analysis of Variance (ANOVA) with repeated measures comparing numbers of female and male alfalfa looper moths captured in treated plots (125 killing stations per hectare) against untreated plots (0 killing stations per hectare). This was done to determine if there were significant reductions in numbers of moths captured in plots treated with 125 killing stations per hectare compared to 0 killing stations per hectare. Statistical comparison between treatments for numbers of larvae collected in field plots was not possible due to low larvae density in these plots.

Oviposition reduction: Due to low alfalfa looper larva numbers in the field experiments during the 2004 growing seasons, a different experimental design was used to address the hypothesis that numbers of alfalfa looper larvae are reduced subsequent to deployment of killing stations. Alfalfa looper moths that had been reared in the laboratory as previously described were released in a screen building measuring 7.4 m wide x 29.3 m long x 4.9 m tall. The number of moths released per replicate was 100; 75 males and 25 females. Moths were placed in screened cages (30.5 cm x 30.5cm x 30.5cm) for 24 hours prior to transport and release into the screened building. All moths used in this experiment were 1 to 3 days old when released into the screen building. Lettuce (Black Seeded Simpson, Ferry Morse Seed Co., Fulton, KY, 42041) grown in pots was used as an oviposition site and for larval feeding. Lettuce was chosen because of its suitability as a host for alfalfa looper larvae (Landolt, unpublished data), and its rapid growth. Lettuce plants were approximately

20.0 cm in height. One hundred lettuce plants in individual pots were used in each experimental replicate. Pots were arranged in the screen building in 2 rows containing 50 plants each. Each row consisted of ten black plastic trays measuring 54.0 cm x 36.0 cm and 7.0 cm tall, each holding 5 potted plants. Four plants were positioned in each corner of the tray and one in the center. Water was added to a depth of 5.0 ± 1.0 cm to each tray daily. Treatments were: (T1) 0 killing stations in the screen building with moths not provided sugar prior to release, (T2) 2 killing station in the screen building (equivalent of 125 killing stations per hectare) with moths provided sugar prior to release, and (T3) 2 killing stations in the screen building (equivalent of 125 killing stations per hectare) with moths not provided prior to release. Each treatment was replicated 3 times during the 2005 growing season. At 7 days following the release of moths, the lettuce plants were removed and eggs and larvae on plants were counted. Numbers of eggs and larvae were combined and the data analysis was performed using Analysis of Variance (ANOVA), followed by a L.S.D. test to compare numbers of eggs and neonate larvae present in each treatment. This was done in order to determine if there was a significant reduction in the numbers of eggs and neonate larvae present when killing stations were deployed in the screened building compared to no killing stations, and to determine if feeding with sugar prior to release impacted the efficacy of the killing stations.

Wind tunnel assays of lure and kill stations: Flight tunnel experiments were conducted in order to compare attraction responses and mortality rates of unmated female and male alfalfa loopers in the presence of killing stations and to assess differences in attraction when moths were provided with sugar prior to testing, compared to the moths not provide sugar. The wind tunnel was made of Plexiglas and measured 0.8 m x 0.8 m x 1.8 m. Air flow was adjusted to 0.22 m/s. Air was pulled trough the tunnel with a squirrel cage fan and a blower motor. The wind tunnel floor was motionless. Room temperature and humidity were artificially controlled at 25.0 ± 2.0 °C and 55.0 ± 3.0% R.H. Tunnel air was vented out and a filter was secured to the effluent vent of the tunnel to remove exhaust odorant. Moths used were reared in the laboratory following the methods described previously. All moths used were 2 or 3 days old, and unmated. Males and females were held in separated cages. Moths were placed in the flight tunnel room one hour before the experiments were begun so that insects could habituate to conditions before the experiments started. Individual moths were placed in 20.0 ml polystyrene vial with an open end and a screened end. One at a time, vials were hung horizontally on a metal stand near the downwind end of the wind tunnel, positioned centrally with the open end of the vial upwind. Each moth was observed for 3 minutes after release and was captured and held for 24 hours in a capped 24 ml polystyrene vial. The following factors were observed and scored: upwind flight, plume tracking, contact with the source and mortality at 24 hours after contacting the killing stations. The wind tunnel room was dark, and moths were held on a 14:10 light:dark cycle with lights on from 7:00 pm until 9:00 am. Wind tunnel assays begun daily at 10:00 am and were conducted until 12:00 pm.

Alfalfa looper moth responses were evaluated for the following treatments: (T1) unmated female alfalfa looper moths held without sugar prior to testing in response to a blank control, (T2) unmated female alfalfa looper moths held without sugar in response to a killing station with a phenylacetaldehyde/beta-myrcene lure, and (T3) unmated female alfalfa looper moths provided sugar prior to testing, in response to a killing station with a phenylacetaldehyde/beta-myrcene lure. These tests were repeated for males. Six replications with five moths per replicate were conducted. The tunnel was aired between sets with the blower on for at least 12 hours, to avoid tunnel contamination with the floral attractants. After data was arcsine transformed, treatment comparisons and data analyses were performed with an Analysis of Variance followed by a L.S.D. test to determine differences between treatments, for both genders. Mortality comparisons between treatments 1, 2 and 3 were performed with an ANOVA test followed by a L.S.D test for mean separation.

RESULTS

2003 Field experiments with killing stations: The number of females captured per day in feeding attractant baited monitoring traps during the 7 day killing station post deployment period was significantly lower in treated plots (125 killing stations/hec.) compared to the number of females captured in non-treated plots (0 killing stations/hec.) (Table 1, n=4, f=6.20, df=7, p=0.05). A significant reduction was also observed when comparing the number of females captured in treated plots before deployment of killing stations (pre-deployment period) to treated plots after killing stations deployment (post deployment period) (Table 1, n=4, df=7, f=8.93, p=0.02). Numbers of females captured in feeding attractant traps in non-treated versus treated plots before deployment of killing stations (pre-deployment period) was not significantly different (Table 1, n=4, df=7, f=3.29, p=0.12). Finally, no significant reduction was observed when comparing the number of female alfalfa looper moths trapped in feeding attractant baited monitoring traps in non-treated plots during pre-deployment period versus post-deployment period (Table 1, n=4, df=7, f=0.29, p=0.61).

The number of males in feeding attractant traps in treated and non-treated plots was not different during the post-deployment period (Table 1, n=4, df=7, f=0.58, p=0.48). The number of males captured in feeding attractant baited monitoring traps in treated plots was significantly lower during the post-deployment period compared to the pre-deployment period (Table 1, n=4, df=7, f=5.96, p=0.05).

No significant difference was observed between the number of males captured in feeding attractant baited traps in non-treated plots versus treated plots before killing stations deployment (pre-deployment) (Table 1, n=4, df=7, f=0.21, p=0.66). Finally, the number of male alfalfa looper moths captured in feeding attractant traps in non-treated plots was not significantly different during the pre-deployment period compared to the post-deployment period (Table 1, n=4, df=7, f=2.29, p=0.18).

There was no significant difference between the number of male alfalfa loopers captured in pheromone traps in non-treated plots versus treated plots for both periods of the experiment, pre and post-deployment (Table 1, n=4, df=7, f=0.01, p=0.92 for pre-deployment and f=0.01, p=0.97 for post-deployment). There was no significant difference in the number of male alfalfa looper moths captured in sex pheromone traps in both non-treated and treated plots when comparing pre-deployment to post-deployment periods (Table 1, n=4, df=7, f=0.45, p=0.53 for non-treated plots and f=84, p=0.39 for treated plots).

The graphical representation of female moths captured per day (Figure 4) demonstrates that after the first day the number of female alfalfa loopers captured daily in feeding attractant traps before deployment of killing stations (pre-deployment period), was numerically higher in treated plots versus non-treated plots. After the 8th day of the experiment (post-deployment period) the number of females trapped was always numerically lower in treated plots compared to non-treated plots.

2004 field experiments with killing stations: The number of female moths captured in feeding attractant baited traps in treated plots (125 killing station/hectare) was significantly lower than the number of females captured in non-treated plots (0 killing station/hectare) (Table 2, n=4, df=39, f=41.58, p<0.01). Although a numerical reduction was observed, no significant statistical difference was observed in the number of males captured in feeding attractant baited traps in treated plots compared to non-treated plots (Table 2, n=4, df=39, f=3.68, p=0.06).

A numerical reduction was also observed in the number of moths captured in sex pheromone traps in treated versus control plots. However, there was no significant statistical difference between males captured in treated plots versus non-treated plots (Table 2, n=4, df=39, f=1.90, p=0.17).

The numbers of female moths captured in traps baited with the feeding attractant was numerically higher in every trap collection in non-treated plots (0 killing stations/hectare) compared to treated plots (125 killing stations/hectare) (Figure 5).

2005 screened building test: A significant difference among treatments was observed in oviposition reduction trials (Table 3, n=3, df=8, f=6.05, p=0.04). The number of eggs and neonate alfalfa loopers found on the 100 lettuce plants was significantly lower (4.33 $\pm$ 3.38) in treatment 3 (killing stations present, sugar not provided to moths) when compared to both treatment 2 (killing stations present, sugar provided to moths) and treatment 1 (no killing stations present, sugar not provided to moths). There was no significant difference in eggs and larvae on plants for treatment 1 versus treatment 2. The mean (± S.E.M.) numbers of eggs plus larvae for these treatments were 287.67 ± 93.97, and 238.33 ± 50.1 respectively (Table 3). A higher number of larvae than eggs was observed in treatment 2 while in treatment 1 more eggs than larvae were encountered (Figure 6).

Wind tunnel assays of killing stations: A greater number of starved female moths was attracted to, and contacted, the killing-stations compared to the control (killing station not baited with feeding attractants) (Table 4, $n=6$, $f=183.82$, $p<0.01$ for attraction and $f=72.46$, $p<0.01$ for contact). Significant contact and attraction responses were also seen for fed female moths (Table 4, $n=6$, $f=41.74$, $p<0.01$ for attraction and $f=121.0$, $p=0.01$ for contact). When comparing starved and fed moths, a significantly greater number of alfalfa looper female moths that were not provided with sugar were attracted to, and contacted the killing-stations compared to female moths that were provided with sugar (starved) (Table 4, $n=6$, $f=57.80$ $p<0.01$ for attraction and $f=47.24$, $p<0.01$ for contact).

Similar results were obtained for male alfalfa looper moths response to killing stations in the wind tunnel. A greater number of starved males were attracted to, and contacted, the killing stations compared to the control (Table 5, $n=6$, $f=135.0$, $p<0.01$ for attraction and contact). Male alfalfa looper moths that were provided with sugar had significantly greater attraction and contact rates in response to the killing stations (Table 5, $n=6$, $f=45.0$, $p<0.01$ for attraction and $f=7.50$, $p<0.01$ for contact). The number of starved male alfalfa looper moths attracted to, and contacting the killing stations was significantly greater than the number of fed males attracted to and contacting killing stations (Table 5, n=6, f=19.28, $p<0.01$ for attraction and f=15.63 p=0.01 for contact).

A mortality rate of 90.9% for all starved female moths that contacted killing stations was observed, and 73.3% of tested starved female moths contacted killing stations. For starved male moths a mortality rate of 87.5% that contacted killing stations was observed, and 53.3% of tested starved male moths contacted killing stations. The mortality rate of female moths tested to killing stations in the wind tunnel was significantly greater with starved rather than fed moths (Figure 7, n=6, f=2.47, $p<0.01$). The mortality rate of female moths contacting killing stations was not significantly different when those moths were starved or fed sugar (Figure 7, n=6, f=2.08, p=0.22). Similar results were obtained for male moths tested to killing stations in the wind tunnel. The mortality rate of all males tested was significantly greater for starved versus fed females (Figure 8, n=6, f=3.71, $p<0.01$), and the mortality rate of contacting males was significantly greater for starved versus fed males (Figure 8, n=6, f=1.74, p=0.04). Mortality rate of male moths that contacted the killing stations was not significantly different when comparing male moths that were not fed sugar during rearing against male moths that had been provided sugar.

DISCUSSION

Monitoring traps in field plots where killing-stations were deployed provided evidence of a reduction in the numbers of alfalfa looper moths present. This was supported by reductions of number of moths captured in feeding attractant baited traps when killing stations were deployed during both the 2003 and 2004 field seasons. Reductions of both female and male moths were observed in these feeding attractant traps used to monitor the experimental plots. These reductions in the numbers of alfalfa looper moths captured in monitoring traps could be interpreted as evidence of reductions in the populations of moths in field plots, as a consequence of mortality of moths at killing stations. However, these reductions in the number of moths captured in the feeding attractant baited traps could also be interpreted as a result of the moths being disoriented because of the number of feeding attractant point sources in the field, and being unable to locate the monitoring traps rather than the result of mortality caused by contacting the killing stations. Even thought it provides only a relative, and not absolute, sampling of insect population in plots, monitoring traps were the most suitable method of demonstrating moths activity in the field due to (1) alfalfa looper moths flight ability and the fact that (2) mortality of moths contacting killing stations does not occur instantly. Other monitoring methods, such as sweep netting or visual counts would be a less appropriate method for this particular study. A comparable approach was used by Charmillot et al. (2000), who used pheromone traps to monitor codling moths in plots treated with an attract-and-kill formulation using sex pheromone. Use of a blacklight trap would help confirm this hypothesis but due to the overhead irrigation systems employed in our experimental fields it was not possible to use it.

The numbers of males trapped were not reduced by the presence of killing stations. The numbers of males captured in sex pheromone traps or in feeding attractant traps were similar in treated and control plots. This may be due to a greater mobility of males compared to females and also, as demonstrated in wind tunnel experiments, a weaker response by males to feeding attractant lures compared to females. Our results then do not demonstrate reductions of males in plots due to the deployment of killing stations. Even though a reduction of males in treated plots would be encouraging, the primary goal of these studies was to reduce the numbers of female moths, thereby lowering the number of eggs laid and the number of larvae produced in the subsequent generation.

Experiments conducted during the 2003 growing season demonstrated the potential for killing stations to rapidly decrease female moth numbers soon after their deployment. The number of female alfalfa loopers in 0 baits/hectare plots remained high and showed no significant difference during pre and post-deployment, sustaining the hypothesis that the reduction of female alfalfa looper moths captured was due to killing station deployment. The knock-down effect on moths after killing stations were deployed in treated plots could be seen within a day or two. Other techniques relying on chemical attractants have previously been noted for their relatively slow or delayed effects on the insect population (Minks, 1997). Our killing stations also performed well over the longer term evaluation during the 2004 season. Efficacy of the killing stations was apparent throughout the entire 20 days duration of the experiment.

Experiments conducted during 2005 that examined the possibility of reducing oviposition by female alfalfa loopers in a screened building showed that killing stations can be effective in reducing reproduction through mortality of female moths, thus lowering the number of eggs and larvae in the subsequent generation. However, reductions of eggs and larvae occurred only when released alfalfa looper moths were not fed sugar. These results, although encouraging, demonstrate a potential drawback of the killing-stations. In the field previously fed moths may not respond and alternative food sources may compete with feeding attractant lures reducing their effectiveness. Loss of efficacy of our killing station might occur either when food is abundant and moths are not seeking food sources, or when flowers are abundant and competing odor sources reduce moth responses to artificial lures. Despite this concern, field experiments indicated a significant reduction of wild female alfalfa looper moths in plots despite potential natural competing food sources.

Wind tunnel experiments also indicated that unfed alfalfa looper moths are significantly more attracted to the feeding attractant than fed moths. This was applicable to both male and female moths. These findings support the interpretation that the failure to reduce reproduction by fed moths released into the screened building with killing stations deployed was probably due to lack of attraction of the fed moths to killing stations, rather than a lack of oviposition by these moths.

Low alfalfa looper numbers in commercial alfalfa fields during 2003 and 2004 season, restricted our ability to assess efficacy of killing stations in reducing larvae activity the damage causing life-stage. Even though results are encouraging, further field assessments will be necessary to provide a better picture of how killing stations can effectively reduce damage in targeted crops. Alfalfa was the most suitable crop to conduct killing stations experiments, due to adult population encountered during the growing season. However, due to alfalfa poor host-suitability to alfalfa looper larvae (Landolt, unpublished data) and low crop value, higher value vegetable crops such as lettuce, broccoli or cabbage might be better candidates for further development of the attract and kill system.

Recently developed attract-and-kill or lure-and-kill technology has shown efficacy in the control of a number of important lepidopteran pests including the pink bollworm, *Pectinophora gossypiela* (Saunders) (Hofer et al, 1996), the light brown apple moth, *Epiphyas postvittana* (Walker) (Suckling and Brockerhoff, 1999), the codling moth, *Cydia pomonella* (L.) (Charmillot et al., 1997, and Charmillot et al, 2000), and lastly the apple maggot *Rhagoletis pomonella* (Bostanian and Racette, 2001). All of these studies used sex pheromones as a lure, causing mortality to the males and thereby reducing the male to female sex ratio with the goal of impeding female mating success. Feeding attractant based attract and kill or lure and kill systems generally affect both sexes, with the female being the primary target. Examples of lure and kill systems include those for apple maggot *Rhagoletis pomonella* (Bostanian and Racette, 2001), and *Diabrotica* beetles (Metcalf et al. 1987). The primary advantage emphasized in these studies is the attraction of both sexes when using feeding attractants. An attract-and-kill system tested in the laboratory for use against the cabbage looper, *Trichoplusia ni* (Hübner) (Landolt et al. 1991) showed attraction and mortality rates similar to that of our experiments. Field experiments using feeding attractants-baited killing stations against Lacanobia fruitworm, *Lacanobia subjuncta* (Grote and Robinson) significantly reduced the numbers of female moths captured in monitoring traps (Landolt, 2002).

A feeding attractant based killing station technique can also have drawbacks when compared to a sex pheromone based system. Sex pheromones are fairly species specific lures. Feeding attractants on the other hand may attract an array of insects including pests and beneficials. Pollinators, including, a wide variety of Lepidoptera and Hymenoptera, were commonly captured in our feeding attractant traps. Bumble bees, *Bombus* spp*.,* honey bees *Apis melifera L.,* and sweat bees (Halictidae)*,* among others, were commonly found in the monitoring traps during field experiments. A large number of bumble bees were captured in traps baited with a similar floral lure in Alaska (Landolt et al. 2006). A severe drawback to the use of feeding attractant based killing stations is the competition with other odorants that might be present in the environment. Flowering plants may disrupt orientation of targeted pests to the killing stations or compete with it. This may be what occurred during 2004 experiments when a numerical reduction in moths captured in monitoring traps was observed after alfalfa plants begun flowering. These reductions in the numbers of moths trapped were seen in both treated and control plots during the 2004 field season between the 14th and 16th day of the test.

There have been a number of plant species discussed in the literature that emit volatile chemicals from flowers that are attractive to insects (Knudsen and Tollsten, 1993). Since Cantelo and Jacobson (1979) determined that phenylacetaldehyde was attractive to numerous species of moths, several other attractive compounds have been identified from several plants. Wiesenborn and Baker (1990) studied odorant in cotton flowers and found possible feeding attractants for the pink bollworm, *Pectinophora gossypiela,* a major insect pest in cotton. The corn earworm, *Helicoverpa zea* (Boddie) a pest of a wide array of crops is attracted to odorants present in flowers of Japanese honey-suckle, *Lonicera japonica* (Pair and Horvat 1997), and *Gaura suffulta* (Englem.) (Beerwinkle et al., 1996). The tobacco budworm, *Heliothis virescens* (F.) was attracted to volatile chemicals in cotton flowers (Tingle and Mitchell 1992). Haynes et al. (1991) and Heath et al. (1992) discovered feeding attractants of the cabbage looper. All of these lures can be improved and further developed so that an application can be derived from them. Characterization of volatile chemicals present in Oregon grape, *Berberis aquifolium* (Landolt and Smithhisler, 2003) yielded a lure that attracts the alfalfa looper, *Autographa californica* (Speyer). This study led the way to the development of a new lure that can be used in the field to capture female moths (Landolt et al., 2006) and now an attract-and-kill system.

In summary, our primary goal of reducing female alfalfa looper moth numbers was achieved during both the 2003 and 2004 field seasons. Our feeding attractant killing station density of 125 per hectare is low compared to other studies using similar attract-and-kill technologies. Charmillot et al. (2000) used 1000 per hectare to control codling moth, and Losel et al. (2000) used 7500 per hectare, also to reduce codling moth populations. Even though our results are encouraging, additional studies are necessary in order to assess effects on larvae in large scale settings. Commercially grown alfalfa fields however, might not be the most suitable site for such studies because of the fast cutting cycle during the summer when moths are flying. We believe that as this technology matures it will lead to an innovative management technique applicable to several lepidopteran pests. This will result as feeding attractants, or other kairomone and pheromonal attractants, are discovered and applied for other moth species. The development and commercial adoption of the attract-and-kill system can provide growers with an alternative to reduce female alfalfa looper moth numbers, thereby reducing reproduction and reducing the amount of pesticide used throughout the growing season.

Looking ahead, experimental design used to assess reduction of oviposition due the deployment of killing station might provide opportunities to investigate ability of moths to learn a specific host plant odor, and the development of a host choice preference by moths. Moths learned host preference can be tested by releasing them into a cage (screen building) in which the moths have more than one host option to lay eggs. A group of female and male moths released will be previously exposed to a host, than oviposition preference will be compared to those of a group of male and female moths released into the building not previously exposed to a host, or exposed to the second host option.

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Figure 1: Killing stations, a shuttlecock (badminton birdie) combined with 8.0 ml Nalgene™ vial.

Figure 2: Universal Moth Trap™ used in field experiments with green lid, yellow cone, and white bucket.

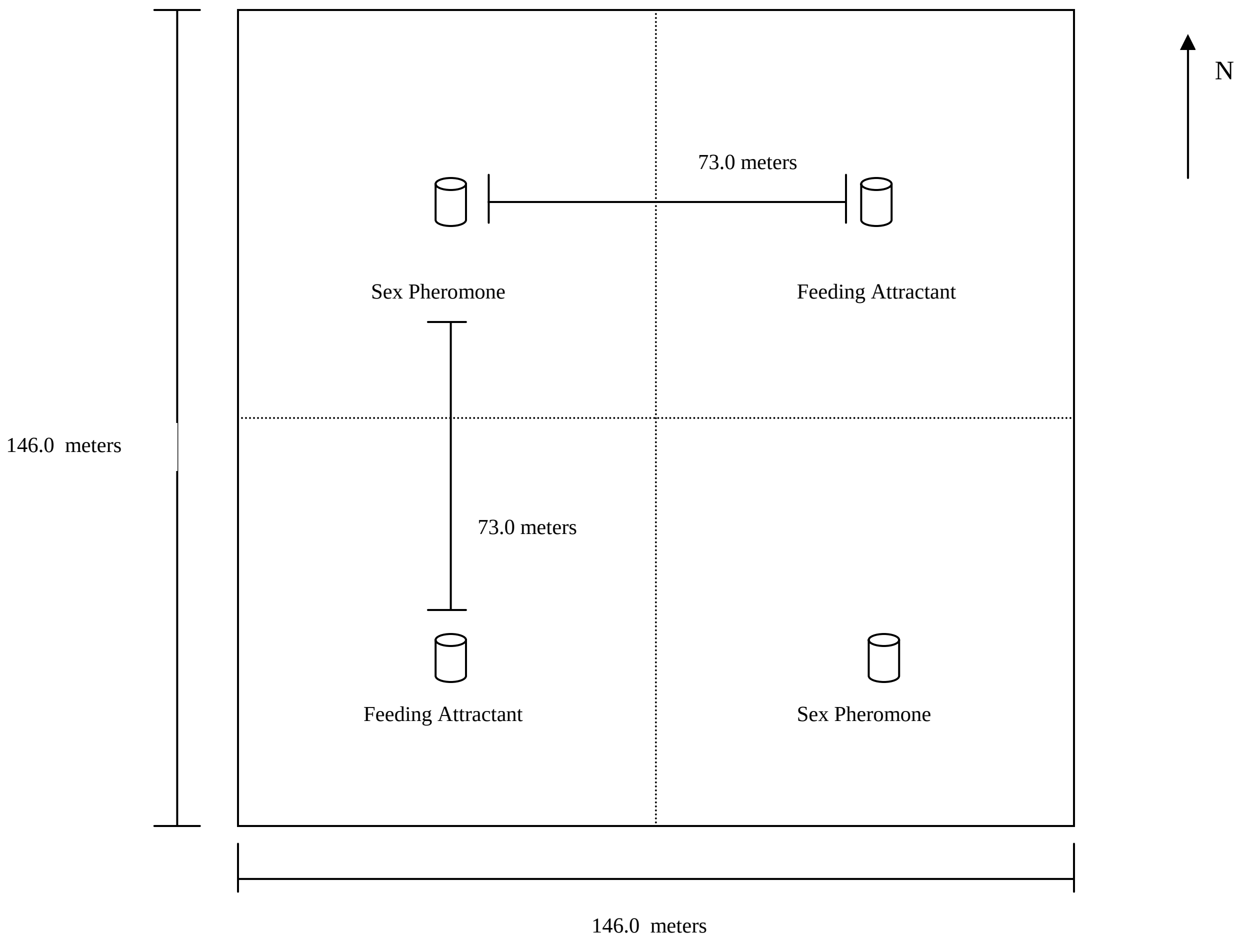

Figure 3 Monitoring traps placement inside plots during killing stations field experiments (2.02 hectare total area).

Table 1 - Mean (± S.E.M.) number of alfalfa looper moths captured per day in alfalfa fields during the 2003 growing season. Female traps were baited with feeding attractant and male traps baited with sex pheromone. Killing stations remained in plots for 7 days.

Feeding attractant	**0 killing stations/hec**	**125 killing stations/hec**
Female pre-deployment	1.86 ± 0.12ar	1.61 ± 0.44ar
Female post-deployment	1.18 ± 0.36ar	0.25 ± 0.11bs
Male pre-deployment	2.32 ± 0.71ar	2.86 ± 0.91ar
Male post-deployment	0.96 ± 0.54ar	0.48 ± 0.35as
Sex pheromone	**0 killing stations/hec**	**125 killing stations/hec**
Male pre-deployment	8.89 ± 3.21ar	9.32 ± 2.44ar
Male post-deployment	14.75 ± 8.08ar	14.39 ± 4.95ar

Means within a row followed by the same letter (a, b) and means within a column followed by the same letter (r, s) are not significantly different (n=4, alpha= 0.05, Pre-test/Post-test with repeated measures, and repeated measures ANOVA within same row).

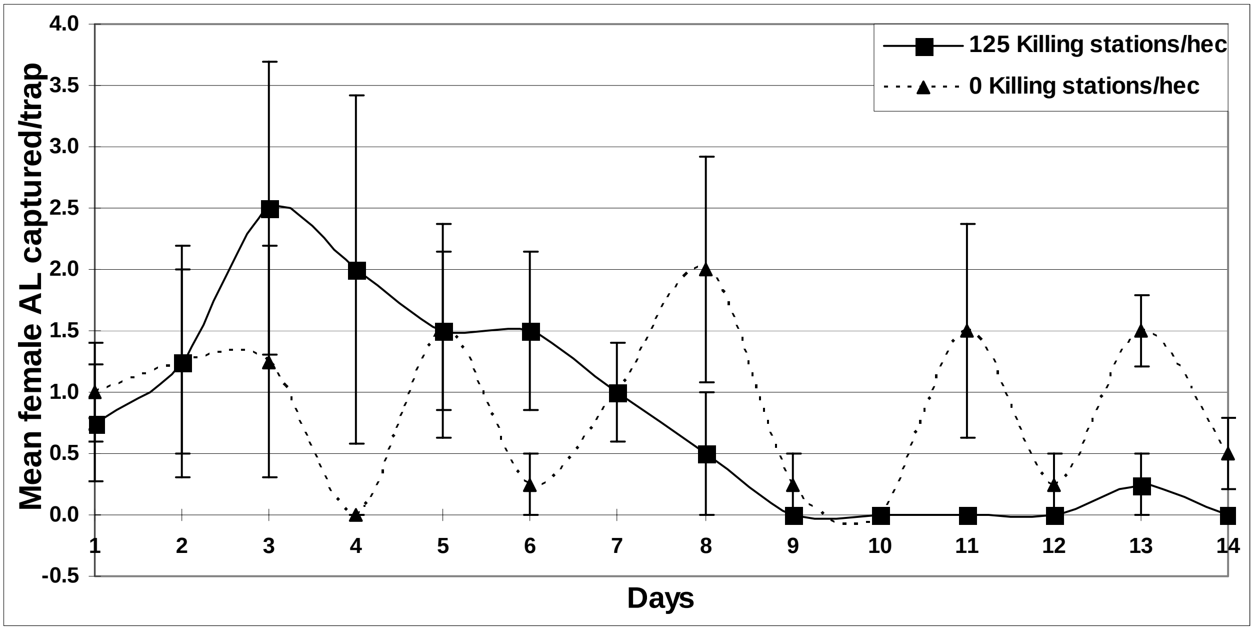

Figure 4 – Mean number (n=4, ± S.E.M.) of female alfalfa looper moths captured with feeding attractant baited traps during the 2003 growing season. Killing stations were deployed 7 days after the start of experiment and remained in the field for 7 days.

Table 2 - Mean (± S.E.M.) number of alfalfa looper moths captured per day in alfalfa fields during the 2004 growing season. Female traps were baited with feeding attractant and male traps baited with sex pheromone. Killing stations remained in plots for 20 days.

Feeding attractant	**0 killing stations/hec**	**125 killing stations/hec**
Females	1.25 ± 0.14a	0.25 ± 0.06b
Males	0.65 ± 0.13a	0.35 ± 0.09a
Sex pheromone	**0 killing stations/hec**	**125 killing stations/hec**
Males	31.45 ± 2.48a	27.09 ± 1.98a

Means within a row followed by the same letter (a, b) are not significantly different (n=4, alpha= 0.05, ANOVA with repeated measures).

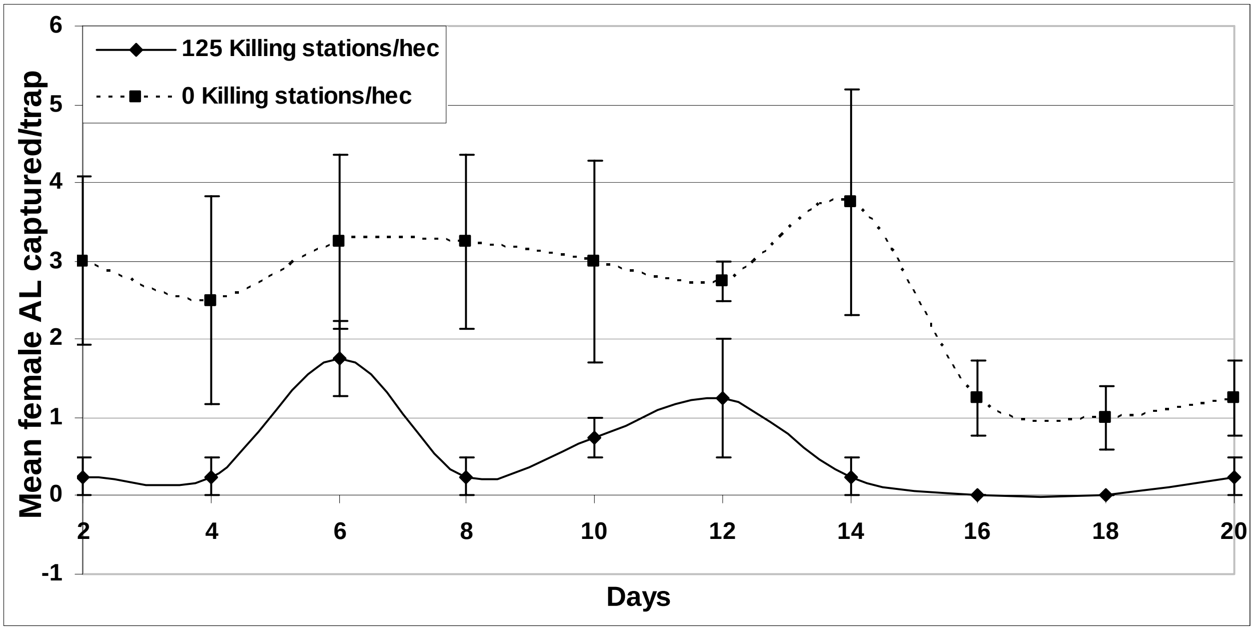

Figure 5 – Mean number (n=4, ± S.E.M.) of female alfalfa looper moths captured with feeding attractant baited traps during the 2004 growing season. Killing stations were deployed at the beginning of the experiment and remained in the field for 20 days.

Table 3 - Number of alfalfa looper eggs and larvae per 100 potted lettuce plants (n=3, Mean ± S.E.M.). Moths were released and allowed to oviposite in a screen building for 7 days. T1 – Zero killing stations per building and moths not provided sugar during rearing, T2 – 2 killing stations per building and moths provided sugar during rearing, and T3 – 2 killing stations per building and moths not provided sugar during rearing.

Treatment	Eggs and larvae
(T1) Control starved	287.67 ± 93.97a
(T2) Killing stations fed sugar	238.33 ± 50.1a
(T3) Killing stations starved	4.33 ± 3.38 b

Means followed by the same letter (a, b) are not significantly different (n=3, alpha= 0.05, L.S.D test).

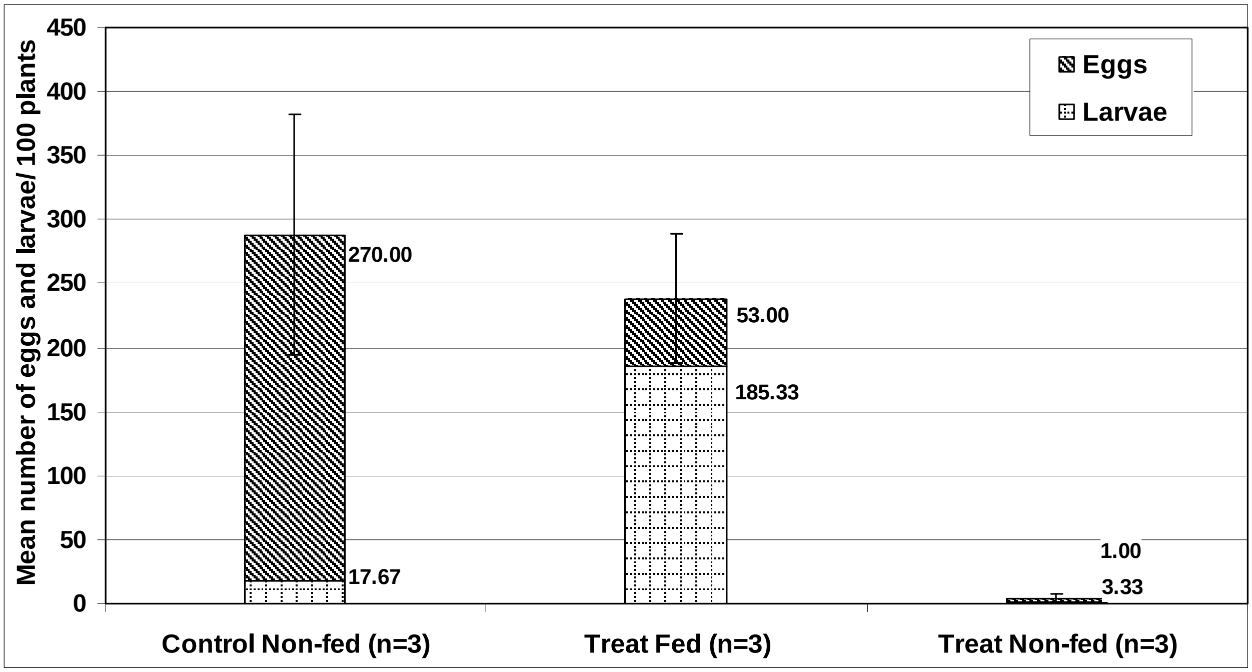

Figure 6 – Number of alfalfa looper eggs and larvae per 100 potted lettuce plants (n=3, Mean ± S.E.M.). Seven days after moths were released in screened buildings. T1 – zero killing stations per building and moths not provided sugar prior to release, T2 – 2 killing stations per building and moths provided sugar prior to release, and T3 – 2 killing stations per building and moths not provided sugar prior to release.

Table 4 – Mean (± S.E.M.) percentage of female alfalfa looper moths that were attracted to and contacted shuttlecock killing stations loaded with floral chemical lure. Comparison between control, moths that were provided sugar, and moths that were not provided sugar before the test.

		% plume tracked			% contacted source		
Treatment comparisons	n[1]	x + SEM	f[2]	p[2]	x + SEM	f[2]	p[2]
(T1) No lure	6	0.0 ± 0.0a	97.8	<0.01	0.0 ± 0.0a	60.62	<0.01
(T2) Moths not fed sugar	6	83.3 ± 6.15b			73.3 ± 6.67b		
(T3) Moths fed sugar	6	26.7 ± 4.22c			20.0 ± 5.16c		

[1]5 moths tested per replicate.

[2]ANOVA test

Means followed by the same letter (a, b) within a column are not significantly different (n=3, alpha= 0.05, L.S.D test). T2 and T3 shuttlecock killing station loaded with tested lure and insecticide.

Table 5 – Mean (± S.E.M.) percentage of male alfalfa looper moths that were attracted to and contacted shuttlecock killing stations loaded with floral chemical lure. Comparison between control, moths that were provided sugar, and moths that were not provided sugar before the test.

		% plume tracked			% contacted source		
Treatment comparisons	n[1]	x + SEM	f[2]	p[2]	x + SEM	f[2]	p[2]
(T1) No lure	6	0.0 ± 0.0a	57.86	<0.01	0.0 ± 0.0a	30.63	<0.01
(T2) Moths not fed sugar	6	60.0 ± 5.16b			53.3 ± 4.22b		
(T3) Moths fed sugar	6	30.0 ± 4.47c			20.0 ± 7.30c		

[1] 5 moths tested per replicate.

[2]ANOVA test

Means followed by the same letter (a, b) within a column are not significantly different (n=6, df= 17, alpha= 0.05, L.S.D test). T2 and T3 shuttlecock killing station loaded with tested lure and insecticide.

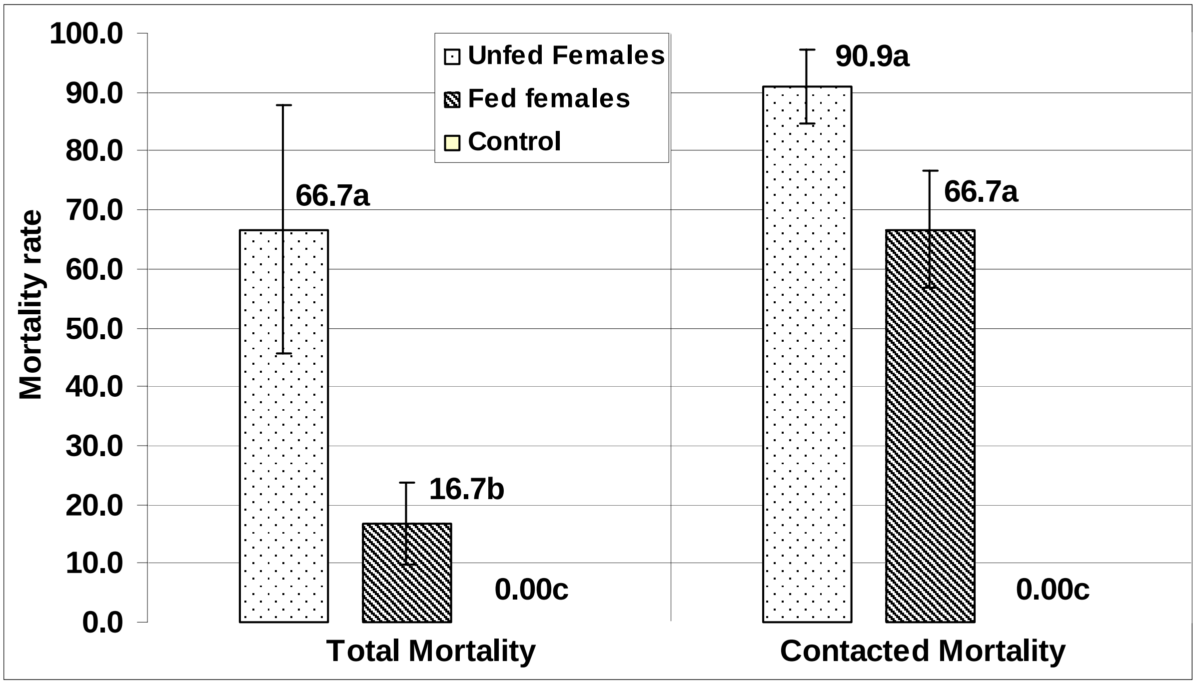

Figure 7 - Mean (± SEM) mortality rate of alfalfa looper female moths attracted to shuttlecock killing stations loaded with floral chemical lure and insecticide. The mortality rate of moths tested and mortality rate of moths that contacted the killing station are presented. Means followed by the same letter (a, b) are not significantly different (n=6, p=0.05, L.S.D. test).

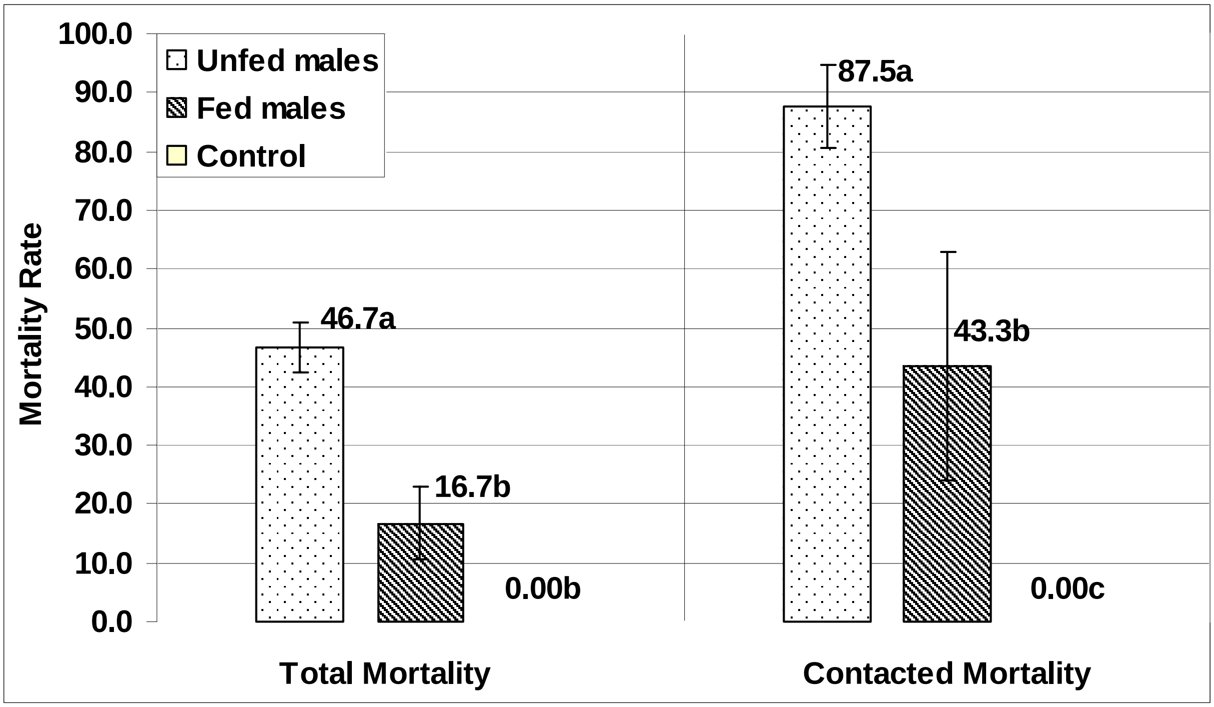

Figure 8 - Mean (± SEM) mortality rate of alfalfa looper male moths attracted to shuttlecock killing stations loaded with floral chemical lure and insecticide. Mortality rate of moths tested and mortality rate of moths that contacted the killing station are presented. Means followed by the same letter (a, b) are not significantly different (n=6, p=0.05, L.S.D. test).

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Chapter 2

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Seasonal activity and field response to floral lures for female and male adult Noctuidae moths in south-central Washington State.

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CHAPTER II

Seasonal activity and field response to floral lures for female and male adult Noctuidae moths in south-central Washington State.

ABSTRACT

Adult seasonal activity of corn earworm, cabbage looper and alfalfa looper were monitored with the use of traps baited with sex pheromone and feeding attractants based on floral compounds. Three floral lures were used, phenylacetaldehyde with methyl-2-methoxy benzoate, phenylacetaldehyde with methyl salycilate, and phenylacetaldehyde with β-myrcene, in south-central Washington State. Corn earworm moths were generally captured from mid June through September. Numbers of corn earworm moths captured in pheromone traps ranged from 268 per week per trap in 2002 to 68 per week per trap in 2003. Numbers of corn earworm moths captured in feeding attractant baited traps were low compared to sex pheromone baited traps, ranging from 3 moths to 4 moths per week per trap. Alfalfa looper moths were trapped from late March or early April, through late October/early November. Cabbage looper moths were trapped from early June, through November in 2004, with few moths captured in 2002 and 2003. Most corn earworm moths trapped with floral lures wire in traps baited with phenylacetaldehyde combined with methyl-2-methoxy benzoate. The most alfalfa looper moths were trapped with the phenylacetaldehyde combined with beta myrcene blend, and the most cabbage looper moths were trapped with phenylacetaldehyde combined with methyl salycilate.

INTRODUCTION

The corn earworm *Helicoverpa zea* (Boddie), cabbage looper *Trichoplusia ni* (Hübner), and alfalfa looper *Autographa californica* (Speyer) are moths in the family Noctuidae that are agricultural pests throughout the south-central region of Washington State. This region has extensive areas devoted to vegetable, fruit, and hay production. The corn earworm is distributed from southern Canada southward to Argentina and Chile and has been introduced into Hawaii (Crumb, 1956, and Hardwick, 1965, 1996). Foliar insecticide applications are the most common method of managing corn earworm in field crops. The corn earworm can cause damage to the region's tomatoes, sweet and field corn, feeding on the fruits and corn ears respectively. In the western part of the United States it overwinters as a pupa in the soil as far north as southern Washington State (Eichmann 1940, and Klostermeyer 1968). This insect is also migratory, undoubtedly contributing to regional pest pressures (Hardwick 1965).

The cabbage looper is a pest of a variety of vegetable crops of several distinct taxonomic groups (Sutherland and Greene 1984) including Crucifers, Solanaceae, Curcubitaceae, Umbelliferae, Amaranthaceae, and others. Both, cabbage looper and alfalfa looper can feed on cole crops and lettuce (McKinney, 1944) damaging local crops between May through late fall. Overwintering by the cabbage loopers occur in southern parts of North America in the pupa stage, attached to host materials

(Metcalf and Flint 1962), and the moths can migrate into the northern States and into southern Canada.

The alfalfa looper is a widely distributed and polyphagous foliage feeder in western North America. Even though, it is considered to be a less serious pest than both the cabbage looper (Vail et al., 1989), and the corn earworm. Damage caused by the larvae includes the destruction of the heads of cole crops, and defoliation of potatoes, peas, sugarbeets, alfalfa, beans, mint, spinach and other minor crops (Brewer, 1995). Once the variable economic threshold is reached foliar insecticide applications that target the early instars of these species are the most common management technique used to prevent damage (Godfrey et al., 2005; Baird and Homan, 1996).

All three of these species occur in central Washington State. The warm and dry climate during the growing season allows for the production of a variety of vegetable and field crops. The large acreage devoted to vegetable crops in the area supports a large population of larvae of these species during the growing season. Although several studies are present in the literature reporting seasonal abundance and damage by these Lepidopteran pest, the relative abundance, importance and seasonal patterns of adult activity for each species varies with geographic location (Harrison and Brubaker 1943, Reid and Bare 1952, Harcourt et al. 1955, Wilson 1957, Todd 1959, Radcliffe and Chapmen 1962, Bonnemaison 1965, Oatman and Platner 1969, Wieres and Chiang 1973, Chalfant et al. 1979, Hamilton 1979, Andaloro et al. 1982, Shelton et al. 1982). Thus a better understanding of the seasonal occurrence of these three species through the growing season in south-central Washington State would be helpful to pest management efforts. Adams (2001) provided seasonal monitoring data for the corn earworm in south-central Washington State. However, that study was limited to sex pheromone traps.

The use of attractants to aid pest management and monitor insect population activity is not an innovative approach. Attractive materials have been studied as a method for better management of agricultural and urban pests for an extended period of time (Dethier, 1947), and monitoring systems have long been in place. The use of sex pheromones for trapping adult male insects is commonly adopted in agricultural settings. Currently female sex pheromones are available for most Lepidopteran pests and they attract males only. Field sampling of the female population would provide information on the gender responsible for laying eggs that will ultimately cause damage to crops in the larval stage. Other than the use of blacklight traps, there are no available methods for sampling adult females of these species.

Floral derived lures that are moth feeding attractants have long been studied. Grant (1971), Cantelo and Jacobson (1979), Haynes et al. (1991), Heath et al. (1992), Pair and Horvat (1997), Lopez et al. (2000), and Landolt and Smithhisler (2003) have described floral odors that are recognized as attractants to both sexes of a handful of Lepidopteran pest species. Phenylacetaldehyde is one of the volatile compounds emphasized from these studies and long used as an attractant. This chemical is attractive to several Noctuid moths, including agricultural pests such as the corn earworm, cabbage looper, alfalfa looper, celery looper *Anagrapha falcifera* (Kirby), and the soybean looper *Pseudoplusia includens* (Walker), among others. Heath et al. (1992) also demonstrated that cabbage looper attraction to floral lures was not limited to male moths. Floral chemical lure combinations have been tested in the field and female and male moths of corn earworm, alfalfa looper, and cabbage looper were captured in Universal Moth Traps™ (Landolt et al., 2001). Gender ratio observed in these studies was approximately 1/1. Female adult attraction to floral scents can be explained by its reward of increased reproductive potential when feeding on sugar as demonstrated by Landolt (1997). Sugar feeding probably also fuels flight in some moths. Migrating corn earworm moths for example are found with pollen from flowers of various plant species (Lindgren et al., 1988). Strong female attraction can be a great asset for the development of monitoring systems. According to Haynes et al. (1991), Landolt et al. (1991), Hitchcox (2000), and Landolt and Higbee (2002) there is no indication to date that the responsiveness of Noctuidae pests to feeding attractants is limited to early or late in life.

In this study, population levels of adults of corn earworm, alfalfa looper, and cabbage looper in the central region of Washington State were monitored throughout the growing season for 3 years. Populations were monitored with sex pheromone and with feeding attractant baited traps. The primary objective of this study was to provide a seasonal picture of the adult activity of these three species in the irrigated agricultural area of south-central Washington State. We also sought to test the hypotheses that (1) corn earworm moths are preferably attracted to phenylacetaldehyde with methyl-2-methoxy-benzoate, that (2) alfalfa looper moths are preferably attracted to phenylacetaldehyde with beta-myrcene and that (3) the cabbage looper moths are preferably attracted to phenylacetaldehyde with methyl salycilate compared to the other blends. Lastly, we sought to compare the trapping of corn earworm moths with sex pheromone versus the floral based feeding attractants.

MATERIALS AND METHODS

Seasonal activity of adults of corn earworm, cabbage looper and alfalfa looper were monitored during a 3 year period in 2002, 2003, and 2004 in selected locations of south-central Washington State's Yakima Valley, Yakima and Benton Counties. Adult male corn earworms were monitored using Universal moth traps™ with green lids, yellow cones and white buckets (Figure 1), loaded with commercial sex pheromone lures (Trecé, Adair, CA). Pheromone lures were placed in a small plastic basket located at the center of the interior of the top of the trap. Sex pheromone lures were replaced every two weeks, and traps were replaced with clean ones every four weeks. Adult male and female moths of all three species were monitored with feeding attractant lures. Feeding attractants were dispensed with 8.0 ml polypropylene vials (Nalgene™ 2006-9025, Fisher Scientific, Pittsburg, PA), loaded with 5.0 ml of the attractant. The chemical attractants were added to cotton balls inside the vials using a plastic, graduated syringe. A single hole 3.0 mm in diameter was drilled into the lid of each vial to provide the desired rate of release. Vials containing each component blend were attached in the upright position to the inside of monitoring traps using a thin wire. Feeding attractants were replaced every two weeks, and traps replaced for clean ones every four weeks. For better preservation of collected specimens a 3.0 cm^2 piece of Vaportape® (Hercon Environmental, Emigsville, PA) and a 12.0 cm x 12.0 cm piece of tissue paper were placed inside of each bucket trap. The Vaportape® was stapled into the internal wall of the bucket. Traps with each lure were located at four sites each year, always near fields of corn and alfalfa. Vaportape® was replaced every month. Traps were emptied weekly throughout the monitoring season. Captured specimens were placed into Ziplock® plastic bags (S.C. Johnson and sons, Racine, WI) and transported to the laboratory and stored in a freezer until they were sorted, identified, and separated by gender and then counted.

During 2002 two trapping sites were selected neat Wapato, WA, one near Granger, WA, and one near Toppenish, WA, all within Yakima County. Four monitoring traps were set up in each site. The first trap was baited with commercial corn earworm sex pheromone. The second trap was baited with a feeding attractant optimal for the corn earworm, phenylacetaldehyde with methyl-2-methoxy benzoate (PAA+MMB). The third trap was baited with a feeding attractant optimal for the alfalfa looper, phenylacetaldehyde with beta myrcene (PAA+BM). The fourth trap was baited with a feeding attractant optimal for the cabbage looper, phenylacetaldehyde with methyl salycilate (PAA+MS). These feeding attractant lures were found to be variably attractive to these three different species in prior comparative trapping experiments (Landolt et al, 2001, and unpublished data). Each of these 2-component lures was loaded in one vial as a 1 to 1 mixture, at a 5.0 ml dose. During 2003 the four trapping sites were in Moxee, WA, Toppenish, WA, Prosser, WA, and Paterson, WA. These sites were within Yakima and Benton Counties. Monitoring trap lures were the same as in the 2002 experiment described above. During 2004 monitoring sites were near Moxee, Prosser, Paterson, and

Toppenish, WA. Monitoring traps were baited with commercial sex pheromone for the corn earworm and a 4-component blend feeding attractant (PAA+MMB+BM+MS) for trapping corn earworm, cabbage looper and the alfalfa looper. The load amount for the mixture was 10.0 ml per vial. Feeding attractant lures were blended together at a 1:1:1:1 ratio, and the mixture was loaded into a 15.0 ml Nalgene® vial with a single hole 6.0 mm in diameter. For all sites the order of traps was randomized every week when traps were emptied. Traps were placed on fences or on poles about 1.0 meter high and 90 to 100 meters apart in a north-south orientation.

For seasonal monitoring graphic representation data analyses were done by calculating the mean number of moths per trap per week and the standard error of the mean (Microsoft Excel™). For comparisons of moth responses to different lures, data analyses were done by calculating number of moths captured per trap per week for the season, followed by an Analysis of Variance (ANOVA), as a complete randomized block design with repeated measures. Mean comparisons were followed with an LSD test.

RESULTS

During the 2002 growing season the first catch of corn earworm in sex pheromone baited traps occurred on the 31st of May and the last catch of corn earworm moths on October 17th, for a seasonal duration of slightly less than five months (Figure 2). There were two principal flight periods for corn earworm, the first lasting from mid June through late July and the second from late August through mid September. However after the first trap catch in late May, the number of moths captured per trap per week never reached zero until late October. The peak for corn earworm numbers in 2002 occurred during the second flight with an average trap catch of 268 moths per trap per week on August 22nd.

In 2003 the first trap catch of corn earworm using sex pheromone baited traps occurred on the 23rd of May and the last trap catch on October 30th, for a seasonal duration of slightly longer than five months (Figure 3). Again there were two principal flight periods. However, these were not as distinct as in 2002. The first flight occurred from mid June to early July and the second from early August through mid September. As in 2002 trap catches never reached zero moths per trap per week after the first moths were captured in late May, until the season over in late October. The peak for corn earworm numbers in 2003 occurred during the second flight with an average trap catch of 68 moths per trap per week on August 21st.

During the 2004 season the first trap catch of corn earworm using sex pheromone baited traps occurred on the 21st of May and the last trap catch was on October 29th, for a seasonal duration of slightly longer than five months (Figure 4). Distinct generations were not evidenced in 2004. Numbers trapped generally were increased into late August and generally decreased there after. Again trap catches never reached zero moths per trap per week after the first males were trapped until the seasonal activity was over in late October. The peak for corn earworm numbers in 2004 was an average trap catch of 100 moths per trap per week on August 20th.

During the 2002 growing season the first capture of corn earworm moths using feeding attractant traps occurred on May 31st and the last trap catch on October 31st, for a seasonal duration of five months (Figure 5). Feeding attractant trap catches were numerically low when comparing to sex pheromone baited traps. After the first moths were caught in late May, numbers did reach zero moths per trap per week at several points during the season. The peak for corn earworm numbers in feeding attractant traps in 2002 occurred in late August with an average trap catch for males and female corn earworm moths combined of 3.5 per trap per week on August 15th.

In 2003 the first captures of corn earworm moths using feeding attractant traps occurred on 6 of June and the last trap catch on October 23rd (Figure 6). As in 2002 trap catches were numerically low when compared to sex pheromone traps. As in 2002 numbers of moths per trap per week were zero at several intervals during the season. The peak for corn earworm numbers in feeding attractant traps in 2003 occurred in late August with an average trap catch for male and female moths combined of 2.5 per trap per week on August 28th and again on October 2nd.

A low number of corn earworm moths were captured using the floral attractant blend (PAA+MMB+BM+MS) baited traps during 2004. Thus, it was not possible to assess the seasonal activity of this species that year.

Alfalfa looper moth catches during the 2002 season in feeding attractant traps started on April 5th and lasted until October 24th, for a seasonal duration of slightly less than six months (Figure 7). There was one principal period of activity, in May, with fewer moths captured the rest of the season. After the first catch of moths in early April, alfalfa looper moths were trapped every week until early August. The peak for alfalfa looper numbers in feeding attractant traps in 2002 occurred in mid May with an average trap catch of 71 moths per trap per week on May 17th.

During the 2003 growing season the first trap catch of alfalfa looper moths in feeding attractant traps occurred on April 4th and the last was catch on November 5th, for a seasonal duration of slightly over seven months (Figure 8). There were two principal flight periods for alfalfa looper moths, the first lasting from early April through late July and the second period from late August through late October. After the first trap catch in early April, alfalfa looper moths were trapped every week of the season. The peak for alfalfa looper numbers in 2003 occurred in early April with an average trap catch of 34 moths for males and female moths per trap per week on April 3rd.

In 2004 the first catch of alfalfa looper moths in feeding attractant traps occurred on April 9th and the last trap catch on November 5th, for a seasonal duration of slightly less than seven months (Figure 9). After the first moths were captured in early April, numbers did not reach zero before the end of the summer. Distinct periods of activity were not evident, but numbers trapped were higher from early April trough late May and fewer from early June through early November. The peak for alfalfa looper numbers trapped in 2004 occurred in early April with an average trap catch of 10.5 male and female moths combined per trap per week on April 9th.

Numbers of cabbage looper moths captured during 2002 in feeding attractant traps were very low (Figure 10). The first moth was captured on May 15th and no more moths were captured until September 5th, and the last moth was captured on October 3rd.

During 2003, the first catch of cabbage looper moths in feeding attractant traps occurred on June 13th and the last moth trapped was on October 30th, for a seasonal duration of slightly less than five months (Figure 11). A principal period of activity was evident from mid August to mid September. The peak for cabbage looper numbers trapped in 2003 occurred in late August with an average trap catch for male and female moths combined of 56.5 per trap per week on August 21st.

In 2004 the first cabbage looper moths trapped in feeding attractant traps occurred June 18th and the last captured was on October 30th, for a seasonal duration of slightly less than five months (Figure 12). After the first moths were captured in late June, numbers were zero until August. The peak for cabbage looper numbers trapped in 2004 occurred in early September with an average trap catch for male and females combined of 5.8 moths per trap per week on September 10th.

In the comparison of 2-component blends significantly greater numbers of corn earworm moths were captured with phenylacetaldehyde plus methyl-2-methoxy-benzoate (PAA+MMB) compared to phenylacetaldehyde plus beta-myrcene (PAA+BM) or phenylacetaldehyde with methyl salycilate (PAA+MS) ($p<0.01$, table 1). Significantly great numbers of alfalfa looper moths were captured in the PAA+BM traps than in the PAA+MMB or the PAA+MS traps ($p<0.01$, table 1). No significant differences were observed among chemical lures for the numbers of cabbage looper moths captured ($p=0.21$, table 1). However, numerically more cabbage looper moths were caught in PAA+MS traps than in PAA+MMB traps or PAA+BM traps.

In 2003, significantly great numbers of corn earworm moths were captured in the PAA+MMB traps compared to PAA+BM or the PAA+MS traps ($p<0.01$, table 2). Significantly greater numbers of alfalfa looper moths were captured in the PAA+BM baited traps than in the PAA+MMB or the PAA+MS baited traps ($p<0.01$, table 2). No significant differences were observed among feeding attractants baited traps for numbers of cabbage looper moths captured ($p=0.12$, table 2). However, numerically more cabbage looper moths were caught with PAA+MS than with PAA+MMB, or PAA+BM baited traps.

Throughout experiments in 2002, 2003 and 2004, sex pheromone baited traps captured male moths only, while feeding attractant baited traps captured both genders in a ratio of approximately 1 male to each female.

DISCUSSION

During two of the three years, increased numbers of corn earworm moths were captured in monitoring traps from mid June through September, about the same time that corn is available for oviposition in the region's cultivated field and sweet corn. In 2004, this period of activity was somewhat delayed. The seasonal patterns of corn earworm moths trapped for the 3 years were similar, but the relative abundance of moths during these years varied. In 2002 during peak numbers 268 moths per week were collected compared to 68 in 2003 and 100 in 2004. The abundance of corn earworm moths could be due to a number of factors, including weather affecting overwintering, summer flight and reproduction, and migration from the south. Additionally, changes in planting patterns and Integrated Pest Management programs may impact regional population levels. Warmer temperatures on average may permit a larger percentage of corn earworm to overwinter successfully (Eichman 1940, Klostermeyer 1968), causing a variation in the abundance of moths in the subsequent year.

There are 1 to 3 generations of corn earworm per year in Washington State (Mayer, 1987). The 2002 and 2003 data indicate two flight periods, similar to results obtained by Adams (2001). Also, there may have been a migratory contribution to the corn earworm population. In other areas of North America corn earworm are known to migrate, from Texas into Arkansas and Oklahoma (Hendrix et al 1987, Lingren et al. 1993, 1994) as well as from Mexico into Texas and Arkansas (Harstack et al. 1982).

Migration of corn earworm from an area to a source of corn ears several hundred miles away have been recorded using radar (Wolf et al. 1990). We expect that the corn earworm in the western United States migrates south to north, as it does in the Midwest. These factors could contribute to seasonal variance in the abundance of corn earworm in south central Washington State. Further studies would be necessary to clearly understand the corn earworm migration patterns in the western U.S., and the relative contribution of overwintering and migration moths to species seasonal phenology in Washington State.

The abundance of corn earworm moths in feeding attractant traps was low compared to sex pheromone baited traps, with peak average of 2.5 moths per trap per week in 2002 and 3.5 in 2003, compared to 268 moths per week in 2002, 68 in 2003, and 100 in 2004 in pheromone traps. Peak periods were similar for both years, occurring during mid August, for both monitoring trap systems. Even though the total numbers of captured moths were different, seasonal flight patterns were similar for both types of lures. First catches of moths for this season were the same for 2002, on May 31st. The feeding attractant trap however collected its last moth of the season on October 24th, while the last moth in the sex pheromone trap was captured a week earlier on October 17th. As expected, sex pheromone baited traps captured male moths only, while feeding attractant baited traps captured both genders in a ratio of approximately 1 male to each female, similar to other Noctuid feeding attractant trapping experiments (Landolt et al 1991, Lopez et al. 2000, Landolt and Higbee, 2002). Landolt (personal communication) captured corn earworm moths with the PAA+MMB blend, which proved to be in this experiment more efficient for trapping corn earworms than either the PAA+BM and PAA+MS floral blends.

The phenylacetaldehyde plus beta-myrcene blend demonstrated, as expected, efficiency in attracting both genders of alfalfa looper moths into monitoring traps. During the peak of the season an average 71 moths were captured in 2002 and 34 in 2003. During all three years alfalfa looper flight activity started either in late March or early April, and went through late October/early November. A steep increase of moths captured was observed in 2002 in early May and in 2003 and 2004 in early April. Weather conditions and the availability of host plants in the region are factors that contributed to this initial peak. In 2002, 71 moths were captured per trap per week, 34 were captured during 2003's initial peak and finally 11 in 2004. Initial peak capture variation can be explained by weather differences between 2002 and 2003, and the use of the floral blend in 2004. A late flight peak for alfalfa looper moths was observed in all three years between early to late October. During the late season peak fewer moths were captured than the initial flight peak. During 2002 later peak 9 alfalfa looper moths were captured per average per trap per monitoring period, in 2003, 26 and in 2004 5 moths were captured per average per trap per monitoring period. Alfalfa looper moth captures never reached zero in between the two peak periods for all years. These results are similar to those obtained by Vail et al. (1989) for alfalfa looper adult activity in California. No studies to assess alfalfa looper seasonality have been described in the literature addressing the south-central Washington State region.

As expected both genders of alfalfa loopers were attracted to the phenylacetaldehyde and beta myrcene mixture. The overall sex ratio for all experiments combined was 46.2% females to 53.8% males. This is similar to results obtained by Landolt et al. (2001) trapping alfalfa loopers with different feeding attractant combinations that included phenylacetaldehyde. Creighton et al. (1973) obtained a gender ratio of 55.6% females and 44.4% males trapping cabbage loopers with phenylacetaldehyde. Pair and Horvat (1997) found that both sexes of several species of moths, including the cabbage looper and soybean looper, are attracted to floral odorants. These findings reinforce the idea of the possibility for using floral derived feeding attractants lures as aid on pest management tools. In addition, similarly to alfalfa loopers, the gender ratio for cabbage looper moths captured was also close to 1:1.

During 2002, an average of 2.0 cabbage looper moths was captured per week per trap during the entire season with traps baited with phenylacetaldehyde combined with methyl-salycilate. These results were not expected considering that the cabbage looper is commonly known to be attracted to floral derived feeding attractants (Grant 1971, Haynes et al. 1991, Heath et al 1991, and Landolt et al. 1992). Once again weather, conditions, migratory behavior, and possible environment influences, such as availability of host plants, may have been a cause for low cabbage looper trap captures during 2002. During 2003 and 2004 a stronger abundance, and better seasonal picture of cabbage looper was observed. In both years cabbage looper flight activity started in early June, and went through early November. One major peak was observed in 2003 and 2004, the steep increase of moths captured was observed for 2003 in mid August and in 2004 in early September. These results are different than the seasonality of cabbage looper observed by Vail et al (1989) using sex pheromone baited traps. In the Imperial Valley, CA cabbage loopers occur in highest numbers from mid February trough mid June. Warmer Spring temperatures in southern California contribute to an earlier increase in numbers of cabbage looper moths. In Washington State colder temperatures in the winter contribute to a later seasonality. No studies of cabbage looper seasonality are present in the current literature addressing Washington State. Greatest abundance of alfalfa looper moths were captured, followed by cabbage looper moths, and lastly corn earworm moths. Overwintering success, wild host and crops availability, and responsiveness to floral lures might all have played a role in the numbers of moths captured during the different years.

Weather conditions and planting patterns make it difficult to assess seasonal patterns with precision, and a clear distinction of the different generations within a season for all three species is unclear, with much overlapping occurring throughout the year. Higher and lower numbers within a year might have been caused by warm weather or cooler temperatures. Most trapping sites were near agricultural fields in Yakima and Benton Counties. A successful Integrated Pest Management program may have caused crashes in regional abundance of moths. Overwintering success, year to year variation of temperature during the Spring, and variable migration of corn earworm and cabbage looper moths, may have caused variation in numbers from year to year. Migration by cabbage looper moths depends in part on suitable southwestern populations, as well as wind currents to carry the moths.

These studies provide further evidence of which chemical component mixtures are more attractive to each moth species. During 2002 and 2003 a significantly greater number of corn earworm moths were attracted to the PAA+MMB blend than PAA+BM, or PAA+MS. These results are similar to previous studies on trapping corn earworm with volatile floral chemicals (Landolt, unpublished data). Lopez et al. (2000) used a five component blend from *Gaura suffulta* flowers to attract corn earworm moths, which included phenylacetaldehyde and methyl-2-methoxy benzoate. As expected the PAA+BM blend attracted a significantly greater number of alfalfa loopers compared to the PAA+MMB, and PAA+MS blends. These results are similar to those obtained by Landolt et al. (2006) which evaluated field responses of alfalfa looper moths to floral blends, and found that the combination PAA+BM attracted more alfalfa loopers than six other floral blend combinations, all of which contained phenylacetaldehyde. Previously, Landolt et al. (2001) found the blend phenylacetaldehyde and cis-jasmone to be more attractive to alfalfa looper moths in comparison to other floral blends; PAA+BM was not used in those experiments. Beta myrcene was isolated and identified from Oregongrape flowers, following a study in which alfalfa looper moths were the most abundant species captured over flowering Oregongrape bushes. This plant was found to release both phenylacetaldehyde and beta myrcene along with other chemicals (Landolt and Smithhisler, 2003). Further studies to better understand species preferences to flowers and attractants are necessary. As a pest management tool multiple blends that attract different species can be used to ones advantage combined or alone, to target single or multiple species.

No differences were observed in the numbers of cabbage looper moths responding to the three component blends. The cabbage looper and other Noctuidae species attraction to phenylacetaldehyde are well studied (Smith et al., 1943; Creighton et al., 1973, Cantelo and Jacobson, 1979; Haynes et al, 1991; Heath et al. 1992). This strong attraction explains the lack of a significant difference in attractants of the three floral blends to cabbage looper moths, since every blend contained phenylacetaldehyde. These results are comparable to cabbage looper trapping experiments conducted by Landolt et al (2006) in which a greater number of moths were captured using the PAA+MS blend compared to phenylacetaldehyde alone, however the difference was not statistically different.

These results provide south central Washington State growers and pest management personnel with critical knowledge on alfalfa and cabbage looper moth seasonality, and reinforce corn earworm seasonality data for the area already available (Adams, 2001). It also demonstrates preferential attraction of species to different floral blends allowing for monitoring systems for females to be optimized. The attraction of female moths can contribute to the development of pest management tools, such as attract and kill, mating disruption, and mass trapping of females.

Figure 1: Universal Moth Trap™ used in field experiments with green lid, yellow cone, and white bucket.

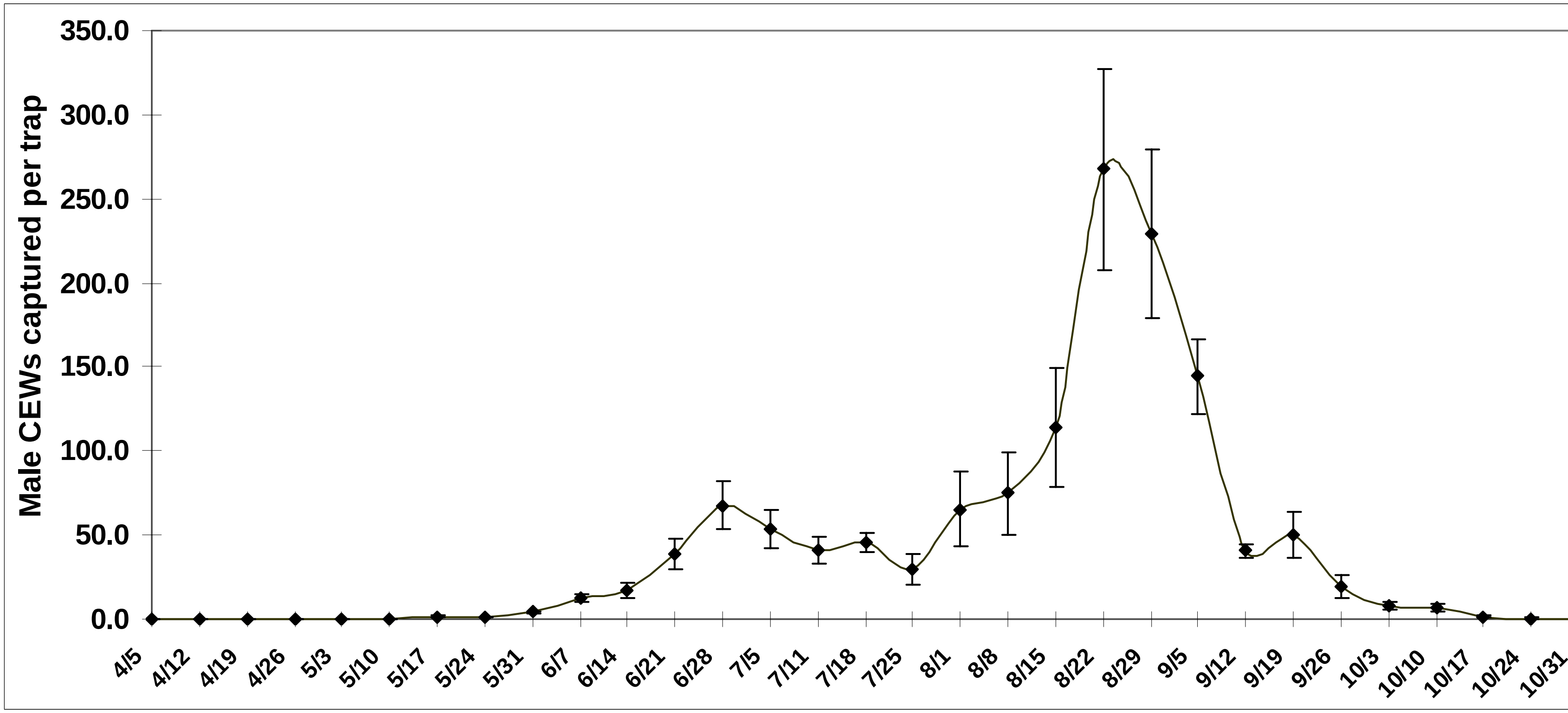

Figure 2: Corn earworm male activity in south-central Washington State during 2002. Mean ± S.E.M. captures per trap per sampling period in sex pheromone baited traps.

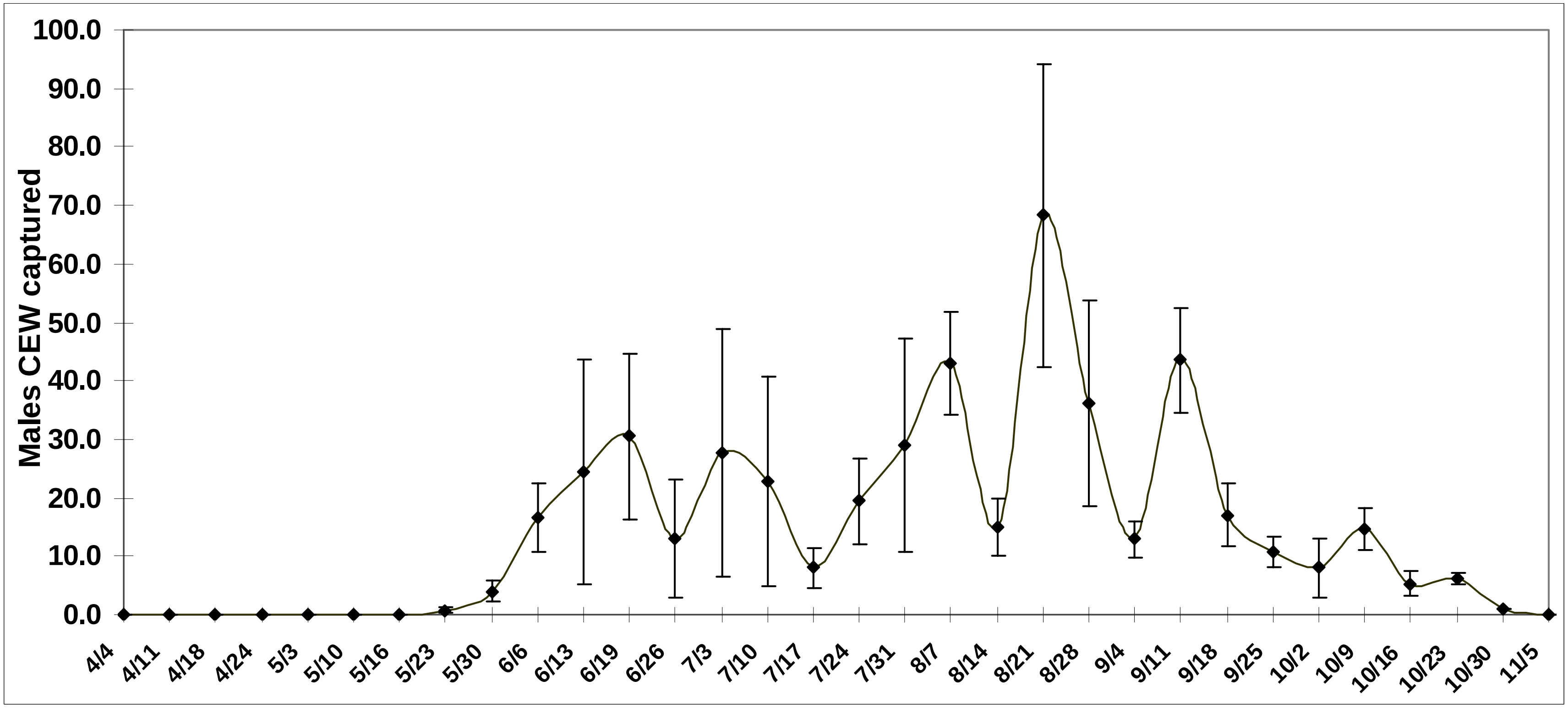

Figure 3: Corn earworm male activity in south-central Washington State during 2003. Mean ± S.E.M. captures per trap per sampling period in sex pheromone baited traps.

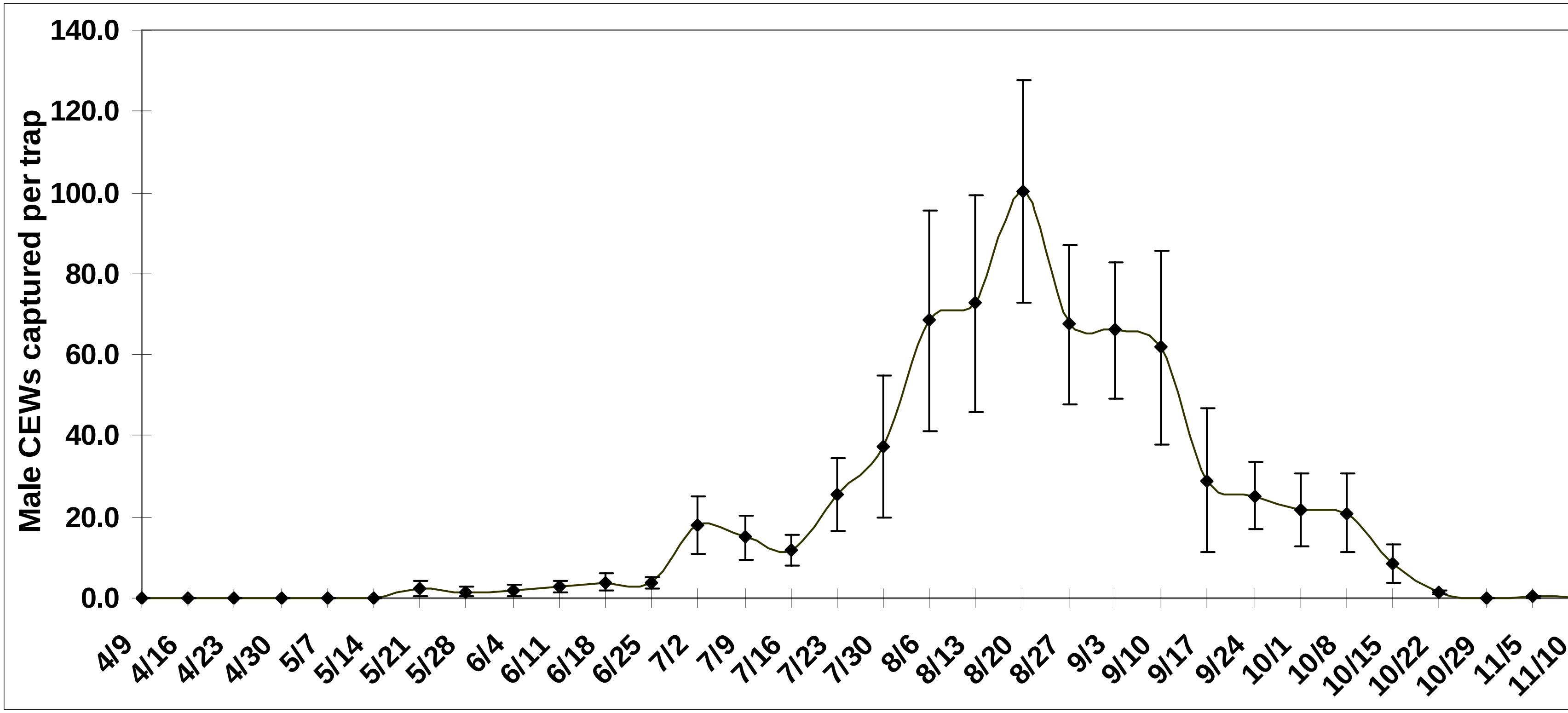

Figure 4: Corn earworm male activity in south-central Washington State during 2004. Mean ± S.E.M. captures per trap per sampling period in sex pheromone baited traps.

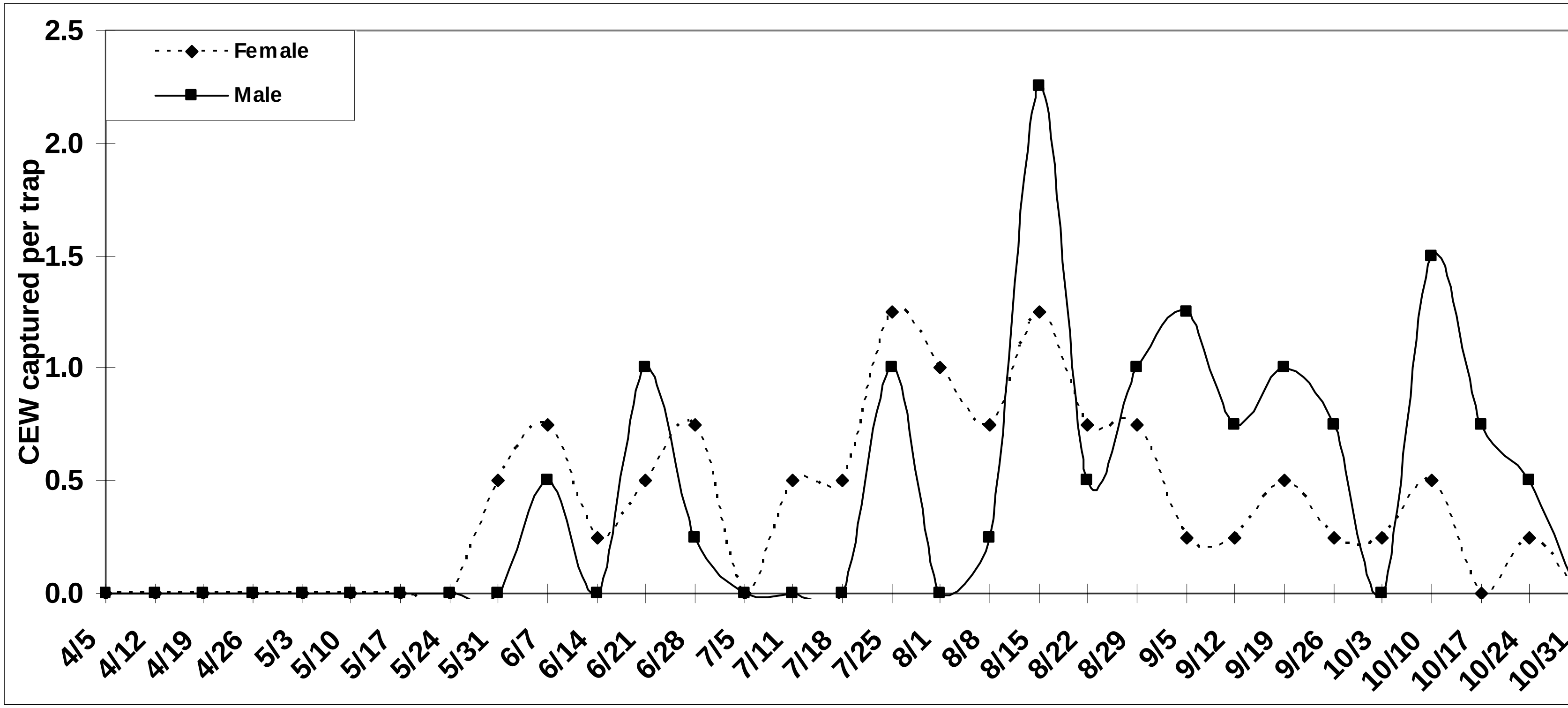

Figure 5: Corn earworm female and male activity in south-central Washington State during 2002. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (phenylacetaldehyde plus methoxy-2-methyl-benzoate).

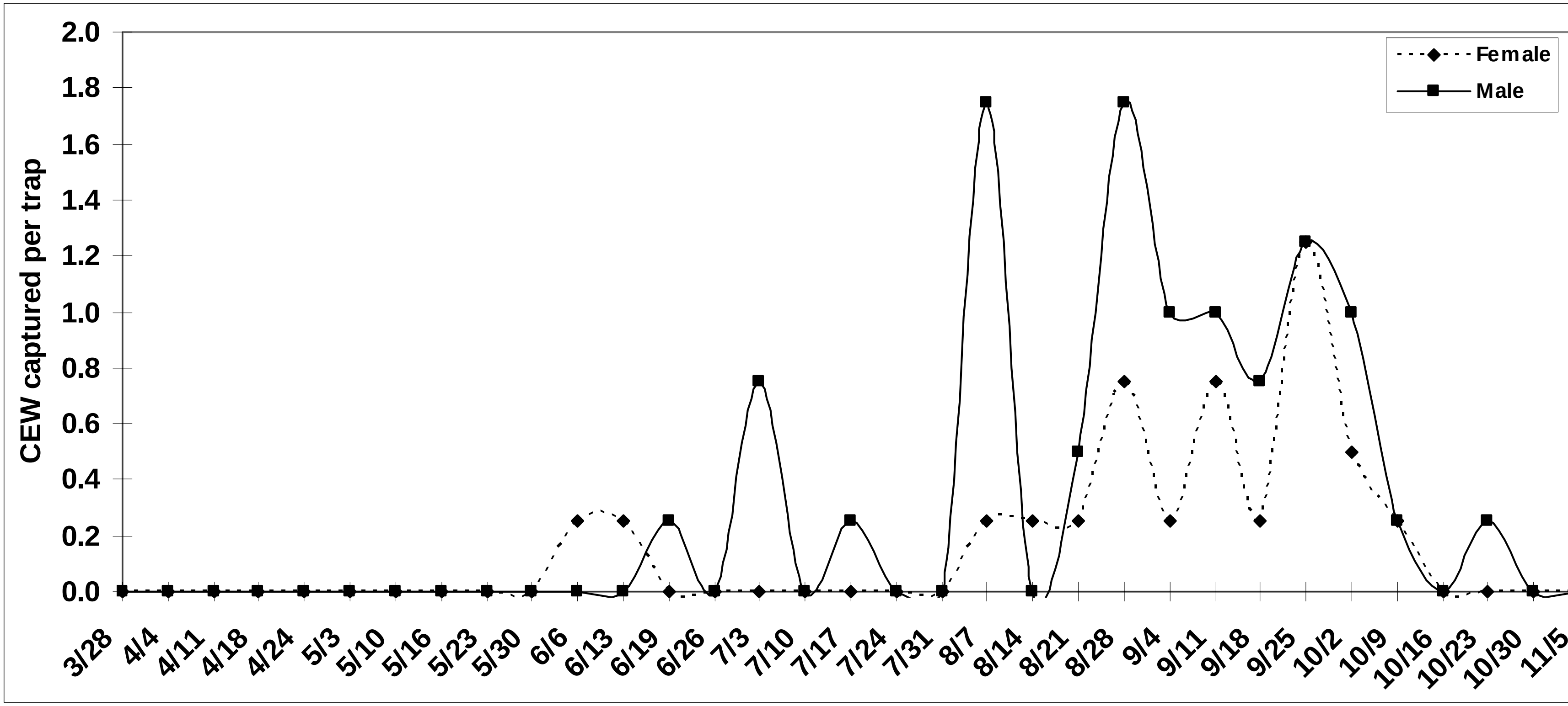

Figure 6: Corn earworm female and male activity in south-central Washington State during 2003. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (phenylacetaldehyde plus methoxy-2-methyl-benzoate).

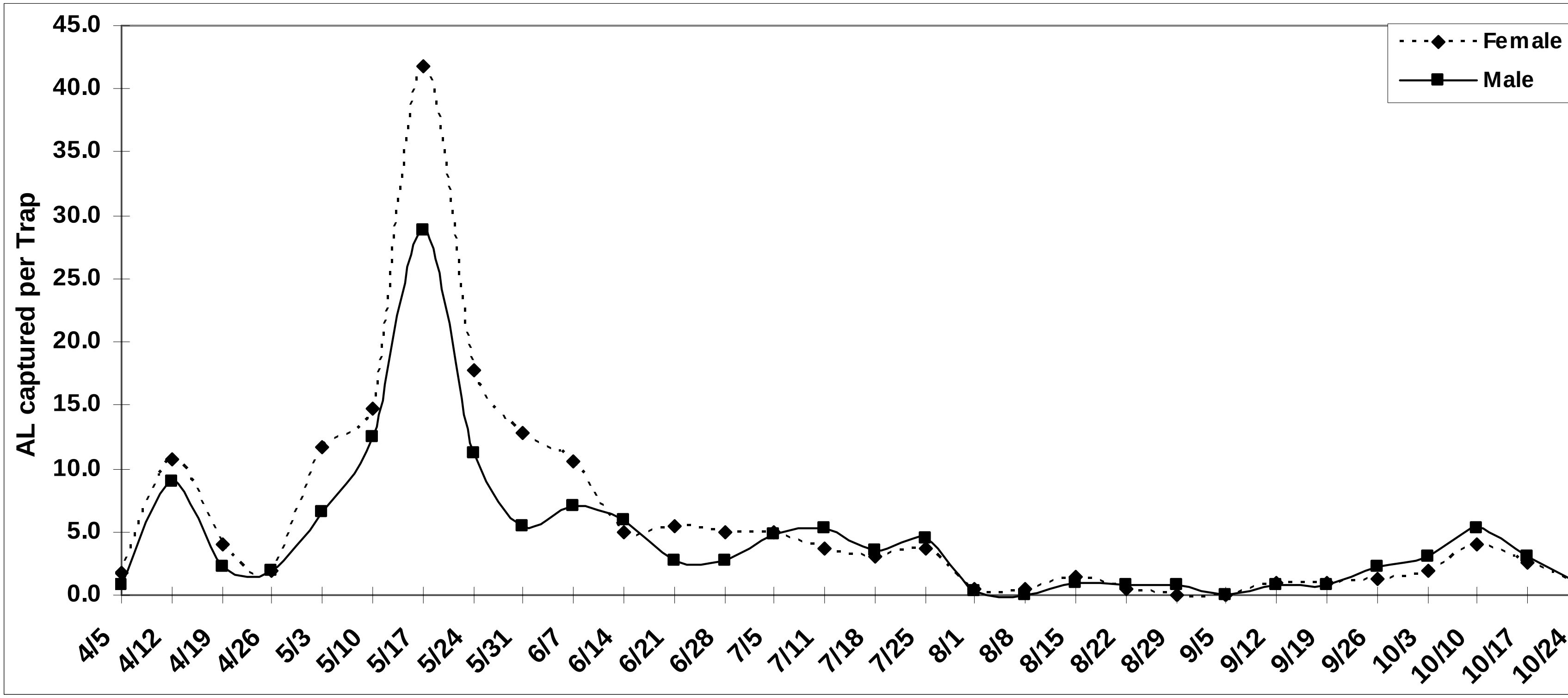

Figure 7: Alfalfa looper female and male activity in south-central Washington State during 2002. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (phenylacetaldehyde plus beta-myrcene).

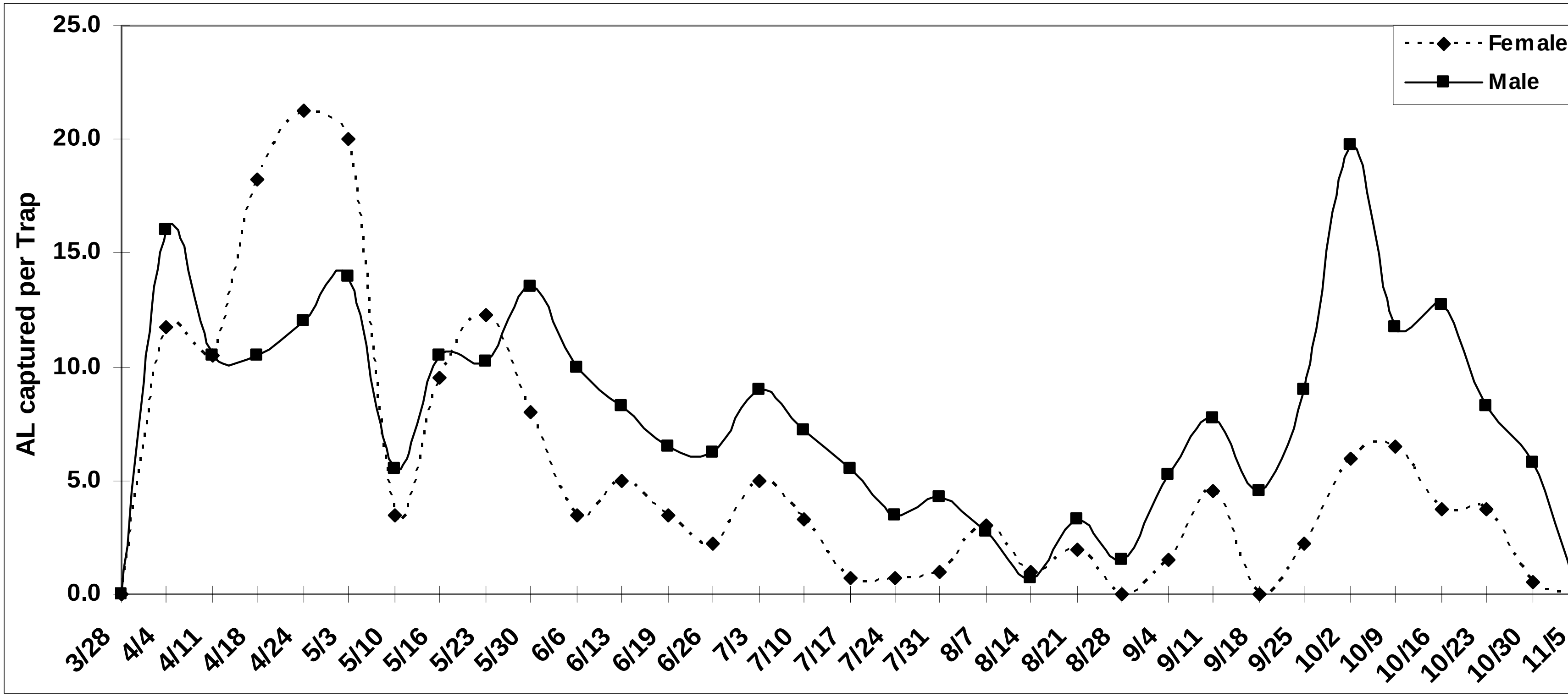

Figure 8: Alfalfa looper female and male activity in south-central Washington State during 2003. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (phenylacetaldehyde plus beta-myrcene).

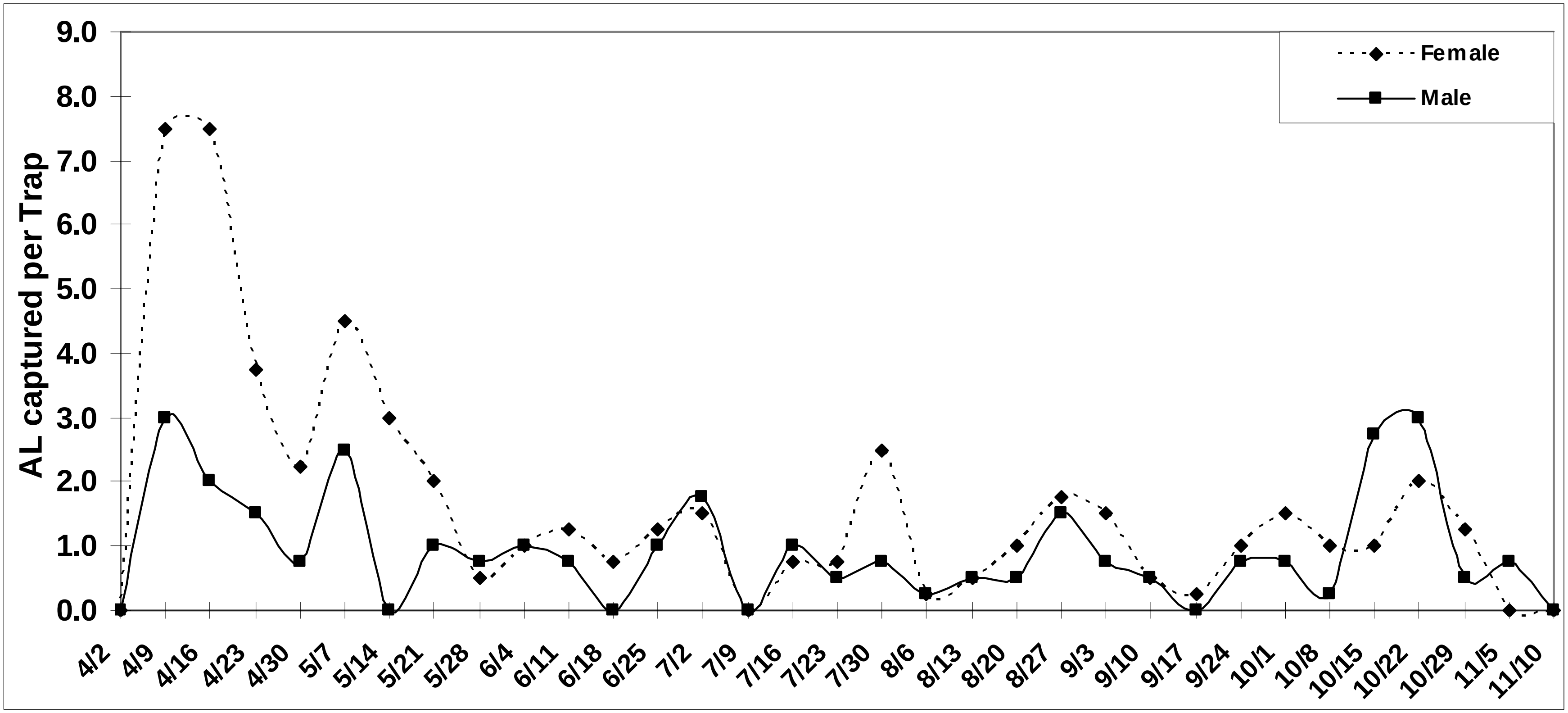

Figure 9: Alfalfa looper female and male activity in south-central Washington State during 2004. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (Floral blend).

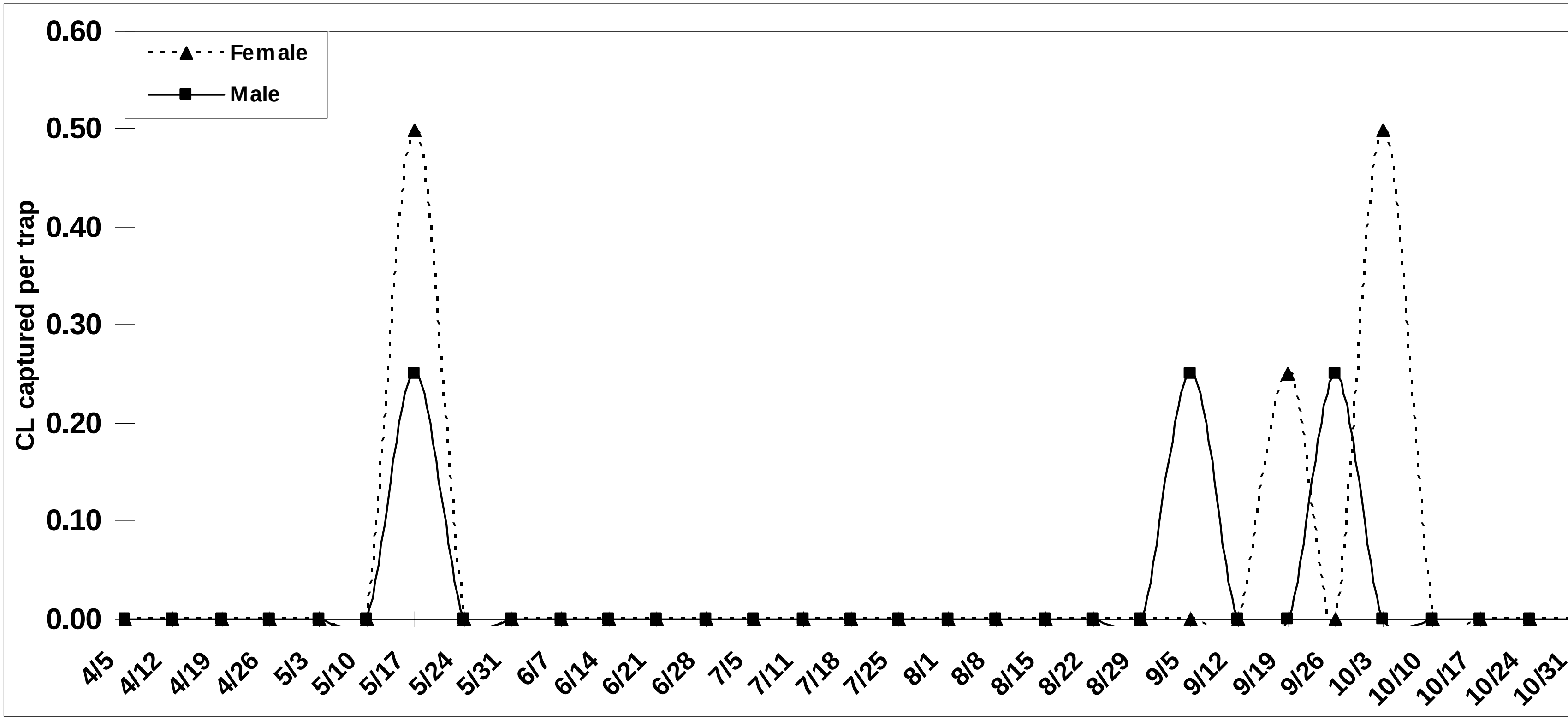

Figure 10: Cabbage looper female and male activity in south-central Washington State during 2002. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (phenylacetaldehyde plus ms).

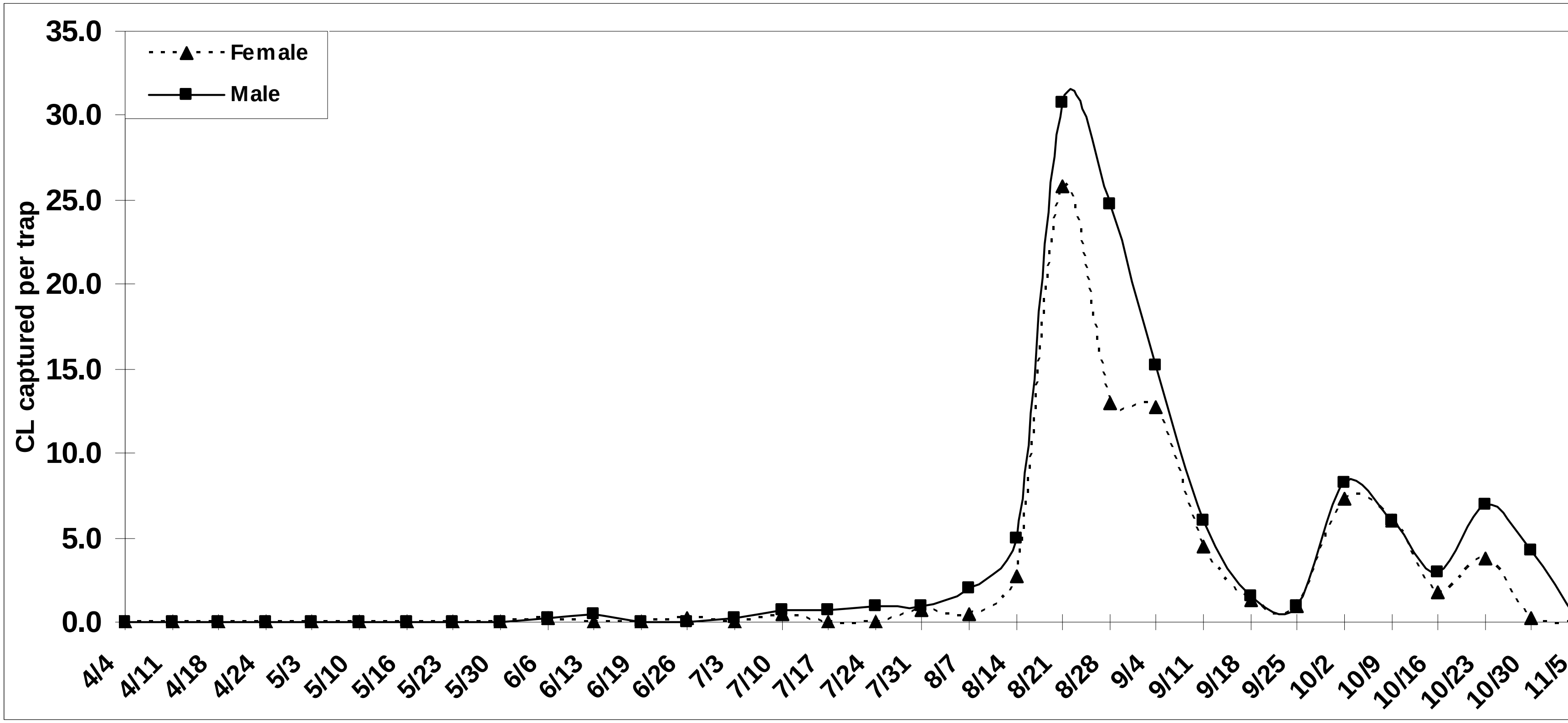

Figure 11: Cabbage looper female and male activity in south-central Washington State during 2003. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (phenylacetaldehyde plus ms).

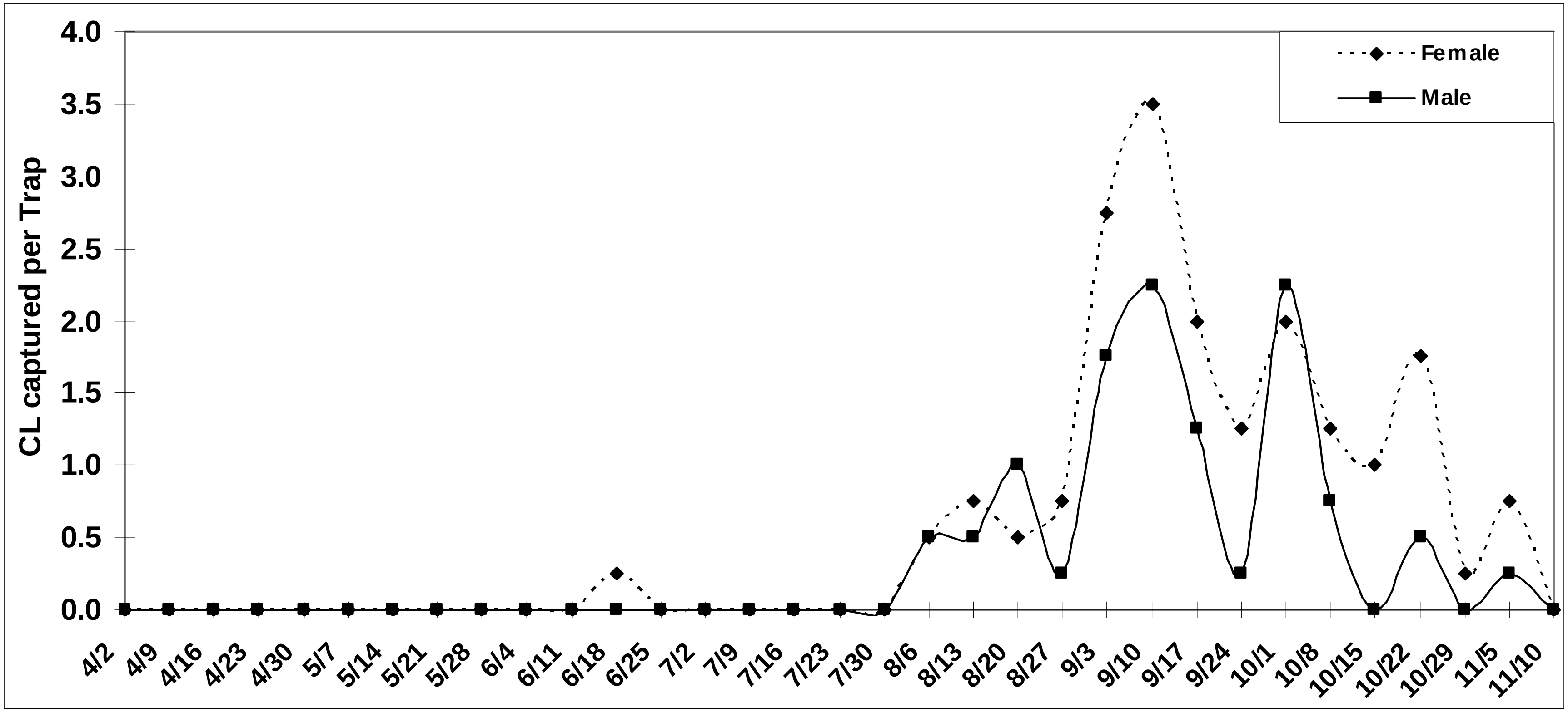

Figure 12: Cabbage looper female and male activity in south-central Washington State during 2004. Mean ± S.E.M. captures per trap per sampling period in feeding attractant baited traps (Floral blend).

Table 1: Number of corn earworm, alfalfa looper, and cabbage looper moths captured per trap per week during 2002 with floral attractants (Mean ± S.E.M.).

	Feeding attractant blend		
	paa + mmb	paa + bm	paa + ms
Corn earworm	**0.81 ± 0.15ar**	0.34 ± 0.08br	0.21 ± 0.06br
Alfalfa looper	2.86 ± 0.61as	**9.94 ± 2.46bs**	2.56 ± 0.54as
Cabbage looper	0.01 ± 0.01ar	0.02 ± 0.02ar	**0.06 ± 0.03ar**

Means within a row followed by the same letter (a, b) and means within a column followed by the same letter (r, s) are not significantly different (n=4, alpha= 0.05, LSD test).

Table 2: Number of corn earworm, alfalfa looper, and cabbage looper moths captured per trap per week during 2003 with floral attractants (Mean ± S.E.M.).

	Feeding attractant blend		
	paa + mmb	paa + bm	paa + ms
Corn earworm	**1.35 ± 0.46bx**	0.22 ± 0.07ax	0.17 ± 0.06ax
Alfalfa looper	2.14 ± 0.47axy	**13.07 ± 1.81by**	2.61 ± 0.43axy
Cabbage looper	4.08 ± 1.17ay	5.47 ± 1.93az	**6.30 ± 2.22ay**

Means within a row followed by the same letter (a, b) and means within a column followed by the same letter (x, y, z) are not significantly different (n=4, alpha= 0.05, LSD test).

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Chapter 3

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Attraction and mortality of female and male cabbage loopers *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) responding to a feeding attractant based killing station.

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CHAPTER III

Attraction and mortality of female and male cabbage loopers *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) responding to a feeding attractant based killing station

ABSTRACT

We have incorporated a floral chemical lure from compounds derived for "moth-visited" flowers into a killing station. This feeding attractant is dispensed from polypropylene vials that provide controlled release rate of the attractant for extended periods of time. The killing station was tested in a wind tunnel for use in combination with this lure as an "attract and kill" system against the cabbage looper *Trichoplusia ni* (Hübner). In the wind tunnel, cabbage looper moths demonstrated a high attraction rate to lure, which released phenylacetaldehyde we saw an 89.0% mortality rate of female moths that contacted the killing station, while 92.0% of males died. Moths were less likely to be attracted to lures when fed sugar during the adult stage and fed moths were less affected by killing stations in wind tunnel trials, compared to starved moths. This system has potential to be adopted for cabbage looper population reduction in vegetable and other field crops, and the use of other female or bisexual attractants may permit its application to other Lepidopteran pests.

INTRODUCTION

The cabbage looper, *Trichoplusia ni* (Hübner) is an economically important pest of a wide variety of crops (Sutherland and Greene 1984). It can be found feeding on foliage of cultivated crops of several distinct taxonomic groups including Crucifers, Solanaceae, Curcubitaceae, Unberlliferae, Amaranthaceae, and others. As an adult, cabbage loopers feed at flowers (Grant 1971) presumably to obtain nectar. Moths locate flowers in part by responding to their scent (Cantelo and Jacobson, 1979 for example). Although cabbage loopers populations can be kept low by natural enemies (Elsey and Rabb, 1970), in agricultural settings population outbreaks are often experienced and foliar insecticides applications are the common method used for control (Kirby and Slosser, 1984). Current U.S. federal legislation, environmental concerns, and worker safety issues have highlighted the need for the development of alternative approaches for managing this and other insect pests.

The use of attractants as a management strategy has a long history (Dethier 1947, Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, Charmillot et al. 2000). A method that relies on insect attractants to manage pests is attract-and-kill, where insects are attracted to a source where they contact a lethal dose of a killing agent. The killing agent of attract-and-kill, also known as lure-and-kill, may include sticky materials, insecticides or pathogenic microorganisms (Pedigo 2002). Other management systems that use insect attractants include mass trapping, poisoned baits, and mating disruption, with much research effort on mating disruption and the use of poisoned baits (Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, and Charmillot et al. 2000). Poisoned baits have been defined as a formulation that combines an edible substance with a pesticide (Pedigo 2002). Mass trapping and lure and kill, for example, have been previously proved to be an effective method against the boll weevil (Lloyd et al. 1981, Villavaso et al. 1998, respectively), bait sprays effective against tropical fruit flies (Steiner et al., 1961), and lure and kill effective against the codling moth (Charmillot et al. 2000) although, success against other Lepidopteran pests has been somewhat limited. Generally, these methods have been successful in tree fruit, field, and forest crops as well as with stored products and household pests (Lloyd et al. 1981, Roelofs 1981).

Several problems have been observed in attract and kill systems that utilize sex pheromone as an attractant, including a delay in efficacy in reducing larvae numbers in the field, caused by the male only attraction to female sex pheromones (Minks, 1997). Removal of males to prevent or reduce female mating is an indirect approach to reduce reproduction and damage to crop by larvae. This approach takes time, because killing males to prevent female mating and oviposition requires planning, and treatment weeks before the targeted pest problem. Also, removal of water does not directly reduce female mating when males mate multiple times, thus a high percentage of males must be removed so that reproduction can be reduced. Charmillot et al. (2000) demonstrated that in some cases applications should be made at least one week before the flight season, and that the application of sex pheromone based attract and kill systems should occur only in areas where the initial pest population is low and there is sufficient isolation from external sources of infestations. The attraction and management of female adult moths would directly impact the reproductive potential of a pest species, reducing the number off eggs laid and consequently reducing the number of larvae infesting the field.

Floral derived lures that are moth feeding attractants have long been studied. Grant (1971), Cantelo and Jacobson (1979), Haynes et al. (1991), Heath et al. (1992), Pair and Horvat (1997), Lopez et al. (2000), and Landolt and Smithhisler (2003) have described floral odors that are recognized as attractants to both sexes of a handful of Lepidopteran pest species. Phenylacetaldehyde is one of the volatile compounds emphasized from these studies and long used as an attractant. This chemical is attractive to several Noctuid moths, including agricultural pests such as the cabbage looper, the corn earworm *Helicoverpa zea* (Boddie), alfalfa looper *Autographa californica* (Speyer), celery looper *Anagrapha falcifera* (Kirby), and the soybean looper *Pseudoplusia includens* (Walker), among others. Heath et al. (1992) also demonstrated that cabbage looper attraction to floral lures was not limited to male moths. Floral lure combinations have been tested in the field and female and male moths of corn earworm, alfalfa looper, and cabbage looper were trapped with the use of Universal Moth Traps™ (Landolt et al., 2001, 2006 and unpublished data). Gender ratio observed in these studies was approximately 1/1. Female adult attraction to floral scents can be explained by its reward of increased reproductive potential when feeding on sugar as demonstrated by Landolt (1997). Sugar feeding probably also fuels flight in some moths. The corn earworm moths for example are found with pollen from flowers of various plant species (Lindgren et al., 1988). Strong female attraction can be a great asset for the development of monitoring systems. According to Haynes et al. (1991), Landolt et al. (1991), Hitchcox (2000), and Landolt and Higbee (2002) there is no indication to date that the responsiveness of Noctuidae pests to feeding attractants is limited to early or late in life.

Strong female attraction can be a great asset for the development of attract-and-kill techniques for pestiferous insects. The annihilation of female moths using an attract-and-kill system might directly reduce the number of eggs that can be potentially laid and consequently, decrease the numbers of larvae present in the field during the subsequent generation. As part of an effort to determine if these floral-based feeding attractants might be used in an attract and kill technology to manage populations of cabbage loopers in agricultural crops, we evaluated a proto-type killing station in combination with a floral lure, using a flight tunnel bioassay. We report here results of tests designed to determine efficacy of such a system not only to lure male and female cabbage loopers, but to kill a high percentage of moths that are attracted to the lure. We also compared attraction rates of moths that were fed sugar during rearing to moths that were starved.

MATERIALS AND METHODS

Killing stations: Killing stations were adapted from what of Dr. Everett Mitchell (Deceased - USDA-ARS, Gainesville, FL) and Landolt (2002) first developed, these consisted of: (1) a white cone shaped target simulating a flower for the moth to contact, (2) a chemical lure at the base of the cone, and (3) a killing agent applied to the cone surface, all integrated into a unit (Figure 1). The cone shaped target is a badminton birdie, also known as a shuttlecock. This is a white plastic cone shaped lattice measuring 7.6 cm tall and with a 6.5 cm wide opening. The original red rubber base of the shuttlecock (2.5 cm wide) was replaced with a 4 ml polypropylene vial (Nalgene 2006-9025, Fisher Scientific, Pittsburg, PA) as the dispenser for the attractant. The vial was loaded with 1 ml of the feeding attractant lure, phenylacetaldehyde purchased from Sigma-Aldrich Chemicals (Milwaukee, WI). The component was added to cotton balls inside the vial with a 10 ml plastic graded syringe. A single 1.0 mm diameter hole was drilled into each vial lid with the use of an electric drill providing the desired release rate of phenylacetaldehyde. The vial was than attached to the base of the shuttlecock, with the lid of the vial facing into the cone. The internal and external surfaces of the cone were coated with Teflon® Hitch Ball Lube grease (Reese Products Inc., Elkart, IN) mixed with 7% by weight technical grade Permethrin® (FMC Corp., Princeton, NJ).

Insect rearing: For experiment one moths used were from a colony started with a single female moth captured mid-March in a black light trap at the USDA-ARS laboratory in Wapato, WA (Yakima County, Washington). For experiment two cabbage looper moths used were obtained from a colony originated from the USDA-ARS laboratory in Gainesville, FL and subsequently maintained at the Department of Entomology, University of Kentucky (Louisville, KY). Eggs laid by females in plastic cups were surface sterilized with a 0.1% bleach solution. Newly hatched larvae were transferred to individual 30.0 ml plastic cups (Solo cup company, Highland, IL) with a small piece of artificial Lima bean diet for Lepidoptera (Southland Products, Lake Village, AR). Larvae were reared at ± 22° C., 60% H.R. to the pupal stage. Pupae were removed from cups, separated by gender, and placed in cages (20.0 cm x 20.0 cm x 20.0 cm) for moth emergence. Water and sugar-water was made available for emerging moths as soaked cotton balls in open plastic Petri plates. Three male cabbage looper moths were placed in a 16 oz. plastic cup with 1 female cabbage looper (water and sugar-water provided) for mating and to obtain eggs for rearing.

Attraction on flight tunnel: Flight tunnel experiments compared attraction responses and mortality rates of unmated female and male cabbage loopers in the presence of killing stations, and assessed differences in attraction when moths were provided with sugar prior to testing, compared to starved moths. The wind tunnel was made of Plexiglas measuring 0.8 m x 0.8 m x 1.8 m (Figure 2). The air flow was set at 0.22 ± 0.02 m/s. Air flow was measured with the release of a small quantity of smoke inside the tunnel, and the time spent for the smoke to travel 1.0 m inside the tunnel. Air was pulled trough the tunnel with a squirrel cage fan and a blower motor. Wind tunnel floor was motionless. Room temperature and humidity were 25.0 ± 2.0 °C and 55.0 ± 3.0% R.H. Tunnel air was vented outside the room and a filter was secured over the effluent duct of the tunnel to remove exhausted odorant. Pupae were moved daily to new cages to provide moths of separate age groups. All moths used were 2 or 3 days old, and unmated. Males and females were held in separated cages. Moths were placed in the flight tunnel room 1 hour before the experiments begun so that insects could get acclimated to conditions before experiments started. Individual moths were placed in a 20.0 ml polystyrene vial with an open end and a screened end. One at a time, vials were hung horizontally on a metal stand near the center of the downwind end of the wind tunnel, positioned with the open end of the vial upwind. Each moth was observed for 3 minutes after introduction into the tunnel and was captured and held for 24 hours in a capped 24 ml polystyrene vial following assay. The following factors were observed and scored: upwind flight, plume tracking, contact with the source and mortality after contacting killing stations. Mortality was scored 24 hours after moths had contacted the killing-stations. Release rate of phenylacetaldehyde was assessed in laboratory with the use of a Super Q trap system (5 x 500 µl $MeCL_2$).

Cabbage looper Moth responses were evaluated for the following treatments: (T1) unmated female cabbage looper moths held without sugar prior to testing in response to a blank control, (T2) unmated female cabbage looper moths held without sugar in response to a killing station with a phenylacetaldehyde lure, and (T3) unmated female cabbage looper moths provided sugar prior to testing, in response to a killing station loaded with the phenylacetaldehyde lure. These tests were repeated for males. Six replications with five moths per replicate were conducted. The tunnel was aired between sets with the blower on for at least 12 hours to avoid tunnel contamination with the floral attractants. After data was arcsine transformed, treatment comparisons and data analyses were performed with an Analysis of Variance followed by a L.S.D. test to determine differences between treatments, for both genders. Mortality comparisons between treatment 1 and 2 were performed with a t-test. A second experiment was also conducted to compare attraction of cabbage looper moths to shuttlecock killing stations coated with the Teflon® grease, versus the killing station not coated with the grease. This experiment was conducted to assess effects of Teflon® grease in female cabbage looper moths attraction and contact rate to killing station. After data was arcsine transformed, treatment comparisons and data analyses were performed with an ANOVA test followed by an L.S.D. test for mean separation.

RESULTS

A significantly greater number of starved female cabbage looper moths were attracted to, and contacted, the killing-stations compared to the control (killing station not baited with feeding attractants) (Table 1, n=6, f=55.59, $p<0.01$ for attraction and f=84.0, $p<0.01$ for contact). Significant contact and attraction responses were also seen for fed female moths (Table 1, n=6, f=9.31, p=0.01 for attraction and f=7.50, p=0.02 for contact). When comparing starved versus fed moths, a significantly greater number of cabbage looper female moths that were not provided with sugar were attracted to, and contacted the killing-stations compared to female moths that were provided with sugar (starved) (Table 1, n=6, f=8.78, p=0.01 for attraction and f=5.00, p=0.05 for contact).

Similar results were obtained for male cabbage looper moth responses to killing stations in the flight tunnel. A greater number of starved males were attracted to, and contacted, the killing stations compared to the control (Table 2, n=6, f=45.45, $p<0.01$ for attraction and f=35.59, $p<0.01$ for contact). Male alfalfa looper moths that were provided with sugar had significantly greater attraction and contact rates in response to the killing stations, when compared to control (Table 2, n=6, f=15.0, $p<0.01$ for attraction and f=5.00, p=0.05 for contact). The number of starved male alfalfa looper moths attracted to, and contacting the killing stations was significantly greater than the number of fed males attracted to killing stations (Table 2, n=6, f=17.5, $p<0.01$ for attraction). However, no differences were observed in the numbers of male cabbage looper moths that contacted the stations (Table 2, n=6, f=2.36, p=0.16 for contact).

A mortality rate of 96.7% for all starved female cabbage looper moths that contacted killing stations was observed, and 40.0% of tested starved female moths contacted killing stations. For starved male moths a mortality rate of 73.3% that contacted killing stations was observed, and 37.0% of tested starved male moths contacted killing stations. The mortality rate of female moths tested to killing stations in the wind tunnel was significantly greater with starved rather than fed moths (Figure 3, n=6, t=2.71, p=0.02). The mortality rate of female moths contacting killing stations was not significantly different when those moths were starved or fed sugar (Figure 3, n=6, t=1.46, p=0.10). The mortality rate of all male cabbage looper moths tested was not significantly different when comparing starved versus fed moths (Figure 4, n=6, t=0.93, p=0.20). Mortality rate of male moths that contacted the killing stations was not significantly different when comparing fed male moths versus starved male moths (Figure 4, n=6, t=0.54, p=0.31).

No differences were observed in the number of female cabbage looper moths that were attracted to, and contacted, shuttlecock killing stations when comparing killing stations coated with Teflon® grease (T1) versus killing stations not coated with Teflon® grease (T2) (Table 3, n=6, t=0.79, p=0.23 for attraction, and t=0.65, p=0.27 for contact).

DISCUSSION

Our results indicated that female cabbage looper moths attracted by the floral lure (phenylacetaldehyde) can be killed by contacting the Teflon® grease (containing the killing agent) coating in the shuttlecock killing stations. Results of flight tunnel tests also confirmed the attraction of cabbage looper moths to phenylacetaldehyde. The 40 % overall response was slightly lower than the 55 % average obtained by Landolt et al. (1991). Similar attraction and mortality rates were observed for male cabbage looper moths. The 39% average attraction rate observed for male cabbage loopers is also slightly lower than the 55.0% rate observed by Haynes et al. (1991). The difference in attraction could be due to several different experimental conditions or due to the moths itself. Mortality rates of moths that contacted killing stations were high for starved moths: 96.7% for females and 73.3% for males. These results are encouraging and are similar to results obtained by Camelo et al. (in preparation) in a similar study evaluating alfalfa looper *Autographa californica* (Speyer) moths, responses to floral lure baited killing stations.

The Teflon® grease coating in killing stations did not have a repellent effect on female cabbage looper moths during flight tunnel assays. Moths are as likely to be attracted to, and contact killing stations, with or without the coat of grease. However, moths attracted to the killing station not coated with the Teflon® grease (containing Permethrin®) spent a longer amount of time inside shuttlecocks cones, and landed more times when compared to time spent inside shuttlecock cones when the Teflon®

grease was present. The grease might have a role in the reduction of contact rate compared to attraction rate. Moths are being attracted in high percentages but contact at lower percentages, reducing the total number of moths that are killed by the killing stations. These assessments need to be further investigated and, even though mortality rates observed were acceptable, alternative methods of delivering the pesticide might increase the moth mortality rate. Further flight tunnel assays to quantify amount of time spent at killing stations and number of touches, comparing grease-coated killing stations versus grease free killing stations, would provide the necessary information on efficiency of the grease in delivering the killing agent.

Female and male cabbage looper moths demonstrated autotomy of thoracic legs, a sign of poisoning by Permethrin®, and evidenced by cabbage looper moth legs found unattached to the insect abdomen inside observation vials. These occurred within the 24 hour period but were not evidenced in all specimens tested. According to Krupke et al. (2002) in codling moths *Cydia pomonella* this symptom increases after 72 hours after contacting the pyrethroid. Self-amputation has been proposed by other researches as an adaptive trait to avoid uptake of toxins (Moore et al. 1989). Another symptom of poisoning by a pyrethroid was the rapid and intense shaking of thoracic legs, wings and antennae. These symptoms are in accordance with published accounts of general effects of pyrethroids (Coats, 1982).

The observed decrease in attraction rate of moths due to the feeding status was expected (starved compared to fed). However, according to Haynes et al. (1991), Landolt et al. (1991), Hitchcox (2000), and Landolt and Higbee (2002) there is no indication to date that the responsiveness of female Lepidoptera to feeding attractants is limited to early or late in life. For other noctuid species unmated females and mated females with eggs readily respond to the feeding attractant lures in laboratory and field studies (Smith, 1943), making the use of feeding attractants appealing for novel pest management strategies. Male sex pheromone odors are believed to be similar in chemistry to floral scents (Lenczewski and Landolt, 1991). Males may deceptively employ a scent based on host on food plants to attract females as a mating strategy (Krebs and Dawkins, 1984). Landolt et al. (1996) demonstrated that attraction in a wind tunnel by male and female cabbage loopers to male hairpencil extracts (male sex pheromone source) also increased in starved versus fed moths. A drawback of the use of this chemistry for pest management is that floral lure based technologies would be susceptible to competition from natural odors present in the environment (flowers, and others).

The benefits of sugar for cabbage looper moths and other Noctuid species are well known. Fecundity of cabbage looper moths is increased significantly by availability of water, sugar and other sources of food (Landolt, 1997). Sugar feeding is an integral aspect of Lepidoptera biology, possibly increasing mobility, migration capabilities, and others. Sugar feeding in moths is not limited to females, with both genders known to visit flowers of different plants (Cantelo and Jacobson, 1979). These proven benefits of sugar feeding are evidence that moths of different species would visit and contact killing stations deployed in field settings. A possible drawback of floral attractant based pest management tools is that, they may be less effective in when sugar sources in readily available. This may be flowering crops, weeds, insect honeydew, extra-floral nectarines, or fruits (Norris, 1935).

Recently developed attract and kill technology has shown efficacy in the control of a number of important Lepidopteran pests including the pink bollworm, *Pectinophora gossypiela* (Saunders) (Hofer et al, 1996), the light brown apple moth, *Epiphyas postvittana* (Walker) (Suckling and Brockerhoff, 1999), and the codling moth, *Cydia pomonella* (L.) (Charmillot et al., 1997, and Charmillot et al, 2000). All of these studies used sex pheromones as a lure, causing mortality to the males and thereby reducing the male to female sex ratio with the goal of impeding female mating success. Feeding attractant based attract and kill systems like the one described here affect both sexes, with the female being the primary target. Examples of lure and kill systems described in the literature include those for apple maggot R*hagoletis pomonella* (Bostanian and Racette, 2001), and *Diabrotica* beetles (Metcalf et al. 1987). The primary advantage emphasized in these studies is the attraction of both sexes when using feeding attractants. An attract and kill system tested in the laboratory for use against the cabbage looper, *Trichoplusia ni* (Hübner) (Landolt et al. 1991) showed attraction and mortality rates similar to that of our experiments. Field experiments using feeding attractant baited killing stations against Lacanobia fruitworm, *Lacanobia subjuncta* (Grote and Robinson) significantly reduced the numbers of female moths captured in monitoring traps (Landolt, 2002). An advantage to this system is that the shuttlecock killing station provides chemical are as well as a visual target to attract cabbage looper moths. This species is active at dusk, and uses chemical and visual stimulus when foraging at flowers.

After contacting the killing stations female and male cabbage looper moths flew randomly inside the flight tunnel for 60 to 120 seconds, before being captured with the use of a plastic vial. These moths did not die immediately and were held 24 hours to document mortality. This behavior prevents the retrieval of dead moths that contacted the shuttlecock killing stations in the filed, making it difficult to assess mortality rates due to deployment of killing stations.

The 1.0 mm hole size in top lid of vials provided a release rate of 2.07 ± 0.21 μg/h of phenylacetaldehyde. In previous studies, using the same dispenser release system, a 43 μg of phenylacetaldehyde/hour rate was observed when using a 3.0 mm diameter hole in the field, and a 97 μg of phenylacetaldehyde/hour rate when using a 6.3 mm diameter hole (Landolt et al., 2001). In comparison, the release rates of phenylacetaldehyde from flowers range from 4 to 5 μg/day for *Abelia grandiflora* (Haynes et al. 1992), and 52.8 ± 4.5 ng/hour for night-blooming Jessamine (Heath et al., 1992). Even though measured release rates from flowers are lower, shrubs of either species in those studies may possess numerous flowers emitting odorants at the same time. Increasing trap catches with increasing phenylacetaldehyde release rate was also noted by Landolt et al., 2001 and 2006, using the same vial dispenser system. This release system and release rates were effective in a field tested attract and kill system against alfalfa looper moths (Camelo et al. in preparation), and a laboratory tested attract and kill system against the cabbage looper moths (Camelo et al., in preparation[b]).

Although some adjustments to the killing stations are possible, we are encouraged with the results observed. Some changes that might improve the killing station efficiency against cabbage loopers include changes in lure release rate, improvement of the lure blend, an alternative insecticide delivery method, enhancement of the visual attractiveness to cabbage loopers, and others. Some flaws of the design and the use of plant floral volatiles incorporated in an attract-and-kill system include the attraction of beneficial insects such as bumble bees, *Bombus spp.,* honey bees *Apis melifera,* and sweat bees (Halictidae). Although the attraction of females is a great advantage of using plant derived odorants, these scents might be competing with natural volatiles present in the environment when killing stations are deployed in the field. We believe this system might be useful for managing cabbage looper moths and other insect pest species that are attracted to phenylacetaldehyde. As other plant and/or flower derived inset attractants are developed this killing station can be adapted to target more pests.

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Figure 1: Killing stations, a shuttlecock (badminton birdie) combined with 8.0 ml Nalgene™ vial.

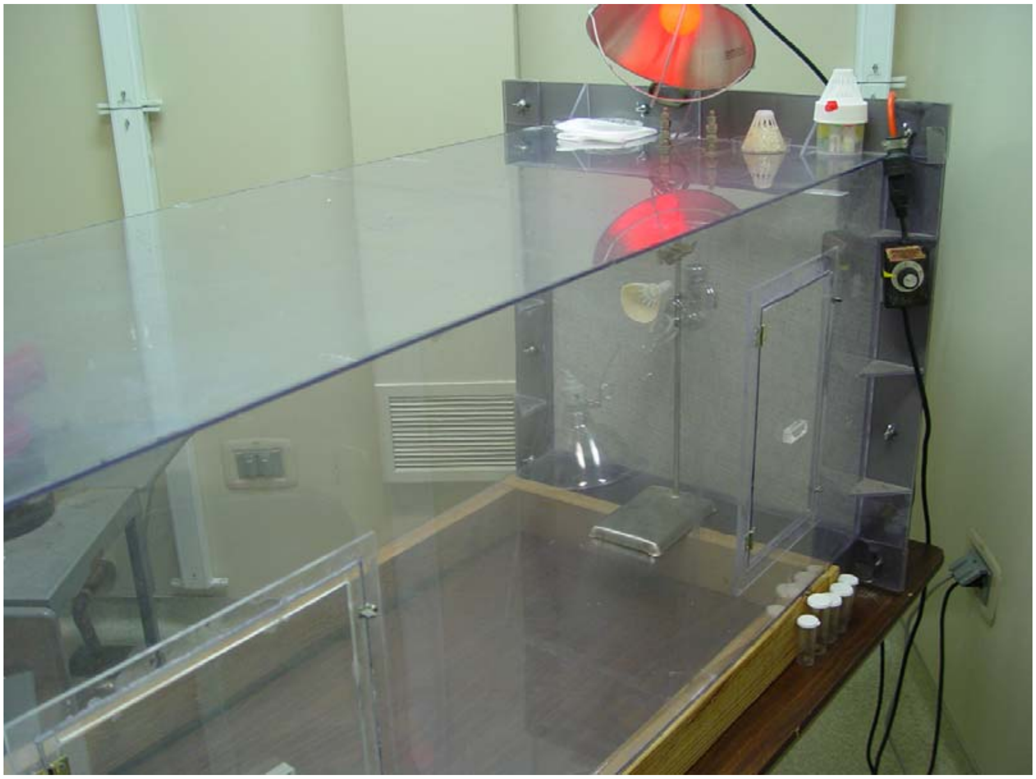

Figure 2: Plexiglas flight tunnel.

Table 1 – Mean (± S.E.M.) percentage of female cabbage looper moths that were attracted to and contacted shuttlecock killing stations loaded with floral chemical lure. Comparison between control, moths that were provided sugar, and moths that were not provided sugar before the test.

	% plume tracked				% contacted source		
Treatment comparisons	n[1]	x + SEM	f[2]	p[2]	x + SEM	f[2]	p[2]
(T1) No lure	6	0.0a	24.21	<0.01	0.0a	15.0	<0.01
(T2) Moths not fed sugar	6	77.0 ± 6.15b			40.0 ± 5.16b		
(T3) Moths fed sugar	6	36.7 ± 12.0c			20.0 ± 7.30c		

[1] 5 moths tested per replicate.

[2]ANOVA test

Means followed by the same letter (a, b) within a column are not significantly different (n=6, df= 17, p= 0.05, L.S.D test). T2 and T3 shuttlecock killing station loaded with tested lure and insecticide.

Table 2 – Mean (± S.E.M.) percentage of male cabbage looper moths that were attracted to and contacted shuttlecock killing stations loaded with floral chemical lure. Comparison between control, moths that were provided sugar, and moths that were not provided sugar before the test.

		% plume tracked			% contacted source		
Treatment comparisons	N[1]	x + SEM	f[2]	p[2]	x + SEM	f[2]	p[2]
(T1) No lure	6	0.0a	28.21	<0.01	0.0a	16.24	<0.01
(T2) Moths not fed sugar	6	67.0 ± 9.89b			37.0 ± 6.15b		
(T3) Moths fed sugar	6	20.0 ± 5.16c			20.0 ± 5.00b		

[1] 5 moths tested per replicate.

[2]ANOVA test

Means followed by the same letter (a, b) within a column are not significantly different (n=6, df= 17, alpha= 0.05, L.S.D test). T2 and T3 shuttlecock killing station loaded with tested lure and insecticide.

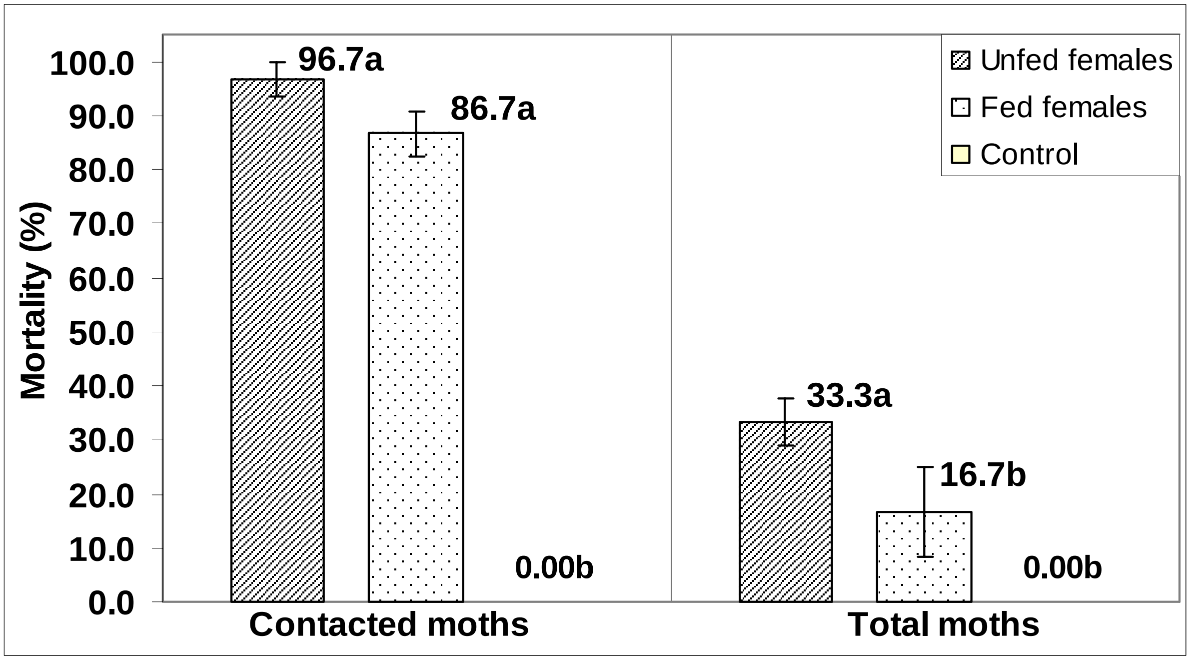

Figure 3 - Mean (± SEM) mortality rate of cabbage looper female moths attracted to shuttlecock killing stations loaded with floral chemical lure and insecticide. The mortality rate of moths that contacted killing station and mortality rate of all moths tested are presented. Means followed by the same letter (a, b) are not significantly different (n=6, p=0.05, L.S.D. test).

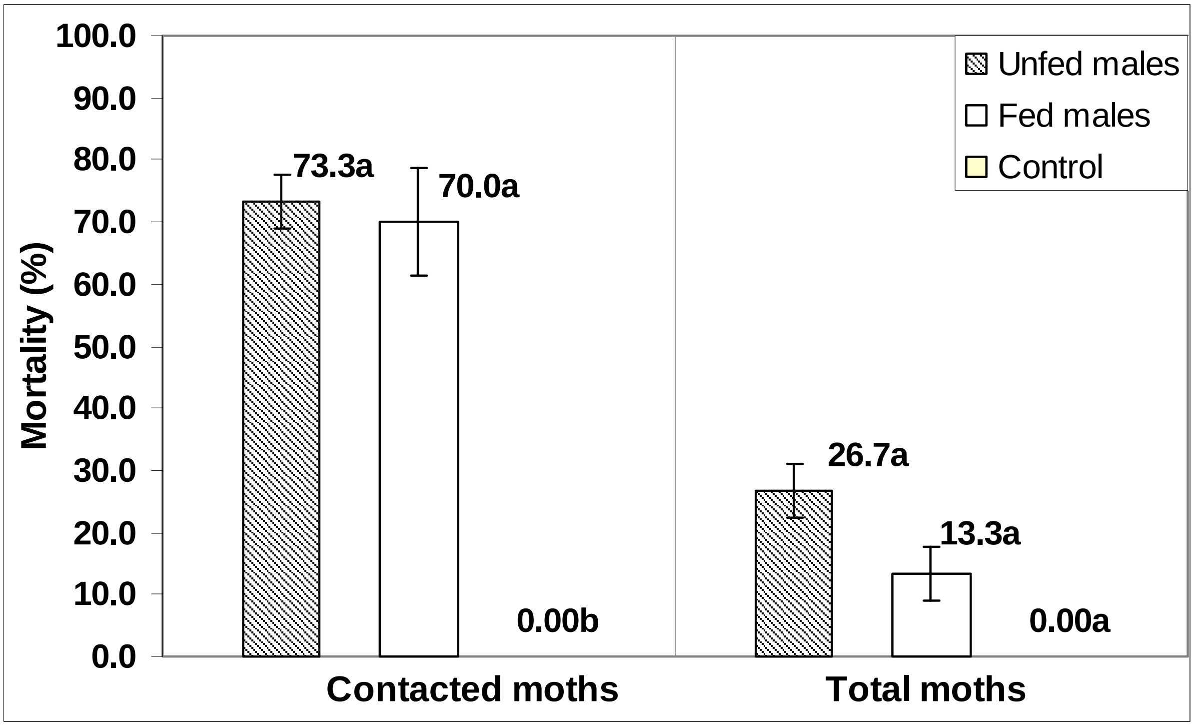

Figure 4 - Mean (± SEM) mortality rate of cabbage looper male moths attracted to shuttlecock killing stations loaded with floral chemical lure and insecticide. The mortality rate of moths that contacted killing station and mortality rate of all moths tested are presented. Means followed by the same letter (a, b) are not significantly different (n=6, p=0.05, L.S.D. test).

Table 3 – Mean (± S.E.M.) percentage of female cabbage looper moths that were attracted to and contacted shuttlecock killing stations loaded with floral chemical lure. Comparison between killing stations coated with Teflon® grease, versus not coated with Teflon® grease.

		% plume tracked			% contacted source		
Treatment comparisons	n[1]	x + SEM	t	p	x + SEM	t	P
(T1) No grease	6	80.0 ± 10.33	0.79	0.23	67.0 ± 8.34	0.65	0.27
(T2) Grease	6	87.0 ± 4.22			57.0 ± 9.55		

[1] 5 moths tested per replicate.

(n=6, alpha= 0.05, t-test).

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Chapter 4

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Field and laboratory tests of floral lure baited killing stations for an attract and kill system against the corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae).

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CHAPTER IV

Field and laboratory tests of floral lure baited killing stations for an attract and kill system against the corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)

ABSTRACT

We have developed a floral chemical lure of compounds derived of "moth-visited" flowers, which attracts both male and female corn earworms. A combination of phenylacetaldehyde and methyl-2-methoxy benzoate is dispensed from polypropylene vials that provides a controlled release rate of the attractant for extended periods of time. Two different designs of killing stations were tested in corn fields for use in combination with this lure as an "attract and kill" system. One consisted of a shuttlecock badminton birdie, while the second was a flat wax-panel fabricated by Suterra LLC. (Bend, OR). Both contained a lure dispenser. Wax-panel killing stations did not significantly reduce captures of female and male corn earworms in monitoring traps. When using the badminton birdie based killing station, a significant reduction in number of female corn earworm moths captured with blacklight baited trap was observed, when comparing treated (125 killing stations per hectare) and control (0 killing stations per hectare) plots. No statistical difference was observed between treatments in the number of male corn earworm moths captured with the use of blacklight baited monitoring trap. The attraction rate (plume tracking) for corn earworm moths flying towards a floral derived lure was 30.0%, with a contact rate of 5.71%, in wind tunnel studies.

INTRODUCTION

The corn earworm is a serious pest on a number of economically important crops in North America. *Helicoverpa zea* (Boddie) is distributed from southern Canada southward to Argentina and Chile and was recently introduced into Hawaii (Crumb, 1956, and Hardwick, 1965, 1996). The most common method of managing corn earworm in non-organic field crops is the use of foliar insecticide applications, which generate issues such as worker safety concerns, environmental contamination, and food residue hazards, among others. These concerns have and restrictions in the use of certain insecticides as a result of the Food Quality and Protection Act have led researchers to investigate alternative methods of managing insect pests that reduce the amount of conventional insecticides applied, with the ultimate goal of lessening environmental and health related concerns.

The use of attractants as a management strategy has a long history (Dethier 1947, Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, Charmillot et al. 2000). A method that relies on insect attractants to manage pests is attract-and-kill, where insects are attracted to a source where they contact a lethal dose of a killing agent. The killing agent of attract and kill, also known as lure and kill, may include sticky materials, insecticides or pathogenic microorganisms (Pedigo, 2002). Other management systems that use insect attractants include mass trapping, poisoned baits, and mating disruption, with much research effort on mating disruption and the use of poisoned baits (Shorey 1976, Lloyd et al. 1981, Roelofs 1981, Villavaso et al. 1998, and Charmillot et al. 2000). Poisoned baits have been defined as a formulation that combines an edible substance with a pesticide (Pedigo 2002). Mass trapping and lure and kill, for example, have been previously proved to be an effective method against the boll weevil (Lloyd et al. 1981, Villavaso et al. 1998, respectively), bait sprays effective against tropical fruit flies (Steiner et al., 1961), and lure and kill effective against the codling moth (Charmillot et al. 2000) although, success against other Lepidopteran pests has been somewhat limited. Generally, these methods have been successful in tree fruit, field, and forest crops as well as with stored products and household pests (Lloyd et al. 1981, Roelofs 1981).

Several problems have been observed in attract and kill systems that utilize sex pheromone as an attractant, including a delay of efficacy in reducing larvae numbers in the field, caused by the male only attraction to female sex pheromones (Minks, 1997). Charmillot et al. (2000) demonstrated that in some cases applications should be made at least one week before the flight season, and that the application of sex pheromone based attract and kill systems should occur only in areas where the initial pest population is low and there is sufficient isolation from external sources of infestations. The attraction and management of female adult moths would directly impact the reproductive potential of a pest species, reducing the number off eggs laid, consequently reducing the number of larvae infesting the field.

Floral derived odors are attractive to both female and male adult lepidopteran moths (Grant 1971, Haynes et al. 1991, Heath et al. 1992, Pair and Horvat 1997, Lopez et al. 2000, Landolt et al 2001, and Landolt and Smithhisler 2003), and can be utilized in an attract and kill pest management program. Phenylacetaldehyde is one of the volatile compounds discussed in these studies and has been used as an attractant. This chemical has shown to be especially effective in attracting several moths in the family Noctuidae (Cantelo and Jacobson, 1979), a taxon that includes insect pests such as the corn earworm *Helicoverpa zea* (Boddie), tobacco budworm *Heliothis virescens* (Fabricius), the cabbage looper *Trichoplusia ni* (Hübner), the alfalfa looper *Autographa californica* (Speyer), the celery looper *Anagrapha falcifera* (Kirby) among others, all of which are considered to be economically important (Crumb, 1956, Kogan et al. 1979, and Hardwick, 1965, Berry, 1998). Corn earworm attraction to and contact with flowers was first documented during migration studies that included identification of pollen found on the moths (Lingren et al., 1993 and 1994). Subsequent studies (Shaver et al. 1997, Pair and Horvat 1997, and Lopez et al. 2000) examined volatiles from plants that are moth-visited and showed that the corn earworm is attracted to several chemicals produced in flowers. Lopez et al (2000), Pair and Horvat (1997) demonstrated that combinations of phenylacetaldehyde and other compounds including methyl-2-methoxy-benzoate, both floral volatiles, were particularly strong in attracting both sexes of corn earworms. Strong female attraction of pestiferous insects can be a great asset for the development of attract-and-kill techniques. There is no indication to date that the responsiveness of females moths to feeding attractant lures is limited to early or late in life, with unmated females and mated females with eggs strongly attracted to the lures in laboratory and field according to Hitchcox (2000), and Landolt and Higbee (2002). The annihilation of female moths using an attract-and-kill system can directly reduce the number of eggs decrease the number of larvae in the subsequent generation.

We hypothesized that female corn earworm populations can be reduced by using a killing station that combines a floral attractant (phenylacetaldehyde and methyl-2-methoxy-benzoate) and a killing agent (Permethrin®). Laboratory assays to assess mortality of corn earworm moths when contacted to wax-panel killing stations, wind tunnel assays to demonstrate attraction rates of corn earworm moths to chemical floral lures, and field implementation of two different designs of killing stations using floral based feeding attractants are reported here.

MATERIALS AND METHODS

Killing stations: Two designs of killing stations were tested against corn earworm moths in corn fields in the Yakima Valley (Yakima and Benton Counties, Washington) during the 2004 growing season. Wax-panel killing stations were comprised of a flat shaped panel (20.3 cm by 12.7 cm) coated with a yellow wax, in combination with a floral lure placed inside two vials that served as controlled release dispenser (Figure 1). During the panel fabrication 7% technical grade Cypermethrin® (FMC Inc.) was incorporated into the wax to serve as a killing agent. The panels were developed by Suterra LLC. (Bend, OR). The controlled release dispensers were two 8 ml polypropylene vials (Nalgene 2006-9025, Fisher Scientific, Pittsburg, PA). One vial was loaded with phenylacetaldehyde (5.0 mg) and the second with methyl-2-methoxy-benzoate (1.0 mg) (Sigma-Aldrich Chemicals, Milwaukee, WI). The components were added to cotton balls inside the vials using a plastic, graded syringe. A single hole with a 6 mm diameter was drilled into the lid of each vial, to allow for the desired release rate. Vials containing each component were attached side by side to the wall of the wax panels using a thin wire. Vial lids faced up. Killing stations were hung in corn fields with the use of a hook-shaped wire. Stations were hung just above the ear in corn plants in a grid format 9.15 meters apart from the adjacent killing station. In treated plots 125 killing stations were used per hectare. Experimental units were 2.02 hectares in size.

Shuttlecock killing stations were adapted from Dr. Everett Mitchell (USDA-ARS Gainesville, Florida, Deceased), and from Landolt (2002) previous designs. These consisted of: (1) a badminton birdie which is cone shaped and which simulated a flower, (2) a chemical lure placed at the base, and (3) a killing agent applied to the inside and out of the cone (Figure 2). The station thus consisted of a white plastic cone-shaped lattice measuring 7.6 cm tall and with a 6.5 cm wide opening. The soft rubber base of the shuttlecock was replaced with an 8.0 ml polypropylene vial (Nalgene 2006-9025, Fisher Scientific, Pittsburg, PA) holding the attractant. The vial was loaded with a mixture of phenylacetaldehyde (5.0 grams) and methyl-2-methoxy-benzoate (1.0 gram). The mixture was added to cotton balls inside the vials with a 10.0 ml plastic, graded syringe. A single hole of 6.0 mm in diameter was drilled into each vial lid in order to provide the desired release rate of the volatilized chemicals. The internal and external surfaces of the cone were coated with Teflon® Hitch Ball Lube grease (Reese Products Inc., Elkart, IN) mixed with 7% by weight technical grade Permethrin® (FMC Corp., Princeton, NJ). Killing stations were placed in the field by manually hanging them with the use of hook-shaped wire in corn plants just above the ear in a grid format 9.15 meters apart from the adjacent killing station. In treated plots 125 killing stations were used per hectare. Experimental units were 2.02 hectares in size.

Plot monitoring and data collection: Monitoring of corn earworm adults monitoring during trials was accomplished with two sex pheromone baited traps, two feeding attractant baited traps, and one blacklight trap in each experimental unit. For males, Universal moth traps or Unitraps™ with green lids, yellow cones and white buckets (Figure 3) loaded with a commercial corn earworm sex pheromone (Trece Inc., Adair, OK) applied to a dark gray rubber septum (West Co., Lionville, PA, cat. no. 1060-0275) were used. Pheromone lures were placed in a small plastic basket located at the center of the interior on the top part of the trap. For better preservation of collected specimens a 3.0 cm^2 piece of Vaportape® (Hercon Environmental, Emigsville, PA) and a 12.0 cm x 12.0 cm piece of two-ply toilet paper were placed inside of each bucket trap. The Vaportape® was stapled to the internal wall of the bucket. Monitoring of female moths was accomplished using Universal Moth Traps loaded with feeding attractant lures. Lures consisted of two 8.0 ml polypropylene vials. Polypropylene vials were constructed and loaded as described previously. A single hole of 6.0 mm in diameter was drilled into each vial lid in order to provide the desired release rate of the volatilized chemicals. This hole size provided a release rate of approximately 43 μg per hour (Landolt et al. 2001). Vials were attached to the internal wall of the bucket trap with a thin wire that held the lure in the upright position. A single 8 watt blacklight (Elko Ltd. F8T5/BL, Top Bulb, East Chicago, IN) monitoring trap was placed in the center of the plot, by hanging it on a metal pole with the light just standing above the corn ear (1.50 meters). Traps were powered by a 12-volt battery that was replaced every 2 to 3 days with recharged batteries. To facilitate counting and identification of specimens, traps were emptied every second day during the trials, where killing stations where implemented for 20 days. Captured specimens were placed into Ziplock® plastic bags (S.C. Johnson and sons, Racine, WI) and transported to the laboratory and stored in a freezer until they were sorted, identified, and separated by gender.

Laboratory assessment of corn earworm moths mortality when contacted to killing stations: Efficacy of wax panel killing stations was tested in the laboratory to determine the mortality rate of corn earworm moths contacting wax panels developed by Suterra®. Corn earworm moths used in this experiment were reared in Laboratory. Colonies were started with eggs obtained from females trapped in black light traps during mid-May. Eggs were surface sterilized with 1.5% bleach and set-up for hatching under room conditions (22° C. ±, 60% H.R.). After eclosion larvae were placed into individual 29.6 ml plastic cups (Solo cup company, Highland, IL) containing a small piece of artificial diet (Southland Products, Lake Village, AR). Second and third instar larvae were transferred into plastic recipients containing 2 inches of soil covered with paper towel. Two pieces of diet were placed on top of paper towels. Larvae were reared throughout the pupa stage. Pupae were removed from the containers, separated by gender and placed into a screen rearing cage (20.0 cm x 20.0 cm x 20.0 cm). Water and a sugar-water mixture were made available to emerging moths. After adult emergence, three male moths were placed in a plastic cup with 1 female (water and sugar-water provided) so that mating could occur. After female moths laid eggs, they were surface sterilized and the rearing process was repeated so that sufficient numbers of adults were obtained for experiments. Moths were reared on a 14:10 scotophase with lights on from 7:00 pm until 9:00 am. Efficacy studies were conducted at room temperature (22 ± 2°C) inside a fume hood. Corn earworm moths were held with forceps and put into contact with wax panel for 5 to 10 seconds. After contact moths were placed in a 20.0 ml glass vial with white plastic lids. Observations were taken at 1, 6, and 24 hours after contact with panel. Moths were assessed for paralysis and death. Ten replicates were conducted for this experiment.

Large scale field trials: We conducted field tests to evaluate both the panel killing station design, and the shuttlecock design as a means to reduce numbers of male and female corn earworm moths present in corn fields. These large scale experiments were conducted in commercial corn fields in Yakima and Benton Counties (Washington State) during 2004. Plots of 2.02 hectares were monitored for corn earworm adults with a single feeding attractant trap beginning in mid-July 2004. Trials started when several moths (5 to 10) were captured in traps per week signaling the start of the corn earworm flight in Central Washington. Each 2.02 hectare plot (square shaped) was divided into 4 quadrants with a monitoring trap was placed in the center of each quadrant. Each plot had 2 sex pheromone traps and 2 feeding attractant monitoring traps. Feeding attractants and pheromone traps were placed in alternated positions. Pheromone traps were placed on the northwest and southeast quadrants. Feeding attractant traps were paced in the northeast and southwest quadrants. Each trap was 73.0 meters from the other trap. Blacklight traps were positioned in the center of the plot (Figure 4). Plots were monitored for 20 days. Corn fields were monitored with a feeding attractant trap, beginning in mid July 2004, to determine the presence of moths for suitable timing of experiments. A set of two replicates was conducted for each killing station design between mid July to mid September 2004, using the same methodology. Treatments consisted of (T1) 0 killing stations per hectare and (T2) 125 killing stations per hectare. Killing stations were deployed at the start of the trial and lasted 20 days.

Experiments were set up as a complete randomized block design with repeated measures. Data analysis was done using Analysis of Variance (ANOVA) with repeated measures. Comparisons were between the numbers of female and male corn earworm moths captured in control plots with 0 killing stations per hectare (T1) against plots treated with 125 killing stations per hectare (T2). We sought to determine if there were significant reductions in numbers of corn earworm moths in plots treated with 125 killing stations per hectare compared to 0 killing stations per hectare (Untreated), for both designs of killing stations.

Flight tunnel assays: Flight tunnel experiments were conducted in order to determine attraction responses of corn earworm moths to shuttlecock killing stations. The wind tunnel used was made of Plexiglas and measured 0.8 m x 0.8 m x 1.8 m. Air flow was adjusted to 0.22 m/s. Air was pulled trough the tunnel with a squirrel cage fan and a blower motor. The wind tunnel floor was motionless. Room temperature and humidity were artificially controlled at 25.0 ± 2.0 °C and 55.0 ± 3.0% R.H. Tunnel air was vented out and a filter was secured to the effluent vent of the tunnel to remove exhaust odorant. Moths used were reared in the laboratory following the methods described previously. All moths used were 2 or 3 days old, unmated, and females. Males and females were held in separated cages. Female corn earworm moths were placed in the flight tunnel room one hour before the experiments were begun so that insects could habituate to conditions before the experiments started. Individual moths were placed in a 20.0 ml polystyrene vial with an open end and a screened end. One at a time, vials were hung horizontally on a metal stand near the downwind end of the wind tunnel, positioned centrally with the open end of the vial upwind. Each moth was observed for 3 minutes after release and was captured and held for 24 hours in a capped 24 ml polystyrene vial. The following factors were observed and scored: upwind flight, plume tracking, and contact with the source. Wind tunnel assays begun daily at 10:00 am and were conducted until 12:00 pm. Wind tunnel room was dark, moths were reared on a 14:10 scotophase with lights on from 7:00 pm until 9:00 am. Fourteen replications with five moths per replicate were conducted. The tunnel was aired between sets with the blower on for 5 minutes and for at least 12 hours, following each day of trials to avoid tunnel contamination with the floral attractants. Treatment comparisons and data analyses were performed with a t-test of the data arcsin converted to determine differences between the control (killing station without lure) and treatment one (killing station with dispenser loaded with phenylacetaldehyde plus methyl-2-methoxy benzoate).

RESULTS

Laboratory assessment of corn earworm moths mortality when contacted to killing stations: Six hours after contact with wax panels, 30.0% of corn earworm moths died, while zero moths in the control group died. Twenty four hours after contact with wax panels 100.0% of corn earworm moths died, while zero corn earworm moths in the control group died (Table 1).

Field efficacy trials of wax panel killing stations: The numbers of female corn earworm moths captured in feeding attractant baited traps in treated plots (125 killing station/hectare) were not significantly different than the numbers of female moths captured in non-treated plots (0 killing station/hectare) (Table 2, n=2, df=3, p=0.16). No males were captured in feeding attractant baited traps in either treated or non-treated plots (Table 2, n=2).

The numbers of male corn earworm moths captured in sex pheromone baited traps in treated plots (125 killing station/hectare) were significantly lower than numbers of male moths captured in non-treated plots (0 killing station/hectare) (Table 2, n=2, df=3, p=0.03).

The numbers of female corn earworm moths captured in blacklight traps in non-treated plots (125 killing station/hectare) were significantly lower than the numbers of female moths captured in treated plots (0 killing station/hectare) (Table 2, n=2, df=3, p=0.02). The number of male moths captured in blacklight traps however, was not significantly different in treated versus non-treated plots (Table 2, n=2, df=3, p=0.08).

The numbers of female corn earworm moths captured daily per blacklight trap in non-treated plots (0 killing stations/hectare) and treated plots (125 killing stations/hectare) are graphically represented in figure 5.

Field efficacy trials of shuttlecock killing stations: the numbers of female corn earworm moths captured in feeding attractant baited traps in treated plots (125 killing station/hectare) were not significantly different than the number of females captured in non-treated plots (0 killing station/hectare) (Table 3, n=2, df=3, p=0.61). Similar results were observed for male corn earworm moths captured in feeding attractant baited traps (Table 3, n=2, df=3, p=1.00).

The numbers of male corn earworm moths captured in sex pheromone baited traps in treated plots (125 killing station/hectare) were not significantly different than the numbers of male moths captured in non-treated plots (0 killing station/hectare) (Table 3, n=2, df=3, p=0.26).

The numbers of female corn earworm moths captured in blacklight traps in treated plots (125 killing station/hectare) were significantly lower than the number of females captured in non-treated plots (0 killing station/hectare) (Table 3, n=2, df=3, p=0.047). The numbers of male moths captured in blacklight traps however, were not significantly different in treated versus non-treated plots (Table 3, n=2, df=3, p=0.09).

The numbers of female corn earworm moths captured daily per trap baited with blacklight in non-treated plots (0 killing stations/hectare) and treated plots (125 killing stations/hectare) are graphically represented in figure 6.

Flight tunnel assays: Corn earworm female moths were significantly more attracted to (plume track flight) a shuttlecock killing station with dispensers loaded with phenylacetaldehyde plus methyl-2-methoxy benzoate, compared to the control (shuttlecock killing station without a lure dispenser) ($n=14$, $p<0.01$, table 4). However, the percentage of moths attracted to the killing stations was of 30.0%. Numbers of corn earworm female moths contacting the shuttlecock killing station were significantly greater in treatment one (killing station loaded with phenylacetaldehyde plus methyl-2-methoxy benzoate) when compared to the control (shuttlecock killing station not loaded with chemical lure) ($n=14$, $p<0.01$, table 4). However, the percentage of moths that contacted the killing station was of 5.71%. A significantly greater number of corn earworm moths were attracted to (plume track flight) the shuttlecock killing stations, than contacted the shuttlecock killing stations ($n=14$, $p<0.01$, table 4).

DISCUSSION

Deployment of wax panel killing stations did not result in a significant reduction of female corn earworm moths in corn fields. Reductions were observed for male moths: the numbers trapped with sex pheromone lures were significantly lower in treated versus non-treated plots. These reductions in males trapped are probably a consequence of mortality of moths attracted to and contacting killing stations. However, these results are not encouraging because the major objective of the deployment of killing stations was to reduce the number of female moths in plots, lowering oviposition and interrupting the reproductive cycle of the corn earworm.

The number of corn earworm moths captured with blacklight traps in plots treated with wax panel killing stations was also not significantly lower for either gender. The number of female moths captured with blacklight traps was significantly higher in treated plots (125 killing stations per hectare) than in non-treated plots (0 killing stations per hectare). Pair and Horvat (1997), and Lopez et al (2000) demonstrated the ability to capture both genders of corn earworm moths with the use of monitoring traps baited with floral derived chemical lures. It is possible that our results demonstrate the power of these feeding attractant lures to attract moths into the experimental plots, evidenced by the increase of moths captured in treated plots. However, it also showed an inability of the wax panel killing stations to produce a significant mortality of corn earworm populations in the plots. That is, the feeding attractant lures may have lured more corn earworm moths into plots than were killed by contact with the panels, providing a net increase in the moth population in the treated plots.

The failure to reduce corn earworm in the field suggests a failure of the panel killing stations to deliver the killing agent or a failure of the stations to elicit contact by moths in the field. Wax panels were tested in the laboratory by manually touching live moths to them. All moths in this test died within 24 hours. However, moths in flight in the field may not have contacted panels because of its flat shape and the positioning of lure dispensers. Additional wind tunnel studies, or field observations of moths approach to the wax panel killing stations, might provide an insight as to why they were not effective at reducing corn earworms populations in our field plots.

Experiments conducted with shuttlecock killing stations demonstrated better potential for reducing female and male moth activity. Table two shows a significant reduction in the number of female corn earworm moths with 125 killing stations per hectare when compared to 0 killing stations per hectare in blacklight traps. The number of female moths were significantly lower in blacklight traps, and reductions in moth captures is interpreted to be evidence of reductions in the female moth population, supporting the hypothesis that reduction of female corn earworms captured is due to mortality of moths contacting the killing stations. Charmillot (2000) and Landolt (1998) used a similar approach to demonstrate reductions for codling moth *Cydia pomonella,* and lacanobia fruitworm *Lacanobia subjuncta* respectively in apple orchards using different attract and kill systems.

It is encouraging that reduction of moths captured in treated plots was observed in the blacklight traps. A reduction only in feeding attractant traps in plots with 125 killing stations per hectare could be interpreted as a disruption of the moths ability to find the feeding attractant lures in the traps, rather than mortality of moths due to contacting the killing stations. Studies conducted by Shorey and Gaston (1965) showed that male cabbage looper *Trichoplusia ni* (Hübner) moths respond stronger to blacklight baited traps rather than chemical lure baited traps when both are positioned close together. Therefore, reductions of female and/or male moths captures after killing stations were deployed is more likely to be a consequence of mortality due to contact with killing stations, than inability of corn earworm moths to find monitoring traps if numbers of moths captured in blacklight traps are significantly reduced in treated plots. It is understood that monitoring traps provide a relative not absolute sample method of moths population within our plots. However, due to moths not dying instantly after contacting killing stations, there is not another suitable sampling method for demonstrating the efficacy of the killing stations deployed in the field.

The number of killing stations per hectare we used (125) is low compared to other attract and kill studies previously described in the literature. For example, 1000 per hectare (Charmillot et al. 2000), and 7500 stations per hectare (Losel et al. 2000), were used against the codling moth. However, Landolt (1998) obtained more effective reductions of lacanobia fruitworm with a similarly low density of killing stations (125 per hectare). These low number of killing stations per hectare may decrease cost and labor of implementation of the attract and kill system.

Corn earworm attraction and contact rate to killing stations was low (30.0 %, and 5.71 % respectively), compared to other wind tunnel studies previously described in the literature for different Noctuidae species attracted to floral volatiles. No flight tunnel studies of corn earworm moths responding to floral attractants are reported in the literature. Tingle and Mitchell (1992) observed an attraction rate of grater than 70.0 % for tobacco budworm *Heliothis virescens* flying towards debracted cotton flowers. Landolt et al (1991) observed an attraction rate grater than 80% for the cabbage looper *Trichoplusia ni* (Hübner) flying towards phenylacetaldehyde in a wind tunnel. Attraction is also evidenced by field trapping experiments that use monitoring traps baited with floral attractants (Pair and Horvat, 1997; Lopez et al., 2000; and Landolt et al., 2006). It is possible that visual and tactile cues are used by these species in order to locate food, rather than chemical cues alone. Further behavioral and physiological studies are necessary to better understand the attraction of these moths to floral volatiles, in order to improve attractions rates and killing station efficacy.

Our primary goal of reducing female corn earworm moths was better achieved with the use of shuttlecock killing stations than with the use of wax-panels killing stations. A similar design was effective against the alfalfa looper *Autographa californica* (Speyer) (Camelo et al., in prep). We expect that a reduction of female corn earworm activity will reduce the number of larvae in the subsequent generation by killing females before they are able to lay eggs. However, experiments need to be conducted to address that hypothesis. Even though the results are somewhat encouraging, additional development of the lures and killing stations are necessary so that better efficacy against corn earworm is achieved.

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Figure 1. Wax panel killing stations combined with two 8.0 ml Nalgene™ vial.

Figure 2: Shuttlecock (badminton birdie) killing stations combined with 8.0 ml Nalgene™ vial.

Figure 3: Universal Moth Trap™ used in field experiments with green lid, yellow cone, and white bucket.

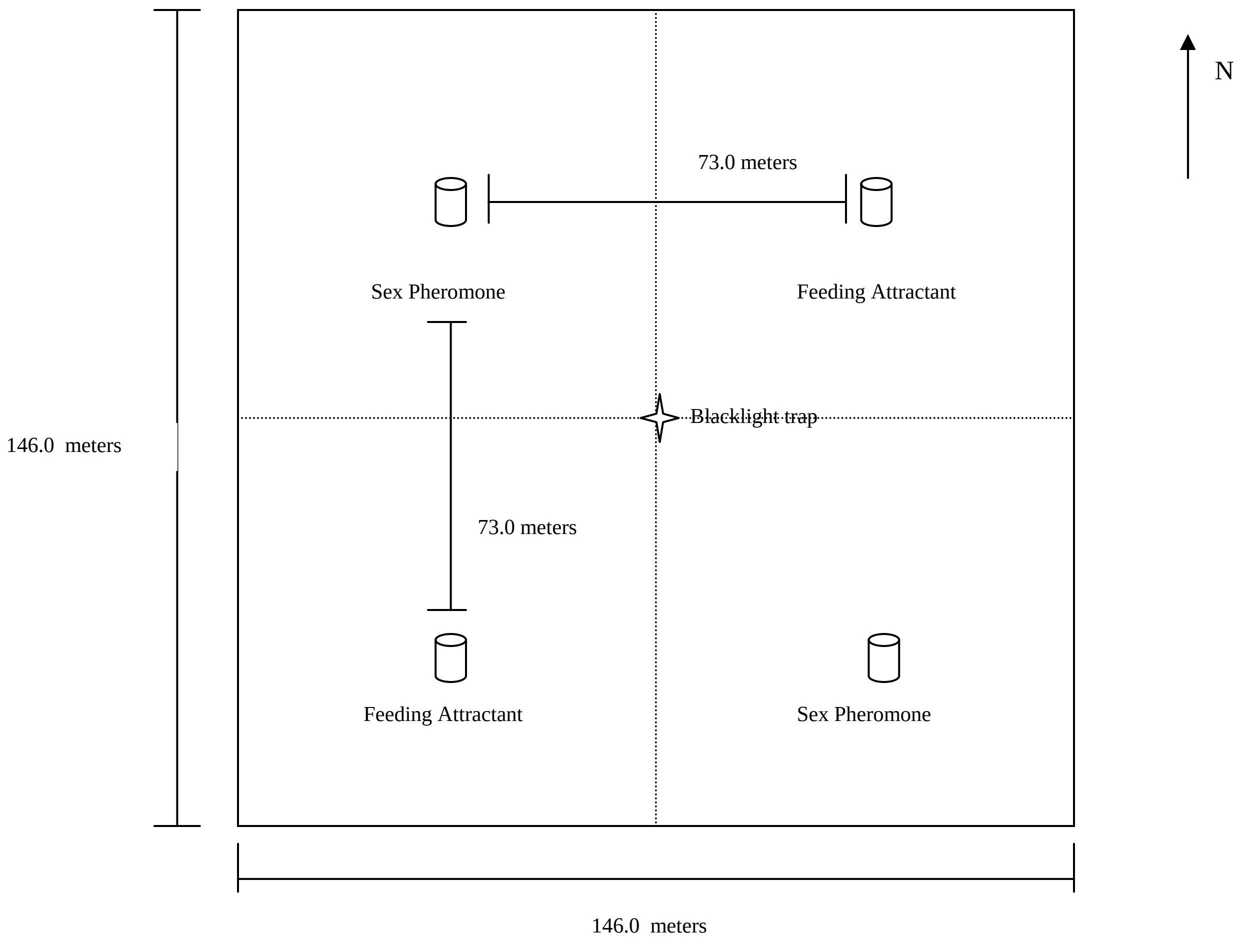

Figure 4: Monitoring traps placement inside plots during killing stations field experiments (2.02 hectare total area).

Table 1 – Mortality rate (%) of corn earworm moths after contacted to wax panel killing stations in Laboratory.

Treatment (n=10)	1 hour	6 hours	24 hours
Control[1]	0.0 %	0.0 %	0.0 %
Wax panel killing station[2]	0.0 %	30.0 %	100.0 %

[1]Moths were held with forceps

[2]Moths were contacted to wax panel killing stations with the help of a forceps

Table 2 - Mean (± S.E.M.) number of corn earworm moths captured per day in corn fields (2.02 hectare plots) treated with wax panel killing stations. Monitoring traps were baited with blacklight, with feeding attractant, and with commercial sex pheromone. Plots were maintained for 20 days in August/September 2004 in Yakima County, WA.

Feeding attractant	**0 killing stations/hec**	**125 killing stations/hec**
Females	0.05 ± 0.03a	0.00 ± 0.00a
Males	0.00 ± 0.00	0.00 ± 0.00
Sex pheromone	**0 killing stations/hec**	**125 killing stations/hec**
Males	2.15 ± 0.30a	1.33 ± 0.23b
Blacklight	**0 killing stations/hec**	**125 killing stations/hec**
Females	0.45 ± 0.17a	1.15 ± 0.24b
Males	3.35 ± 0.42a	5.65 ± 1.20a

Means within a row followed by the same letter (a, b) are not significantly different (p= 0.05, LSD test).

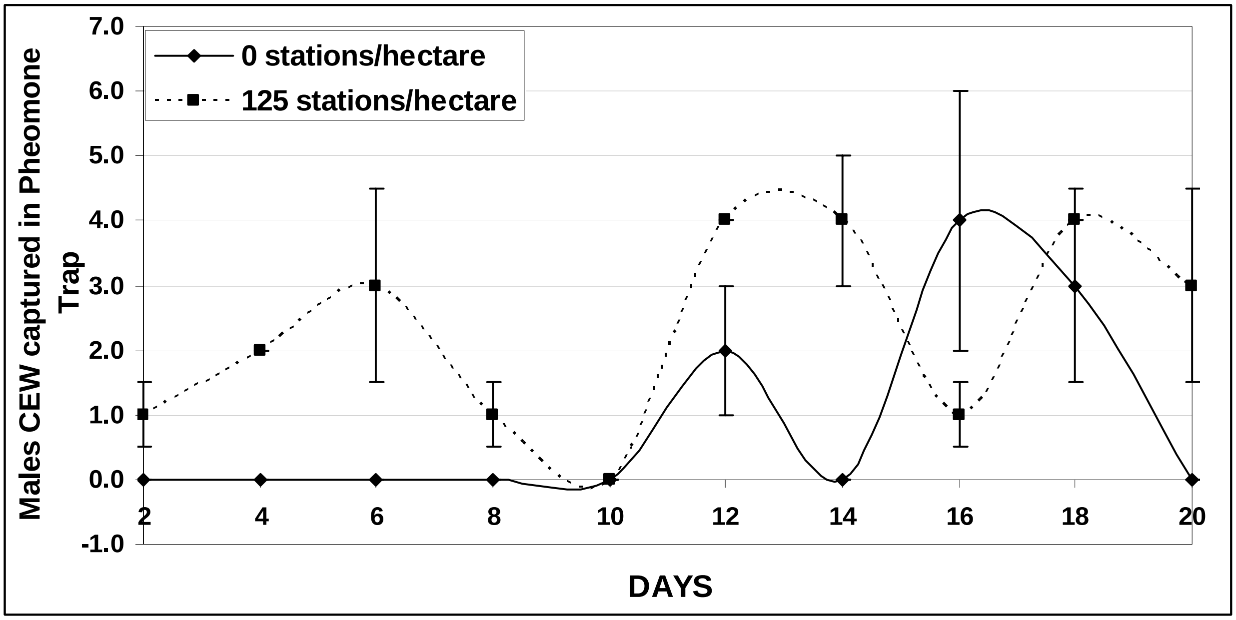

Figure 5 – Mean number (± S.E.M.) of female corn earworm moths captured with blacklight traps during the 2004 growing season. Wax panel killing stations were deployed at the start of the experiment and remained in the field for 20 days.

Table 3- Mean (± S.E.M.) number of corn earworm moths captured per day in corn fields (2.02 hectare plots) treated with shuttlecock killing stations. Monitoring traps were baited with blacklight, with feeding attractant, and with commercial sex pheromone. Plots were maintained for 20 days in August/September 2004 in Benton County, WA.

Feeding attractant	**0 killing stations/hec**	**125 killing stations/hec**
Females	0.23 ± 0.07a	0.18 ± 0.06a
Males	0.10 ± 0.04a	0.10 ± 0.04a
Sex pheromone	**0 killing stations/hec**	**125 killing stations/hec**
Males	1.55 ± 0.33a	1.13 ± 0.18a
Blacklight	**0 killing stations/hec**	**125 killing stations/hec**
Females	1.75 ± 0.41a	0.80 ± 0.20b
Males	8.85 ± 2.19a	4.95 ± 0.56a

Means within a row followed by the same letter (a, b) are not significantly different ($p = 0.05$, LSD test).

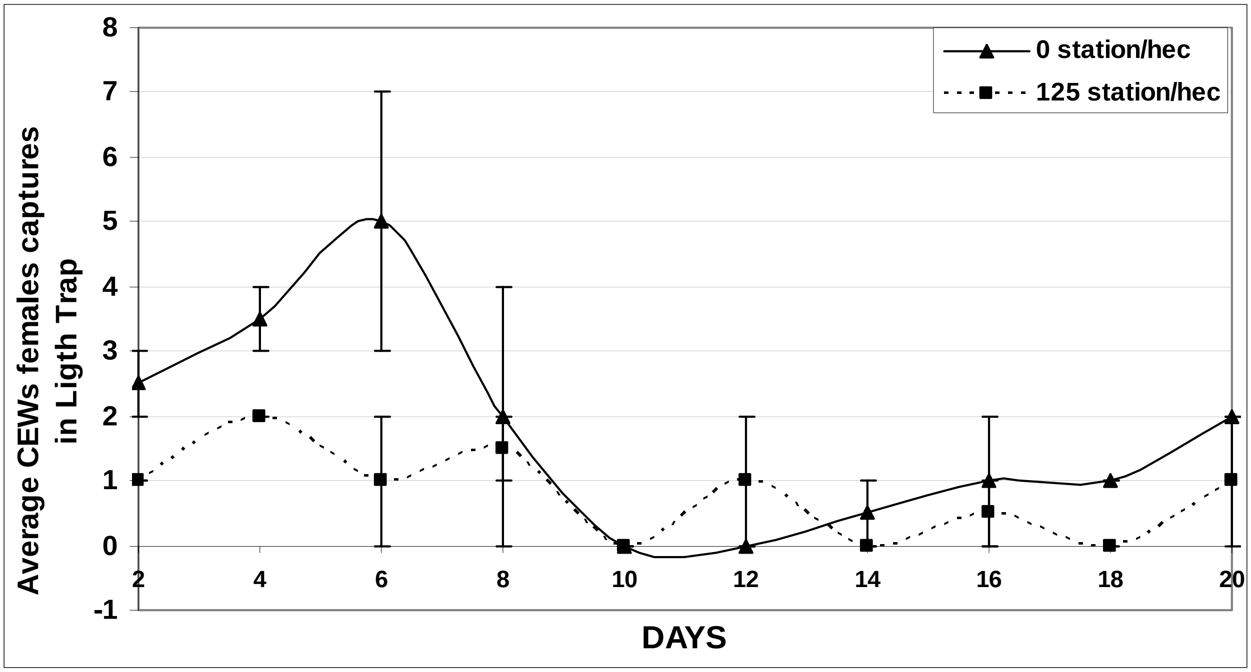

Figure 6 – Mean number (± S.E.M.) of female corn earworm moths captured with blacklight traps during the 2004 growing season. Shuttlecock killing stations were deployed at the beginning of the experiment and remained on the field for 20 days.

Table 4 – Mean (± S.E.M.) percentage of female corn earworm moths that were attracted to and contacted shuttlecock killing stations loaded with floral chemical lure.

		% plume track		
Treatment comparisons	n[1]	Mean (SEM)	t	Pr > t
Untreated[2]	14	0.0 ± 0.0	4.36	0.01
Killing station	14	30.0 ± 6.87		
		% contacted the source		
Treatment comparisons	n[1]	Mean (SEM)	t	Pr > t
Untreated[2]	14	0.0 ± 0.0	2.28	0.03
Killing station	14	5.71 ± 2.51		
	n[1]	Mean (SEM)	t	Pr > t
% plume track	14	30.0 ± 6.87	3.32	0.01
% contacted	14	5.71 ± 2.51		

Means followed by the same letter (a, b) within a column are not significantly different (n=14, alpha= 0.05, t-test). Shuttlecock killing station loaded with tested lure and insecticide. [1] number of replicates with 5 moths tested per replicate. [2] Shuttlecock killing station not loaded with phenylacetaldehyde plus methyl-2-methoxy benzoate.

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Chapter 5

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Improvements to floral odor-based lures for trapping corn earworm and alfalfa looper moths (Lepidoptera: Noctuidae).

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CHAPTER V

Improvements to floral odor-based lures for trapping corn earworm and alfalfa looper moths (Lepidoptera: Noctuidae)

ABSTRACT

This study was conducted to improve field efficiency of floral derived feeding attractants in trapping female and male corn earworm and alfalfa looper moths. For the corn earworm, tests evaluated lure release rates, a variation of lure dispensers, and ratios of components in the lure blend (phenylacetaldehyde with methyl-2-methoxy benzoate). For the alfalfa looper, tests evaluated floral lure release rate (using phenylacetaldehyde with beta-myrcene), purified versus unpurified beta-myrcene in the lure, and lure component ratios. A 25.0 mm diameter vial lid hole provided the most effective release rate for trapping corn earworm moths, and for trapping alfalfa looper moths. No differences were observed for either moth species whether the floral attractants were dispensed in separate or combined vials. When comparing ratios by altering vial hole diameters, more corn earworm moths were captured with phenylacetaldehyde vials dispensers with 3.0 mm diameter holes and methyl-2-methoxy benzoate vials dispensers with 6.0 mm diameter holes, compared to other hole sizes tested. Lastly, numbers of male and female corn earworm and alfalfa looper moths captured during experiments demonstrated that both genders are attracted to floral attractants.

INTRODUCTION

The corn earworm *Helicoverpa zea* (Boddie) is a serious pest on a number of economically important crops in North America. It is distributed from southern Canada southward to Argentina and Chile and was recently introduced into Hawaii (Crumb, 1956, and Hardwick, 1965, 1996). This insect can cause damage to the region's tomatoes, sweet and field corn feeding on the fruits and corn ears respectively. In areas where the corn earworm is not active throughout the year, overwintering occurs in the pupa stage. Sixth instar larvae drop to the ground and burrow into the soil (Metcalf and Metcalf, 1993). Corn earworm however does not overwinter north of the 40th parallel east of the Rocky Mountains, but in the western part of the United States it overwinters as far north as southern Washington State (Eichmann 1940, and Klostermeyer 1968). Even though corn earworm does not overwinter north of the 40th parallel it is still a key pest of vegetables and field crops into southern Canada due to its flight capabilities and its migratory behavior (Hardwick 1965). The most common method of managing corn earworm in non-organic field crops is the use of foliar insecticide applications, which generate issues such as worker safety concerns, environmental contamination, and food residue hazards, among others. These concerns have and restrictions in the use of certain insecticides as a result of the Food Quality and Protection Act have led researchers to investigate alternative methods of managing insect pests that reduce the amount of conventional insecticides applied, with the ultimate goal of lessening environmental and health related concerns.

The alfalfa looper *Autographa californica* (Speyer) is a widely distributed, destructive foliage feeder in North America (Berry, 1998). Damage caused by the larvae includes the destruction of the heads of cole crops to be used as processed foods, and defoliation of potatoes, peas, sugarbeets, alfalfa, beans, mint, spinach and other minor crops (Brewer, 1995). Alfalfa looper distribution includes western United States and parts of western Canada (Metcalf and Flint, 1962). In some locations populations do not reach economic injury level as viral diseases, predators, and parasites lower its numbers (Berry, 1998). Natural enemies, however, may not be sufficient to reduce heavy infestations, particularly in agro-ecosystems. Economic injury thresholds for alfalfa looper vary among cropping systems. Once the economic threshold is reached, foliar insecticide applications that target the early instars are the most common management technique used to prevent damage (Godfrey et al., 2005; Baird and Homan, 1996). Disadvantages of regular insecticide applications as a method of controlling insect pests often include human health hazards, impacts on natural enemies, elevated costs, environmental contamination and residues on food crops.

The use of attractants to aid pest management and monitor insect population activity is not an innovative approach. Attractive materials have been studied as a method for better management of agricultural and urban pests for an extended period of time (Dethier 1947), and monitoring systems have long been in place. The use of sex pheromones for trapping adult male insects is commonly adopted in agricultural settings. Current female sex pheromones available for most Lepidopteran pests attract males only. Field sampling of the female population would provide information on the gender responsible for laying eggs that will ultimately cause damage to crops in the larval stage. Other than the use of blacklight traps, there are no current reliable methods of adult female trapping and sampling for use in agricultural settings.

Floral derived lures that attract lepidopteran pests including those of the family Noctuidae have long been studied. Grant (1971), Cantelo and Jacobson (1979), Haynes et al. (1991), Heath et al. (1992), Pair and Horvat (1997), Lopez et al. (2000), and Landolt and Smithhisler (2003) have described floral odors that are recognized as attractants to both sexes of a handful of Lepidopteran pest species. Phenylacetaldehyde is one of the volatile compounds emphasized from these studies that is an attractant. This chemical is attractive to several Noctuid moths, including agricultural pests such as the corn earworm, alfalfa looper, cabbage looper (*Trichoplusia ni*), celery looper *Anagrapha falcifera*, and the soybean looper *Pseudoplusia includens*, among others. Heath et al. (1992), Lopez el al (2002), Landolt and Smithhisler (2003) demonstrated that moth attraction to flower odorants was not limited to males. Floral lures have been tested in the field and female and male moths of corn earworm, alfalfa looper, and cabbage looper were trapped with the use of Universal Moth Traps™ (Landolt et al. 2001, 2006; Lopez et al 2000). Gender ratio observed in these studies was approximately 1/1. Female adult attraction to floral scents can be explained by the reward of increased reproductive potential when feeding on sugar as demonstrated by Landolt (1997). Strong female attraction can be a great asset for the development of monitoring systems. According to Haynes et al. (1991), Landolt et al. (1991), Hitchcox (2000), and Landolt and Higbee (2002) there is no indication to date that the responsiveness of moths to feeding attractants is limited to early or late in life. For other Noctuid species both unmated females and mated females with eggs readily respond to feeding attractant lures in laboratory and field studies (Smith et al., 1943).

Landolt and Smithhisler (2003), and Landolt et al (2006) demonstrated that the combination of phenylacetaldehyde plus beta-myrcene, both floral volatiles present in *Berberis aquifolium*, were particularly strong in attracting both sexes of adult alfalfa loopers. These studies using single and double component lures found an attraction ratio of 1:2, female to male respectively. Landolt (unpublished data) found similar results for attraction of the corn earworm using the combination phenylacetaldehyde plus methyl-2-methoxy benzoate. Strong female attraction would be a great asset for the development of pest management or monitoring techniques. For the development of management techniques the annihilation of female moths should directly reduce the number of eggs laid, and consequently decrease the numbers of larvae in the subsequent generation.

Even thought floral derived lures that attract Lepidopteran pests have long been described in the literature (Grant, 1971; Cantelo and Jacobson, 1979; Haynes et al., 1991; Heath et al., 1992; Pair and Horvat, 1997; Lopez et al., 2000; and Landolt and Smithhisler, 2003), few efforts have been made to improve or optimize field trapping of female Noctuidae moths with feeding attractant (Landolt et al., 2006). Lure delivery systems, release rates, lure composition, and others, impact attraction rates and consequently affect the number of moths that are trapped. The optimal release rate and component ratios of any given lure needs to be assessed, so that lures are as strong as possible in terms of attraction to moths. Previous field trapping experiments using floral lures used a separate vial for each lure component (Landolt et al., 2001, 2006). Ongoing efforts to develop an attract and kill system combine lure components in one vial. Assessments need to be made to determine differences in attraction between the two delivery methods. Corn earworm floral lure based attract and kill efforts have proven difficult (Camelo et al., in preparation). An increase in number of corn earworm female moths captured using better floral lures might provide more efficient tools for an attract and kill system. Previous work with phenylacetaldehyde and beta-myrcene showed great variance in beta-myrcene enhancement/synergism of alfalfa looper attraction to phenylacetaldehyde. We sought to determine if such variance is due in part to impurities in commercial beta-myrcene.

The objective of this study was to improve field efficiency of previously field tested floral derived feeding attractants (Landolt and Smithhisler, 2003, Landolt et al., 2006), in order to capture higher numbers of both female and male corn earworm and alfalfa looper moths of both genders in monitoring traps. For the corn earworm lure (phenylacetaldehyde plus methyl-2-methoxy benzoate) we conducted a release rate test, lure dispenser method comparison, and evaluation of ratios of phenylacetaldehyde to methyl-2-methoxy benzoate in the lure. For the alfalfa looper lure (phenylacetaldehyde plus beta-myrcene) we compared release rates, compared purified versus unpurified beta myrcene, and assessed a novel lure combination, a mixture of phenylacetaldehyde, beta myrcene, acetic acid, and isoamyl alcohol. Acetic acid and isoamyl alcohol are co-attractants for another group of noctuid moths, based on their responses to fermented sweet baits (Landolt, 2000).

MATERIALS AND METHODS

For all experiments Universal Moth Traps (Unitraps™ Agrisense, Fresno, CA) with green lids, yellow cones and white buckets were used (Figure 1). Traps were baited with floral odorants with the use of 15.0 ml or 8.0 ml Nalgene® vials, depending on the treatments. Individual vials were attached to the internal wall of the bucket trap with a thin wire that held the lure in the upright position. With the use of a 10.0 ml plastic graded syringe the chemical components were added to cotton balls previously placed inside the vials. A single hole drilled into each vial lid provided the desired release rate of the volatilized chemicals. To facilitate counting and preservation of specimens captured, traps were emptied every week. Trap contents were placed into Ziplock® plastic bags (S.C. Johnson and sons, Racine, WI) and transported to the laboratory where they were stored in a freezer. Insects were later sorted, identified, and separated by sex. For better preservation of trapped specimens, a 3.0 cm^2 piece of Vaportape® (Hercon Environmental, Emigsville, PA) and a small amount of tissue paper were placed inside of each bucket trap. The Vaportape® was stapled into the internal wall of the bucket. Traps were hung with the use of metal poles or/and fences at a height of 1.2 meters, at least 12.0 meters apart from adjacent treatments. Phenylacetaldehyde with methyl-2-methoxy benzoate was used as the standard lure for corn earworm moths, and phenylacetaldehyde with beta-myrcene was used as the standard lure for alfalfa looper moths. All lures were dispensed with the use of polypropylene vials (Nalgene®, Rochester, NY 14625). Release of attractants was regulated with different hole sizes on lid of vials. Phenylacetaldehyde (PAA), methyl-2-methoxy benzoate (MMB), and beta-myrcene (BM) were obtained from Sigma-Aldrich Chemicals (Milwaukee, WI).

Experiment 1 (PAA and MMB release rates)

Experiment one was designed to determine the optimum release rate of a floral attractant blend in order to trap the greatest numbers of female and male corn earworm moths. Each 8.0 ml vial was loaded with 5 ml of the feeding attractant lure, a combination of phenylacetaldehyde and methyl-2-methoxy benzoate (Sigma-Aldrich Chemicals, Milwaukee, WI). Treatment variations consisted of different sizes in diameter of hole drilled into the lid, providing different release rates of the volatilized chemicals. Treatments were (T1) 1.5 mm, (T2) 3.0 mm, (T3) 6.0 mm, (T4) 12.0 mm, and (T5) 25.0 mm. This experiment was conducted for three weeks during the 2003 summer season. Each treatment was replicated five times. Treatments were re-randomized every time traps were checked. The experiment was set up as a complete randomized blocked design. Trap blocks were set up adjacent to different corn fields near Mattawa, WA (Grant County, WA). Total numbers of male and female corn earworm moths combined were square root transformed before statistical analysis. Data analysis was done by performing an analysis of variance (ANOVA) of the total numbers of corn earworm moths captured per treatment, in order to determine if there were significant differences in number of corn earworm moths captured by treatment, and followed by an L.S.D. test for treatment means separation.

Experiment 2 (PAA and MMB one vial or 2 vials)

Experiment two assessed differences in numbers of corn earworm moths captured by dispensing the two component floral chemical lure blended together in one vial, compared to releasing each of the chemicals from two different vials. Traps were baited with floral odorants with the use of 8.0 ml Nalgene® vials. Treatments were (T1) a single vial containing both components of the floral lure, and (T2) two vials each containing one component of floral chemical lure. In treatment 1 each vial was loaded with 5 ml of the mixture of phenylacetaldehyde and methyl-2-methoxy benzoate (Sigma-Aldrich Chemicals, Milwaukee, WI), in treatment 2 each vial was loaded with 5 ml of one of the components. This experiment was set up as a completely randomized blocked design, and was conducted for three weeks during the 2003 summer season. Each treatment was replicated ten times. Treatments were re-randomized every time traps were checked Traps were set up adjacent to different corn fields near Mattawa (Grant County, WA). Total numbers of male and female corn earworm moths combined were square root transformed before statistical analysis. Data analysis was done by performing a t-test of the total number of corn earworm moths captured per treatment, in order to determine if there were significant differences in number of corn earworm moths captured by treatment.

Experiment 3 (PAA and MMB ratio)

Experiment three assessed the effects of altering the release rate of methyl-2-methoxy benzoate while maintaining a single release rate of phenylacetaldehyde, on numbers of female and male corn earworm moths trapped. Traps were baited with the floral lure PAA and MMB with the use of two 8.0 ml Nalgene® vials. Each vial was loaded with 5.0 ml of one of the chemicals. Treatment variations consisted of different sizes in diameter of hole drilled into the lid, providing different release rates of methyl-2-methoxy benzoate. All treatments contained an 8.0 ml vial with 3.0 mm hole size for phenylacetaldehyde release and a second 8.0 ml vial for methyl-2-methoxy benzoate release. MMB vial holes were different sizes to provide different release rates, (T1) 1.5 mm, (T2) 3.0 mm, (T3) 6.0 mm, and lastly (T4) 12.0 mm. This experiment was conducted for seven weeks during 2004. Experiment was set up as a completely randomized blocked design with 10 replicates. Treatments were re-randomized every time traps were checked. Traps were set up adjacent to different corn fields near Mattawa (Grant County, WA). Total numbers of male and female corn earworm moths combined were square root transformed before statistical analysis. Data analysis was done by performing an analysis of variance (ANOVA) of the total number of corn earworm moths captured per treatment, in order to determine if there were significant differences in number of corn earworm moths captured by treatment, and followed by a L.S.D. test for treatment means separation.

Experiment 4 (PAA and BM release rate)

Experiment four determined the effects of varying the release rate of the floral attractant for alfalfa looper moths (PAA and BM) on numbers of female and male alfalfa looper moths trapped. Eight ml vials were loaded with 5 ml of attractant.

Treatments were different diameters of holes drilled into the lid, providing different release rates of volatilized attractants. Treatments were (T1) 0.5 mm, (T2) 1.0 mm, (T3) 1.5 mm, (T4) 3.0 mm, and lastly (T5) 6.0 mm. This experiment was conducted for three weeks during the 2003 summer season. Treatments were randomized every time traps were checked. The experiment was set up as a completely randomized blocked design with 5 replicates. Traps were set up adjacent to different alfalfa fields near Prosser (Benton County, WA). Total numbers of male and female corn earworm moths combined were square root transformed before statistical analysis. Data analysis was done by performing an analysis of variance (ANOVA) of the total number of corn earworm moths captured per treatment, in order to determine if there were significant differences in number of alfalfa looper moths captured by treatment, and followed by an L.S.D. test for treatment means separation.

Experiment 5 (PAA and BM higher release rates)

Experiment five repeated the objective of experiment 4, with a higher range of release rates tested in experiment four. Treatments were (T1) 1.5 mm, (T2) 3.0 mm, (T3) 6.0 mm, (T4) 12.0 mm, and lastly (T5) 25.0 mm. Experimental design and data analysis for the experiment was the same for experiment four.

Experiment 6 (PAA and BM, with purified BM)

Experiment six assessed affects of beta-myrcene sample impurities on numbers of alfalfa looper moths trapped. We compared purified beta myrcene and unpurified beta myrcene in the mixture with phenylacetaldehyde. Beta-myrcene was purified on

Agilent's model 1100 series HPLC with automatic fraction collector, using XDB semi-prep C8 column, 9.4 x 250 mm. Mobil phase was 92:8 MeOH: water at 4.0 ml/min. Peak-based fractions were collected. Best fractions were combined and extracted into hexane by partition extraction, concentrated by roto-evaporation and analyzed by GC for purity. The analysis was done on Agilent's 6890 GC using a fused silica DB-1 column (J&W Scientific, Folsom, CA), 0.25 mm x 60.0 m, 250 μm film thickness with temperature program 40°C for 1 min.; 15°C/min to 200°C. Final material was 97,0 % beta-myrcene, 2,9 % phellandrene. Traps were baited with this floral odor blend in 8.0 ml vials with 3.0 mm holes in the lids. Treatments were (T1) one vial with 5.0 ml of phenylacetaldehyde, (T2) two vials, one with 5.0 ml of phenylacetaldehyde and a second with 5.0 ml of unpurified beta myrcene, and (T3) two vials, one with 5.0 ml of phenylacetaldehyde and a second vial with 5.0 ml of purified beta myrcene. This experiment was conducted for 2 weeks during the 2004 summer season and set up as a complete randomized blocked design. Each treatment was replicated ten times. Treatments were re-randomized every time traps were checked. Trap blocks were set up adjacent to different alfalfa fields near Prosser, WA (Benton County). Total numbers of male and female alfalfa looper moths combined were square root transformed before statistical analysis. Data analysis was done by performing a t-test of the total number of corn earworm moths captured per treatment, in order to determine if there were significant differences in number of corn earworm moths captured by treatment, and followed by a L.S.D. test for treatment means separation.

Experiment 7 (PAA and BM with AAIAA)

Experiment seven assessed the affects of combining two types of feeding attractant lures on captures of alfalfa looper moths in traps. This experiment compared the flower derived chemical attractant phenylacetaldehyde with beta myrcene, with or without the fermented sugar derived lure acetic acid and isoamyl alcohol (AAIAA). Attractants were dispensed from 8.0 ml vials with 3.0 ml diameter hole. Treatments were (T1) 5.0 ml of a mixture of phenylacetaldehyde and beta myrcene, (T2) 5.0 ml of a mixture of acetic acid and isoamyl alcohol, and (T3) 5.0 ml of a mixture of phenylacetaldehyde, beta myrcene, acetic acid and isoamyl alcohol. Traps were set up adjacent to different alfalfa fields in Prosser, WA (Benton County). This experiment was set up as a completely randomized blocked design, replicated 10 times, and conducted for two weeks. Treatments were re-randomized every time traps were checked. Total numbers of male and female alfalfa looper moths combined were square root transformed before statistical analysis. Data analysis was done by performing a t-test of the total number of corn earworm moths captured per treatment, in order to determine if there were significant differences in number of corn earworm moths captured by treatment, and followed by a L.S.D. test for treatment means separation.

To assess the male to female ratio of all moths captured, mean numbers of male versus female corn earworm and alfalfa looper moths, were compared for each experiment by performing t-tests.

RESULTS

Experiment 1 (PAA and MMB release rate). Numbers of corn earworm moths captured with phenylacetaldehyde plus methyl-2-methoxy benzoate baited traps were significantly greater in treatment five when compared to all other treatments ($n=15$, $f=5.73$, $p<0.01$, figure 2). The treatment five vial lid hole diameter of 25.0 mm was the largest tested, thus providing the highest attractant release rate. Numbers of corn earworm moths captured with phenylacetaldehyde plus methyl-2-methoxy benzoate were significantly lower in treatment one when compared to all other treatments ($p<0.01$, figure 2). The treatment one vial lid hole diameter of 1.5 mm was the smallest tested, thus providing the lowest release rate of floral attractants. No significant differences were observed in numbers of corn earworm moths captured among treatment two, three and four. ($p=0.12$, $p=0.22$, figure 2). A significantly greater number of male corn earworm moths were trapped during this experiment (35.15 ± 8.44) compared to the number of female corn earworm moths trapped (13.53 ± 3.77) ($t=1.76$, $p<0.01$, table 1).

Experiment two (PAA and MMB one vial versus two vials): Numbers of corn earworm moths trapped with phenylacetaldehyde plus methyl-2-methoxy benzoate were not significantly different when dispensed as a mixture in one vial versus separate vials ($n=30$, $t=1.09$, $p=0.28$, figure 3). No difference was observed in numbers of female corn earworm moths trapped compared to numbers of male corn earworm moths trapped. ($t=1.66$, $p=0.16$, table 1).

Experiment 3 (PAA and MMB ratio): Numbers of corn earworm moths captured with phenylacetaldehyde plus methyl-2-methoxy benzoate increased as the MMB vial hole size increased from 1.5 to 6.0 mm (Figure 4). Numbers of moths were greater in treatment three compared to treatments one (n=70, f=4.73, p<0.01, figure 4), but not to treatments two and four (p=0.07, figure 4). Lastly, no significant differences were observed among treatment one, two, and four (figure 4). A significantly greater number of female corn earworm moths were trapped during this experiment (3.54 ± 0.67) compared to number of male corn earworm moths trapped (1.73 ± 0.42) (t=1.67, p<0.01, table 1).

Experiment 4 (PAA and BM release rate): Numbers of alfalfa looper moths captured were significantly greater in treatment five when compared to all other treatments (n=15, f=3.40, p<0.01, figure 5). The treatment five vial lid hole diameter of 6.0 mm was the largest tested, thus providing the highest release rate. There were no significant differences among other treatments. No difference was observed in numbers of female alfalfa looper moths trapped compared to numbers of male alfalfa looper moths trapped. (t=1.26, p=0.11, table 1).

Experiment 5 (PAA and BM higher release rates): Numbers of alfalfa looper moths were significantly greater with treatment five when compared to treatments one, two and three (n=15, f=9.58, p<0.01, figure 6). No significant difference was observed when comparing treatment five to treatment four (p=0.07, figure 6). Treatment five vial lid hole diameter of 25.0 mm was the largest tested, thus providing the highest release rate. Numbers of alfalfa looper moths captured were not significantly different among treatments one, two, three and four ($p=0.17$, figure 2). No difference was observed in number of female alfalfa looper moths trapped compared to number of male alfalfa looper moths trapped. ($t=1.17$ $p=0.29$, table 1).

Experiment 6 (PAA and BM, purified BM): Numbers of alfalfa looper moths captured were not significantly different when comparing phenylacetaldehyde alone or, with unpurified beta myrcene, or purified beta myrcene ($n=20$, $f=0.13$, $p=0.88$, figure 7). A significantly greater number of male alfalfa looper moths were trapped during this experiment (8.40 ± 0.75) compared to the number of female alfalfa looper moths trapped (5.70 ± 0.66) ($t=1.73$, $p<0.01$, table 1).

Experiment 7 (PAA, BM, and AAIAA): Numbers of alfalfa looper moths captured in traps baited with PAA with BM (treatment 1) and traps baited with the blend of PAA, BM and AAIAA were significantly greater than number of moths captured with AAIAA (treatment 2) ($n=20$, $f=39.44$, $p<0.01$, figure 8). However, there were no significant differences when comparing numbers trapped with the blend of PAA with BM versus PAA, BM, and AAIAA ($p=0.44$, figure 8). No difference was observed in number of female alfalfa looper moths trapped compared to number of male alfalfa looper moths trapped. ($t=1.68$, $p=0.19$, table 1).

The overall ratio for corn earworm moths captured in PAA and MMB traps in these seven studies was 2.15 males to each female. The overall sex ratio for alfalfa looper moths captured in these studies was of 1.29 males to each female.

DISCUSSION

Phenylacetaldehyde and methyl-2-methoxy benzoate are previously known to attract corn earworm moths (Cantelo and Jacobson 1979, Lopez et al. 2000). Both chemicals are released or produced by moth-visited flowers, as well as corn silk (Cantelo and Jacobson 1979, Teranishi et al. 1991, and Schlotzhauer 1996). The combination of phenylacetaldehyde plus methyl-2-methoxy benzoate was shown to enhance corn earworm attraction when compared to phenylacetaldehyde alone (Landolt et al. unpublished data). In these previous studies plastic vials with a hole in the top of the lid provided for the release of volatiles. However, questions regarding release rates, and lure dispensing methods, among others were unanswered. In our study we demonstrated that the use of a 25.0 mm diameter vial lid hole was the most effective among those tested in attracting and trapping corn earworm moths with the PAA and MMB lure. Generally, the larger the size of the hole diameter the greater the release of lure volatiles, and the greater the number of corn earworm trapped. We do not know what release rate provides the maximum optimum trap catch, which may be higher than that provided by a vial with a 25.0 mm hole diameter.

No difference was observed in numbers of corn earworm moths trapped with PAA and MMB dispensed from separate dispensers or together in one dispenser. The results indicate that the use of one vial in the attract and kill station design would not greatly affect attraction rates of corn earworm moths.

A greater number of corn earworm moths were trapped with PAA and MMB in vials with a hole size for the phenylacetaldehyde dispenser of 3.0 mm and for the methyl-2-methoxy benzoate dispenser of 6.0 mm. It is possible that a higher release rate of MMB than that produced by the 6.0 mm hole diameter may provide an even greater trap catch of corn earworm moths. These results indicate that the ratio of components emitted has an important role in the attraction of corn earworm moths.

Phenylacetaldehyde is also known to attract alfalfa looper and cabbage looper *Trichoplusia ni* (Hübner) moths (Cantelo and Jacobson, 1979; Landolt et al., 2001). Landolt et al (2006) also captured alfalfa looper moths in traps baited with phenylacetaldehyde plus beta myrcene. In our study a greater number of alfalfa looper moths were trapped with PAA and BM, when the dispenser hole diameter was 25.0 mm, compared to all other hole size tested. These previous studies (Landolt et al, 2001 and 2006) were conducted using the same method for dispensing the chemicals, using polypropylene vials with 3.0 mm diameter holes in the lids. No differences were seen in the numbers of alfalfa looper moths captured with the use of purified beta myrcene and phenylacetaldehyde versus unpurified beta myrcene and purified beta-myrcene. In this test, beta-myrcene did not enhance alfalfa looper attraction to PAA, in contrast to results of other studies (Landolt et al., 2006). Lastly, the addition of acetic acid with isoamyl alcohol (AAIAA) to phenylacetaldehyde with beta myrcene (PAA+BM) did not increase the number of alfalfa looper moths captured with monitoring traps. Most importantly, no alfalfa looper moths were captured in traps baited with AAIAA. In previous studies of AAIAA, the blend has not proven to be attractive to alfalfa looper or any related Plusiinae species (Landolt and Hammond, 2001 and Landolt et al. in press[b]).

The larger the vial hole diameter, the greater the release of volatilized attractants. Release rates of volatilized attractants from vials need to be assessed in the laboratory in order to determine, with precision, how much of the chemicals (in weight) are being released per period of time. In previous studies, using the same dispenser release system, a 43 μg of phenylacetaldehyde/hour rate was observed when using a 3.0 mm diameter hole, and a 97 μg of phenylacetaldehyde/hour rate when using a 6.3 mm diameter hole (Landolt et al., 2001). In comparison, the release rates of phenylacetaldehyde from flowers range from 4 to 5 μg/day for *Abelia grandiflora* (Haynes et al. 1992), and 52.8 ± 4.5 ng/hour for night-blooming Jessamine (Heath et al., 1992). Even though measured release rates from flowers are lower, shrubs of either species in those studies may possess numerous flowers emitting odorants at the same time. Increasing trap catches with increasing phenylacetaldehyde release rate was also noted by Landolt et al., 2001 and 2006, using the same vial dispenser system. This release system and release rates were effective in a field tested attract and kill system against alfalfa looper moths (Camelo et al. in preparation), and a laboratory tested attract and kill system against the cabbage looper moths (Camelo et al., in preparation[b]).

Numbers of male and female corn earworm and alfalfa looper moths captured during our experiments demonstrate that both genders are attracted to feeding attractants. These results are similar to other feeding attractant trapping experiments for different Lepidoptera species (Lopez et al 2000, Pair and Horvat 1997, Landolt et al 2001, and Landolt et al in press). These results are encouraging due to the fact that attraction of female Lepidopteran pests is a much needed advancement in attractants to be used in aid of pest management and/or monitoring systems. Additional studies need to be conducted to evaluate additional parameters and delivery methods that can improve attraction of moths to floral attractants. Trap design, floral attractants release rates, competition with natural odor sources, lure dispenser methods, chemical stability and durability, and influence of temperature, beneficial insects attraction, among others.

Figure 1: Universal Moth Trap™ used in field experiments with green lid, yellow cone, and white bucket.

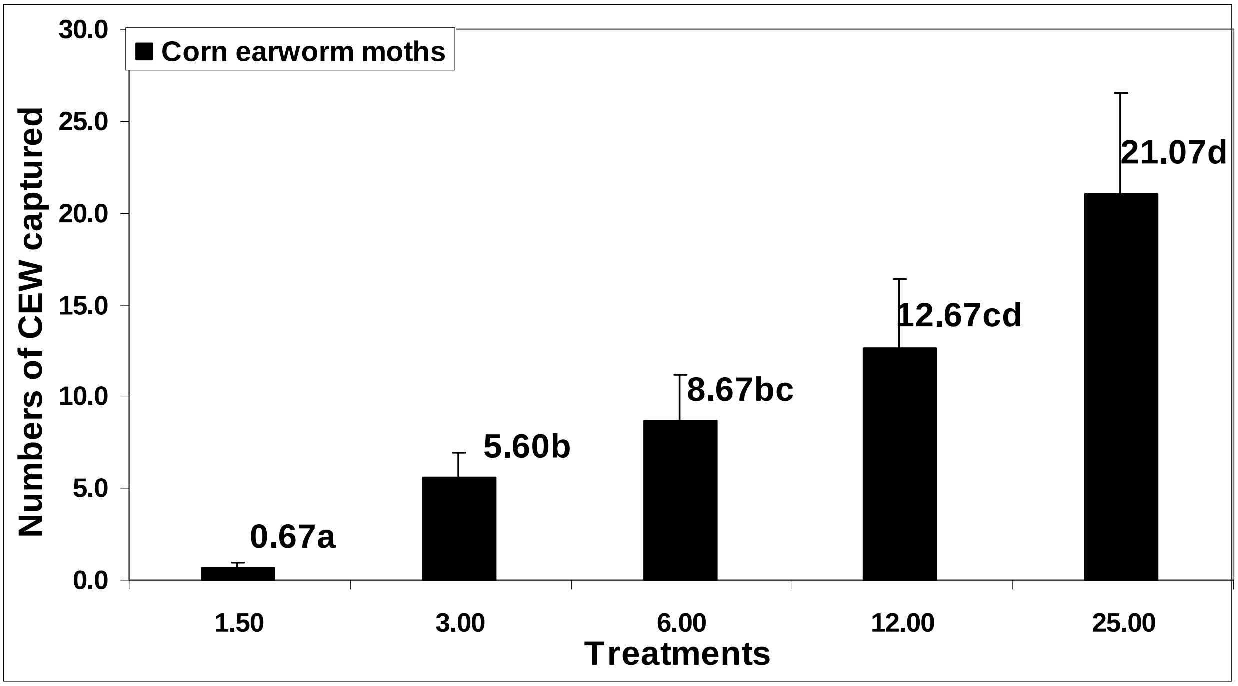

Figure 2. Mean (± S.E.M.) numbers of corn earworm moths captured in traps baited with a range of release rates of phenylacetaldehyde plus methyl-2-methoxy benzoate. Floral odorants emissions was varied by changing vial hole diameter. Means with different letters are significantly different (L.S.D., $p<0.05$).

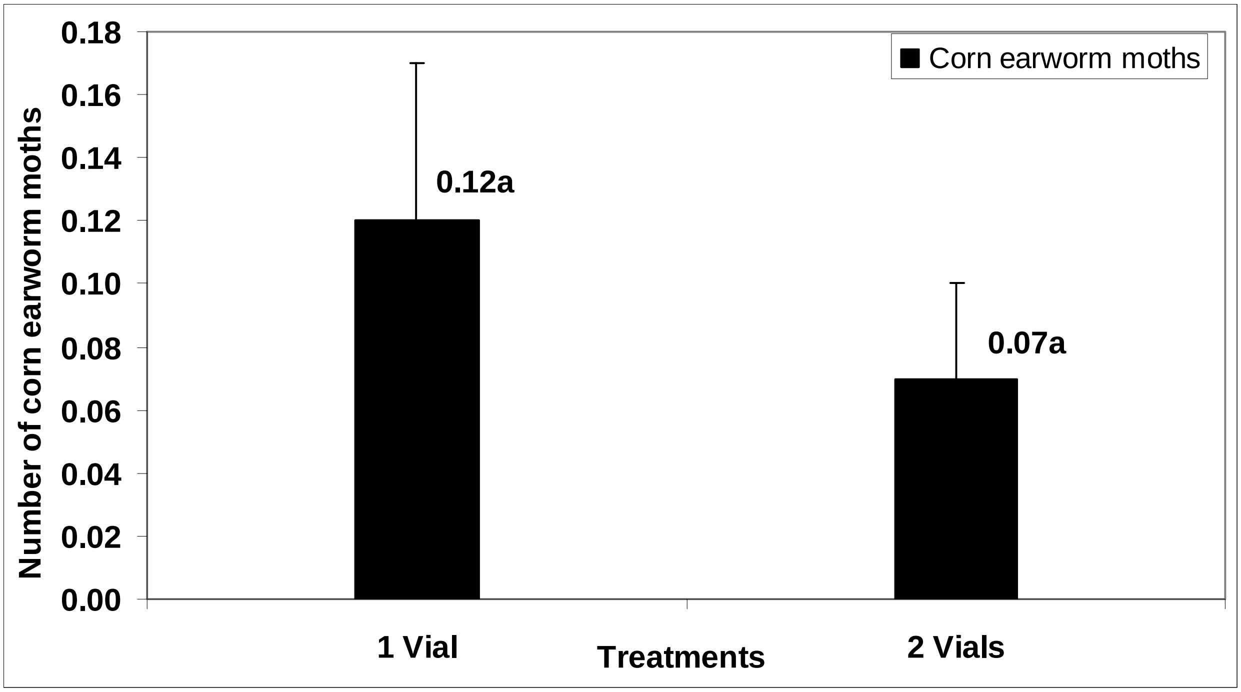

Figure 3. Mean (± S.E.M.) numbers of corn earworm moths captured in traps baited with phenylacetaldehyde plus methyl-2-methoxy benzoate. Floral attractants were dispensed as a mixture in one vial (Treatment 1) or separate in two vials (Treatment 2). Means with different letters are significantly different (t-test, p>0.05).

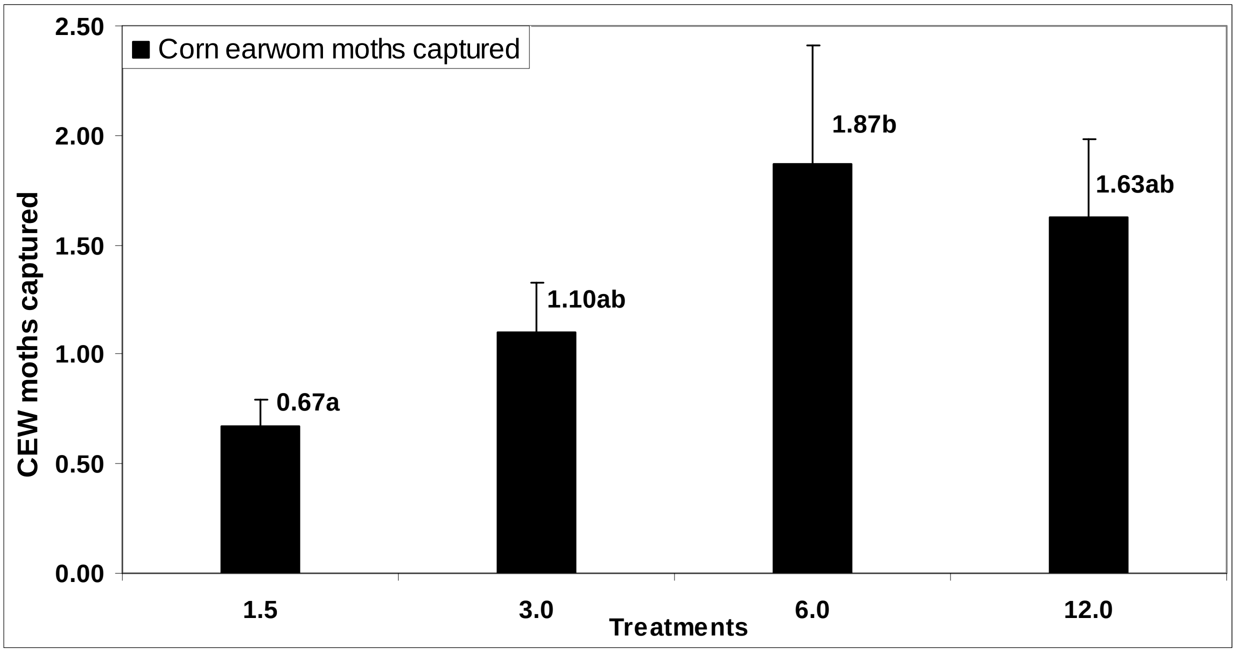

Figure 4. Mean (± S.E.M.) numbers of corn earworm moths captured in traps baited with phenylacetaldehyde plus methyl-2-methoxy benzoate in separate vials. Methyl-2-methoxy benzoate release rate was varied while maintaining phenylacetaldehyde release rate constant. Methyl-2-methoxy benzoate emissions was varied by changing vial hole diameter. Means with different letters are significantly different (L.S.D., $p<0.05$).

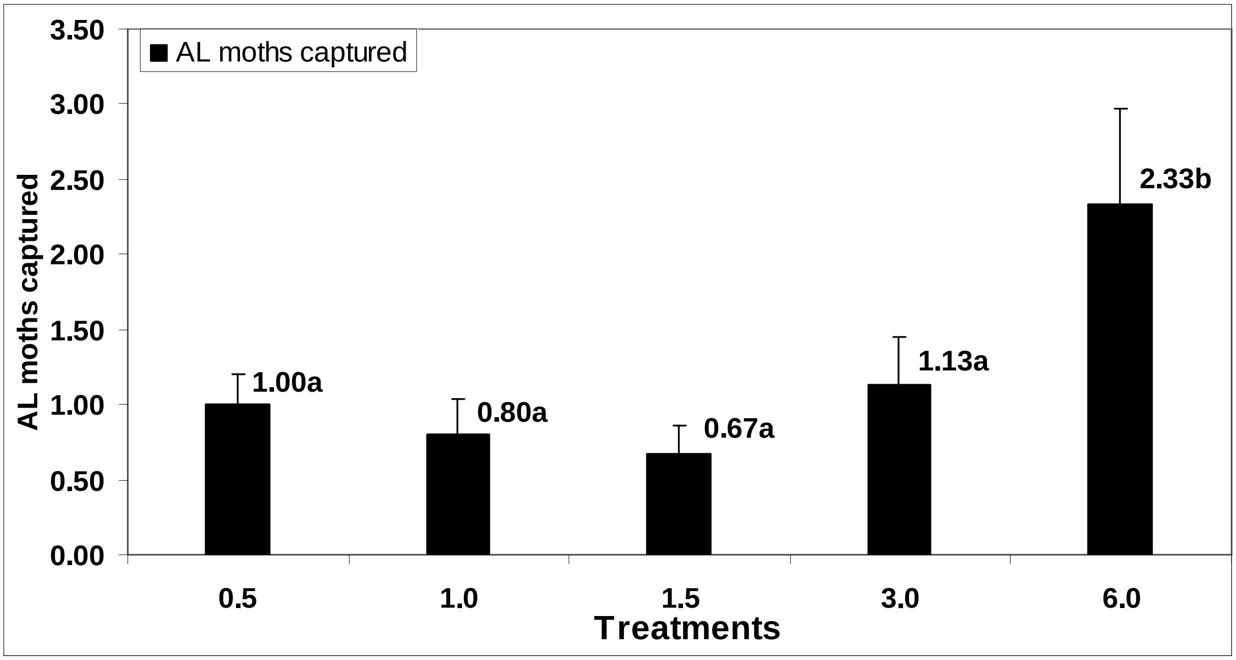

Figure 5. Mean (± S.E.M.) numbers of alfalfa looper moths captured in traps baited with phenylacetaldehyde plus beta myrcene. Floral odorant emissions were varied by changing vial hole diameter. Means with different letters are significantly different (L.S.D., $p<0.05$).

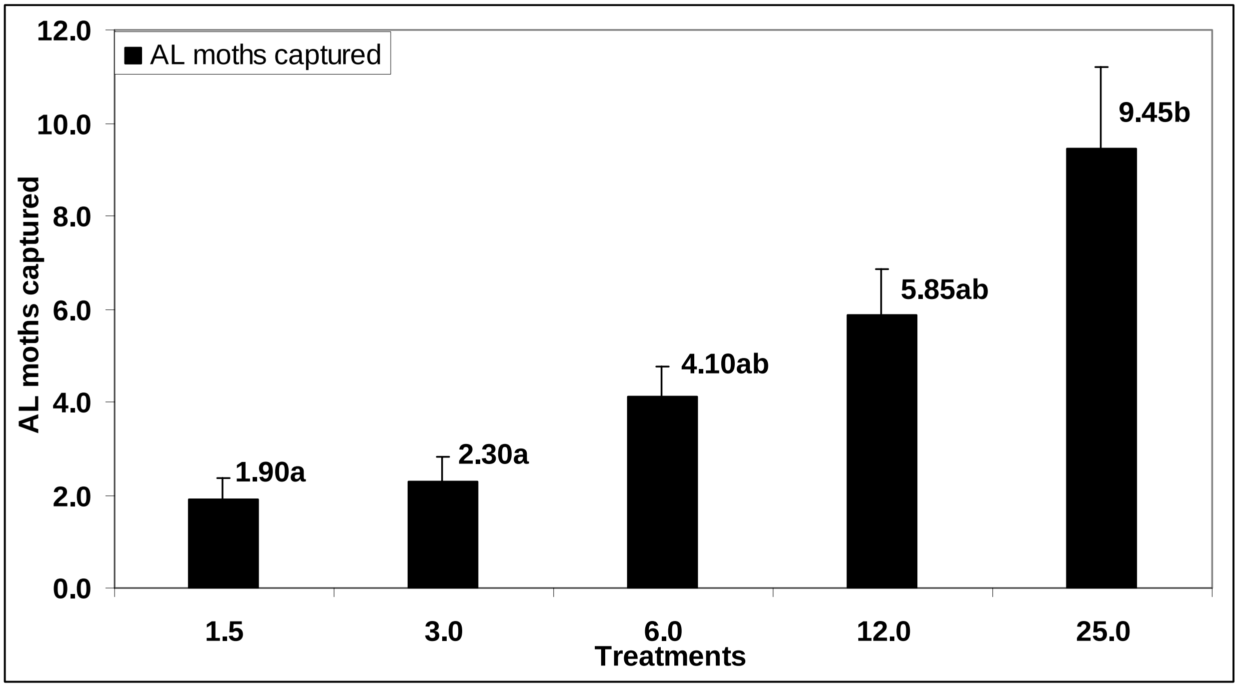

Figure 6. Mean (± S.E.M.) numbers of alfalfa looper moths captured in traps baited with phenylacetaldehyde plus beta myrcene. Floral odorant emissions were varied by changing vial hole diameter. Means with different letters are significantly different (L.S.D., $p<0.05$).

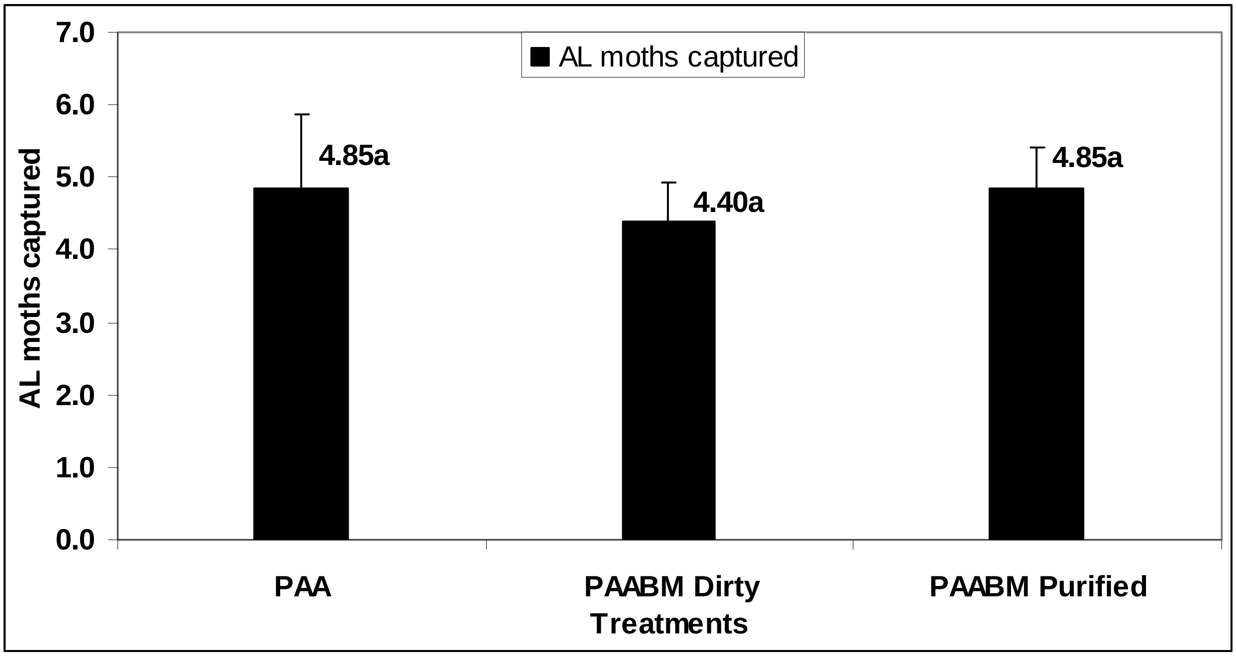

Figure 7. Mean (± S.E.M.) numbers of alfalfa looper moths captured in traps baited with phenylacetaldehyde plus beta myrcene. Treatments consisted of (T1) phenylacetaldehyde, (T2) phenylacetaldehyde plus unpurified beta myrcene, and (T3) phenylacetaldehyde plus purified beta myrcene. Means with different letters are significantly different (L.S.D., $p<0.05$).

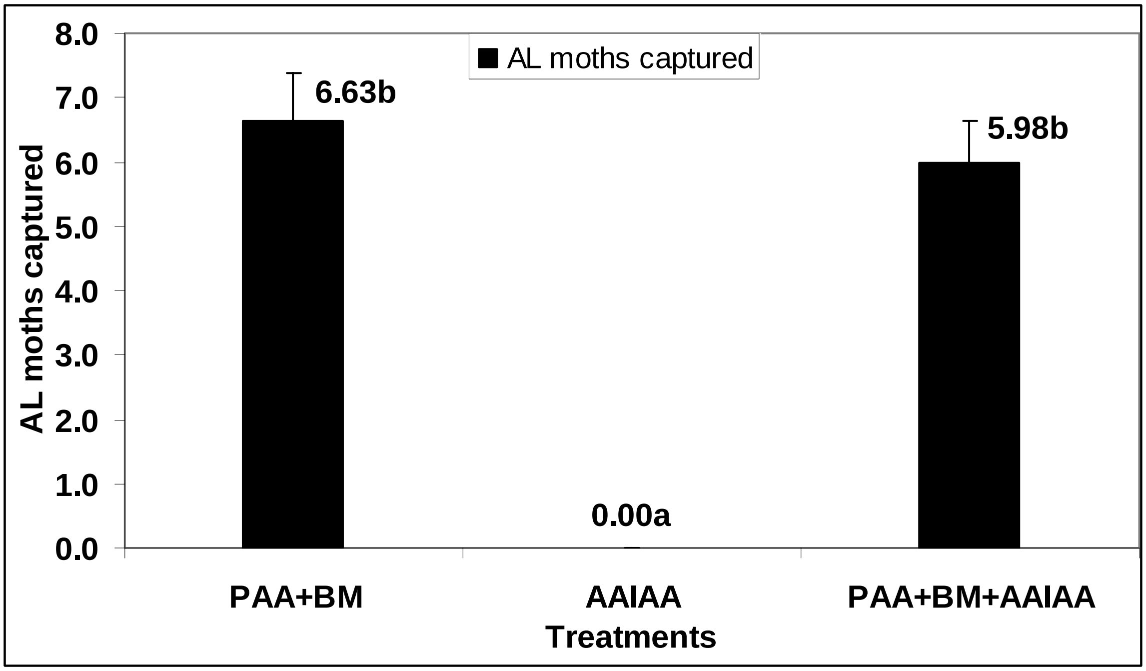

Figure 8. Mean (± S.E.M.) numbers of alfalfa looper moths captured in traps baited with phenylacetaldehyde plus beta myrcene. Treatments consisted of (T1) phenylacetaldehyde plus beta myrcene, (T2) acetic acid plus isoamyl alcohol, and (T3) phenylacetaldehyde plus purified beta myrcene plus acetic acid plus isoamyl alcohol. Means with different letters are significantly different (L.S.D., $p<0.05$).

Table 1. Mean (± S.E.M) numbers of male and female moths captured in experiments with Universal moth traps™ baited with floral attractants.

Experiment	Males (± S.E.M)	Females (± S.E.M)
	Corn earworm moths	
1	35.13 ± 8.44a	13.53 ± 3.77b
2	0.08 ± 0.03a	0.11 ± 0.04a
3	1.73 ± 0.42a	3.54 ± 0.67b
	Alfalfa looper moths	
4	3.33 ± 0.48a	2.60 ± 0.48a
5	12.95 ± 1.65a	10.65 ± 1.35a
6	8.40 ± 0.75a	5.70 ± 0.66b
7	7.00 ± 0.74a	5.60 ± 0.61a

Means within a row followed by the same letter were not significantly different (t-test, $p>0.05$).

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DISCUSSION

One of our primary goals, to reduce female alfalfa looper population activity in plots, was achieved during both years that field experiments were conducted; 2003 and 2004. Even though our results are encouraging, additional studies are necessary in order to assess effects on the larva stage in a large field scale setting. We believe that as this technology matures it will lead to an innovative management technique applicable to several key lepidopteran pests. This will result as feeding attractants, or other kairomone and pheromonal attractants, are discovered and applied for other Lepidoptera species. The development and commercial adoption of the attract and kill system can provide growers with an alternative to reduce female alfalfa looper moth numbers, thereby reducing reproduction and reducing the amount of pesticide used throughout the growing season. The environmental benefits of such technology are evident.

This study provides south-central Washington State critical knowledge on alfalfa looper and cabbage looper moth seasonality, and strengthens corn earworm moth seasonality data already available for this area (Adams, 2001). It also demonstrates preferential attraction of species to specific floral attractant blends, allowing for monitoring systems for females to be optimized for targeted species. The attraction of female moths can contribute to the improvement of pest management tools, such as attract and kill, mating disruption, and mass trapping of females.

Experiments also indicate that female alfalfa looper and cabbage looper moths attracted by the floral lure can be killed by contacting the Teflon® grease (containing the killing agent) coated in the shuttlecock killing stations. Results of flight tunnel tests also confirmed the attraction of alfalfa looper and cabbage looper moths to phenylacetaldehyde. Similar attraction and mortality rates were observed for both male and female alfalfa looper and cabbage looper moths. Mortality rates of moths that contacted killing stations were higher for moths that had not fed on sugar.

Corn earworm population reduction was better achieved with the use of shuttlecock killing stations than with the use of wax panel killing stations. We expect that a reduction of female corn earworm activity will reduce the number of larvae in the subsequent generation by killing females before they are able to lay eggs. However, new experiments need to be conducted to address that hypothesis. Additional improvement of the lures and killing stations are necessary, so that better efficiency against female and male corn earworm moths is achieved.

Numbers of male and female corn earworm and alfalfa looper moths captured during our experiments confirmed that moths are attracted to feeding attractants regardless of gender. These results are similar to other feeding attractant trapping experiments for different Lepidoptera species (Lopez et al 2000, Pair and Horvat 1997, Landolt et al 2001, and Landolt et al, 2006). These results are encouraging due to the fact that attraction of female Lepidopteran pests is a much needed advancement in attractants to be used in aid of pest management and/or monitoring systems. Additional studies need to be conducted to evaluate additional parameters and delivery systems that might improve attraction of moths to floral attractants. Trap design, floral attractants release rates, competition with natural odor sources, lure dispenser methods, chemical stability, chemical durability, influence of temperature, beneficial insect attraction, and others.

Beneficial insects are also attracted to these lures, probably because they are based on odorants of flowers, which pollinators, predators, and parasites also feed. Those include a wide variety of Hymenoptera such as bumble bees *Bombus* spp., honey bees *Apis melifera L.,* and sweat bees (Halictidae), among others. A large number of bumble bees were also captured in traps baited with a similar floral lures in Alaska (Landolt et al., 2006). All these beneficial insects are susceptible to the killing agent present in the killing stations, causing a negative environmental impact. Another drawback to the use of feeding attractant based killing stations is the competition with other odorants that might be present in the environment. Flowering plants may disrupt orientation of targeted pests to the killing stations or compete with it. Both of those issues will need to be addressed before any commercial application of the technology is feasible.

Subsequent studies to improve floral attractant based killing stations are necessary. More specifically, the attract and kill system against alfalfa looper moths needs to be further developed to attain commercial impact and applications. Demonstrations of protection against larval damage with killing stations deployment in commercial fields are necessary. This "product development" process needs to be conducted in partnership with an interested entity that possesses capability and Market reputation in the development of Pest Management products. Killing station designs needs to be economically assessed, and different ways to deliver the killing agent considered. A niche market for the system to be applied needs to be established so that economic viability can be obtained. Possible cropping systems will include high value and high input horticultural commodities in which alfalfa looper moths are a primary or secondary pest. Attract and kill systems against either the corn earworm or cabbage looper moths needs to be further evaluated in laboratory and field, so that efficacy is improved and documented.

This study, as a whole, promotes hypotheses on the interaction of moths with attractants and host plants. Experimental design used to assess reduction of oviposition due the deployment of killing station might provide opportunities to investigate ability of moths to learn a specific host plant odor, and the development of a host choice preference by moths. Learned host preference, can be tested by releasing them into a cage (screen building) in which the moths have more than one host option to lay eggs. A group of female and male moths released will be previously exposed to a host, than oviposition preference will be compared to those of a group of male and female moths released into the building not previously exposed to a host, or exposed to the second host option. Preliminary data demonstrated that a group of male and female cabbage looper moths switched preference to a host when they were previously exposed to it. Flight tunnel studies can be conducted to rank attractiveness of moths to different host plants. Attraction ranking can be correlated to larval performance in host plants (in progress). These hypotheses can be extended to several species including the alfalfa looper, cabbage looper, the celery looper, the corn earworm, and the tobacco budworm, among others. These experiments would provide researches with important information regarding agricultural pests choice for a host over another, and host preference.

The effect of access to sugar on moth responses to floral feeding attractants was noticeable. The relationships between sugar feeding, hunger, and moth response to feeding attractants are not well understood or discussed in the literature. Assessment of sugars present in digestive systems of moths captured in feeding attractant traps versus blacklight traps might provide information to address the hypothesis that moths attracted to feeding attractant traps are hungry. There is no documentation in the literature that clearly demonstrates that these moths acquire nectar from flowers that are sources of feeding attractants. However, this seems highly likely. This can be achieved by placing moths with flowers and comparing crop content to moths that were not placed in flowers (control). Any role of these moths in pollination is also not well documented in the literature. Pollination services provided by moths might be assessed by releasing female alfalfa looper moths into cages with flowering potted Oregongrape plants, and comparing numbers of berry sets between caged plants with and without any moths released inside. This might be best compared also to caged Oregongrape plants with honey bees *Apis melifera*. Effects of sugar feeding on oviposition have been documented for the cabbage looper (Landolt, 1997). That methodology can be expanded to understand the same relationship for the alfalfa looper, corn earworm, celery looper, codling moth and other economically important species.

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