Development of selective management strategies for codling moth in pears

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Abstract

Preliminary studies indicated that two plant volatiles derived from apple and pears could be incorporated into small mini-emitters already developed for dispensing codling moth pheromone. Both the confetti-like flake formulation by Hercon and the microtubule formulation by Scentry successfully emitted the volatiles and attracted codling moth into traps as the first step to developing a complimentary control strategy to pheromone mating disruption. The potential for an "attract and kill" strategy using these plant volatiles may provide a second means to suppress high density situations for codling moth. Secondly, a passive pheromone emitter using a paraffin based formulation was used to produce a presumably higher emission rate per dispense. These dispensers deployed at only 12 dispensers per acre provided a 94% suppression of traps under high pressure conditions. The potential for lower application costs and lower total pheromone costs may help to reduce overall costs for managing codling moth. Finally, using colonies of codling moth collected from an orchard with historically high Guthion resistance and from an organic apple orchard, significant differences in mortality between the two colonies using a larval bioassay were observed for Intrepid, but not for a newer chemistry, novaluron.

Introduction

Pheromone mating disruption programs have proven successful over the past 10 years, but several areas for improvement remain. Program efficacy has been more limited when populations of codling moth are high, which requires supplementation with insecticides that may or may not undermine the selectivity of the pheromone program. Secondly, cost remains an issue as crop production continues to run on a relatively tight margin. Therefore, our efforts in 2005 were focused on 1) developing alternative programs using plant volatiles to supplement pheromone mating disruption without loss of the program benefits (e.g. ease of worker re-entry, no impacts on pre-harvest conditions, no disruption of beneficial insects) and 2) seeking to optimize the pheromone program performance and cost by looking at pheromone emitters that were intermediate in the per unit release rate and in the numbers of units needed per acre. If successful, the hope is that a second means to suppress codling moth using the plant volatiles can be developed that is complimentary of the pheromone program. Secondly, if fewer pheromone emitters can be applied per acre, then the lower costs of application and potentially the lower unit cost can produce a reduction in overall control program costs.

The report is divided into 3 major sections as follows: 1) Codling moth attraction to pheromone and plant volatiles in fiber and flake dispensers 2) Meso-emitters – Pheromone mating disruption and 3) Intrepid and Rimon Insecticides: Baseline resistance levels. Results from both walnut and pear orchards are presented given that the programs are complimentary, yet different insights are provided from each commodity. For example, differences in odor background levels between the types of orchards may prove key to understanding why the apple volatile appears less attractive in walnuts than pears. Similarly, efforts to develop a lower cost hand-applied dispenser with fewer needed dispensers per acre will meet the immediate goals of walnut growers who currently do not have an effective means to deliver codling moth pheromone with hand-applied dispensers, while this approach can also reduce overall management costs in pears, if successful.

Codling moth attraction to pheromone and plant volatiles in fiber and flake dispensers

Supplementation of pheromone programs has proven a necessity in situations where either high densities of codling moth occurred or if secondary pests present a significant risk. Typically, this supplementation relied on the inclusion of either broad or more selective spectrum insecticides. Depending on the time of year, the inclusion of a supplemental spray might present problems with worker re-entry issues or changes in pre-harvest requirement. In addition, if a more broad-spectrum material was used, then one of the advantages of the pheromone program is lost, which is its non-disruption of existing biological control.

Recent advances by USDA researchers (Light, Landolt, and Knight) as well as European researchers (Witzgall or Dorn) have shed light on the potential role of plant volatiles as attractants for codling moth or other lepidopterous insects. The greatest advances have been made with the pear ester as a monitoring tool in apples, pears, and walnuts. However, the opportunities for incorporating these plant volatiles into a management program are still being developed. A variety of plant volatiles have been shown to be attractive including pear and apple volatiles, whereas most of the walnut odors are relatively unexplored.

The overall logic of a plant volatile based program is to use an odor source that is not masked by the pheromone program such that we could potentially have 2 independent attacks that are both selective. The micro-emitters (tubules by Scentry or flakes by Hercon) could be applied to an orchard using a variety of application equipment (fixed wing, helicopter, or modified ground rigs). The small emitters would serve as another attractant and could either include an insecticide on the surface for an attract and kill program or without an insecticide as a false trail following program, which results in the male locating pieces of plastic filled with a plant volatile rather than a female for impregnation. The impact of responses by the female to a specific lure on the overall population trend is largely unknown at this time.

The advantages of this type of approach would include:

a) a highly selective program that is non-disruptive and compatible with the pheromone program
b) the ability to add the treatment later in the season when and if needed, thus giving the growers and their PCAs greater flexibility in overall program costs
c) pending registration, the ability to apply close to harvest if either no insecticide is added or a material with a short pre-harvest interval is used.

Materials and Methods

A series of field trials was conducted in pear and walnut orchards to investigate the possibility of using mini-emitters filled with pheromone and/or plant volatiles to attract codling moth (see Table 1 for outline). A total of 11 trials were conducted in 6 orchards over the growing season. The two types of emitters included the microtubule (or fiber; Scentry Biologicals, Inc., Billings, Montana) and the laminated flake (Hercon Environmental, Emigsville, PA). Pherocon ® Delta VI traps (Trece, Inc., Adair, OK) were loaded with trial dispensers containing either codlemone, (e)-beta-farnesene (apple volatile), ethyl 2,4-decadienoate (pear ester) or a combination of these products. The Hercon laminate of codlemone:apple volatile was formulated with a 1:1 blend of beta-farnesene plus codlemone 4.5% each plus 91% inert ingredients. All Hercon components are 41 mg per square inch initially, but then cut into squares approximately 1 mm^2. All trials were conducted with control references of Codling Moth 1X Biolure (Suterra, Inc., Bend, OR) or CM10X red septa lures (Trece, Inc., Adair, OK) as indicated in specific trial descriptions that follow. Dispensers were placed in the traps as follows: fibers were attached to the upper inside surface with Bio-Tac from Scentry, whereas the flakes were attached to the upper inside surface with Gelva® Multipolymer Emulsion 2333 (Surface Specialties, Inc. Smyrna, GA), and standard methods were used for the commercial 1X and 10X lures. Traps were placed approximately 150 feet apart and hung in the upper half of the canopy. Pears sites used in these studies were located near Walnut Grove (non-pheromone control program) and Sheldon (pheromone program). Walnut sites were located near Hollister, Stockton and Wheatland.

The first set of trials was designed to indicate the rates of codlemone loaded microfiber and flake dispensers that would attract codling moth to a trap. Trials were run in conventional (without any pheromone mating disruption program) as well as in orchards using pheromone mating disruption (product varied by orchard).

Trial 1. Five treatments of codlemone loaded dispensers were deployed at rates of 1-fiber, 10- fibers, 1-flake, 10-flakes or a 1X Biolure per trap. Four replicate blocks were run in each of two commercial pear orchards near under a conventional program for CM control which was not under mating disruption. Traps were read twice at 5 and 6 day intervals and re-randomized following the first reading. Re-randomizing the traps allowed for independent analyses of the data for each reading.

Trail 2. An Isomate treated pear orchard near Sheldon received the same treatments as Trial 1 but used a 10X septa lure as a reference. The trial was run with four replicate blocks in each of two sites in the orchard. Traps were read twice at 5 and 6 day intervals and re-randomized between readings

Trial 3. Based on the results of trial one, codlemone loaded fibers and flakes were modified to reduce the emission rate from an individual unit. The fear was that the emission rate might be too great even at 1 mini-emitter per trap. Therefore, a unit emitting from a single end of the fiber or along only 50% of a flake edge was presumed to have 50% of the emission rate. A second treatment of 2 emitters per trap was also included so as to bound the emission rate of a single emitter on each side with a 2X emission rate. One end of each fiber tube or two edges of the flake square were coated with chemical-resistant glue. Treatments of ½-fiber, ½-flake, 2-fiber, 2-flake, 1X Biolure, or a streak of Gelva alone were placed in traps. Four replicate blocks in each of two conventional pear orchards were set. The traps were read once after 10 days.

Trial 4. Five treatments of codlemone (1-fiber, 1-flake, 5-fiber, 5-flake, or 10X red septa) were placed in traps and set in four replicate blocks in two sites at the pheromone-treated pear orchard. Traps were read twice, after 10 and 9 days, and re-randomized between readings.

Trial 5. This trial set sought to indicate a response of codling moth to the apple volatile, (e)-beta-farnesene, and using the fiber dispenser, find an optimal rate for attraction under pheromone mating disruption. To optimize the emission rate for the apple volatile, traps were loaded with 2, 5, 10, or 20 apple volatile fibers or with a 1X Biolure as the standard. The traps were placed in the conventional pear orchards. Four replicate blocks in each of two sites were evaluated twice after 3 and 4 days, with traps re-randomized between readings.

Trial 6. The next trial set compared trap catches between treatments that included combined pheromone and apple or pear volatile treatments used as trap bait. To compare the attractiveness of the plant volatiles with the pheromone, Scentry fibers loaded with codlemone, pear volatile or apple volatile were set up using a single fiber of each for the following six treatments: codlemone (1 fiber), pear (1 fiber), apple (1 fiber), codlemone plus apple volatile (1 fiber each), codlemone plus pear volatile (1 fiber each), and a 1X Biolure. The combination of plant odors plus the pheromone could be examined for additive effects, inhibitory effects or synergistic effects by comparing the trap counts to the single emitters. Four replicate blocks were set in each of the two conventional pear sites and traps were evaluated once after 2 days.

Trial 7. We again used the Scentry fibers loaded with codlemone, pear volatile, or apple volatile in the following treatments: codlemone (2 fibers), apple volatile (5 fibers), codlemone plus apple volatile (2 plus 5 fibers), codlemone plus pear volatile (2 plus 5 fibers), and a 1X Biolure comparison. Traps were set into four replicate blocks in each of the two conventional orchards and read three times (at 3 days, 4 days and 4 days) with positions re-randomized between each reading. These trials were intended to look for interactive effects between the odor types using the rates that appeared optimal.

Walnuts.
Given the potential differences in the odor signatures for walnuts and pears, many of the trials were repeated in walnuts. However, we attempted to build on the data and experiences from pears in order to optimize our use of time.

Trial 8. Fibers loaded with two rates of either apple or pear volatiles were compared in a walnut block (Ashley variety) near Wheatland. Five treatments were as follows: apple volatile (5 and 10 fibers), pear volatile (5 and 10 fibers) and 1X Biolure. Traps were set in four replicate treatment blocks and read twice. They were re-randomized and re-loaded with fresh fibers and lures between readings.

Trial 9. Five treatments set in a Hollister organic orchard were compared. Treatments were apple volatile formulations (5-fiber), a codlemone plus apple blend (5-flake), pear ester (5-fiber), codlemone (2-fibers) and a 1X Biolure. Three replicates of each treatment were set in the orchard. Traps were read three times.

Trial 10. Trap capture for apple volatile formulations were compared with codlemone formulations in a Stockton site. An existing trial in this Vina block had divided the plot into pheromone and grower standard treatments. We placed two trapping blocks into each treatment plot. Trap treatments were apple volatile (2-fiber), a codlemone plus apple blend (2-flake), codlemone alone (2-fiber) and a 1X Biolure with all traps hung high. Traps were read twice and re-randomized between readings.

Trial 11. Trap capture using different rates of the apple volatile formulation was again compared with codlemone formulations in a Stockton site. An existing trial in this Vina block divided the plot into pheromone and grower standard treatments. We placed two trapping blocks into each treatment plot. Trap treatments were apple volatile (5-fiber), a codlemone plus apple blend (5-flake), codlemone alone (2-fiber) and a 1X Biolure with all traps hung high. Traps were read twice and re-randomized between readings.

Table 1. Lure trials using codlemone and plant volatiles in walnuts and pears.

Crop	Trial	# Sites / Program	Sites* blocks	Treatments	Rates	Times Evaluated
Pears	1	2 conventional	2*4	codlemone	1X Biolure fiber – 1, 10 flake – 1, 10	2 5 days 6 days
	2	2 pheromone	2*4	codlemone	10X septa fiber – 1, 10 flake – 1, 10	2 5 days 6 days
	3	2 conventional	2*4	codlemone Gelva (glue)	1X Biolure fiber – ½, 2 flake – ½, 2 line	1 10 days
	4	2 pheromone	2*4	codlemone	10X septa fiber – 1, 5 flake – 1, 5	2 10 days 9 days
	5	2 conventional	2*4	codlemone apple volatile	1X Biolure fiber – 2, 5, 10, 20	2 3 days 4 days
	6	2 conventional	2*4	codlemone apple volatile pear ester codlemone + apple codlemone + pear	1X Biolure fiber – 1 fiber – 1 fiber – 1 fiber – 1 + 1 fiber – 1 + 1	1 2 days
	7	2 conventional	2*4	codlemone apple volatile pear ester codlemone + apple codlemone + pear	1X Biolure fiber – 2 fiber – 5 fiber – 5 fiber – 2 + 5 fiber – 2 + 5	3 3 days 4 days 4 days
Wal- nuts	8	Pheromone (Wheatland)	1*4	codlemone apple volatile pear ester	1X Biolure fiber – 5, 10 fiber – 5, 10	2 23 days 6 days
	9	Conventional (Hollister)	1*3	Codlemone apple volatile pear ester codlemone-apple blend	1X Biolure fiber - 2 fiber – 5 fiber – 5 flake – 5	3 8 days 10 days 7 days
	10	conventional pheromone (Stockton)	1*2 1*2	Codlemone apple volatile codlemone-apple blend	1X Biolure fiber - 2 fiber – 2 flake – 2	1 2 days

	11	conventional pheromone (Stockton)	1*2 1*2	Codlemone apple volatile codlemone-apple blend	1X Biolure fiber - 2 fiber – 5 flake – 5	1 12 days

Results

Results are only presented for trials which proved successful in catching enough moths to make comparisons useful. Unfortunately, all of the plots in pheromone treated areas failed to have adequate populations, and some of the conventional plots as well.

The attractiveness of single fiber or flake dispenser of codlemone was ca. 50% of the standard 1X lure in a conventional orchard (Fig. 1-Trial 1). Using 10 fibers or flakes proved less attractive at ca 30% of the attraction which is not all that unexpected given that previous work with conventional septa lures has already shown a threshold for the pheromone above which it becomes less attractive. When traps baited with either 2 fibers or flakes or these dispensers modified to have only ½ of the release rate, the results were somewhat variable; they still captured on average ca. 56% of the 1X lures (Fig. 2-Trial 3) with a maximum capture of 82% for the ½ flake dispenser.
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In contrast, when the apple volatile alone was used across a range of fiber numbers, the counts were approximately 4-17%of the 1X lure with the highest counts in traps with 5 or 10 fibers per trap (Fig. 3 from Trial 5). As such, no clear rate response was observed that would result in significant increases in trap captures. The apple volatile may be less attractive within the pear system at this time of year or may operate only over a shorter distance.

The pear volatile appeared more attractive (Fig. 4 from Trial 6) than the apple, yet the counts were fairly low compared to the codlemone in the same emitter type. The pear ester caught approximately 9% of the moths caught in the 1X standard lure compared to 5% by the apple volatile. Both the apple-codlemone and pear-codlemone combinations caught more moths than either volatile alone with percent capture at 30 and 41% compared to the 1X lure.

In figure 5 from Trial 7, the results for traps baited with higher numbers of fibers (and hence higher emission rates) for the different compounds are presented. While the combinations of lure types produced better results than the apple volatile alone, their trap counts were no higher than the pheromone alone. This suggests that no obvious additive effect or advantage was gained with addition of the plant volatile.

For walnuts, the pear ester appeared to be much more attractive alone than the apple volatile (Fig. 6-trial 8). The pear ester caught 3.6 and 4.6 moths per trap for traps baited with 10 or 5 fibers respectively, where the apple volatile was functionally almost 0 with only 0.2 moths caught on average in traps with 10 apple lures.

The same basic pattern was again repeated in Hollister over several weeks (Fig. 7 – Trial 9) with the plant volatiles catching less than the pheromone lures, the apple lure catching less than the pear lure. Finally in Fig 8 (Trial 11), two patterns emerged with the apple volatile having non-detectable attraction in the pheromone plots, whereas the control (conventional treatment) plot had minor counts. No detectable levels were in any lure except the 1X lure for the pheromone treated plots, which again was somewhat surprising. I was expecting the pheromone baited traps to be completely suppressed and the plant volatiles to retain some level of attraction.

In short, the positive news was that different types of dispensers were successful in emitting the plant volatiles and both volatiles were attractive. No clear rate response was observed using additional emitters per trap, but it is possible that the aggregation of several dispensers may not simulate higher emission rates from a single fiber. The lack of sufficient counts in pheromone treated trials prevents us from making any conclusions about their utility in pheromone treated orchards. Similarly, the attractiveness of the compounds within an orchard as the orchard matures remains unclear. The apple volatile does present one option to the pear ester lure, but other volatiles need to be further explored for greater attractiveness.

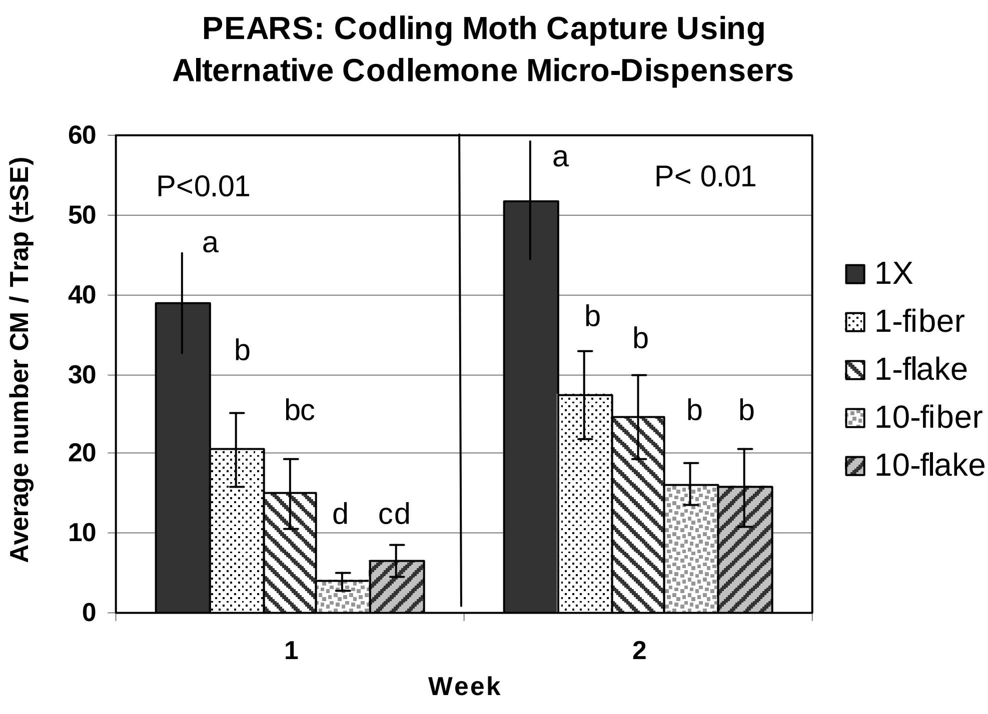

Figure 1. Pear Trial 1. Codling moth capture in traps baited with codlemone in rates of 1 or 10 flakes or fibers or a standard lure in a conventional orchard.

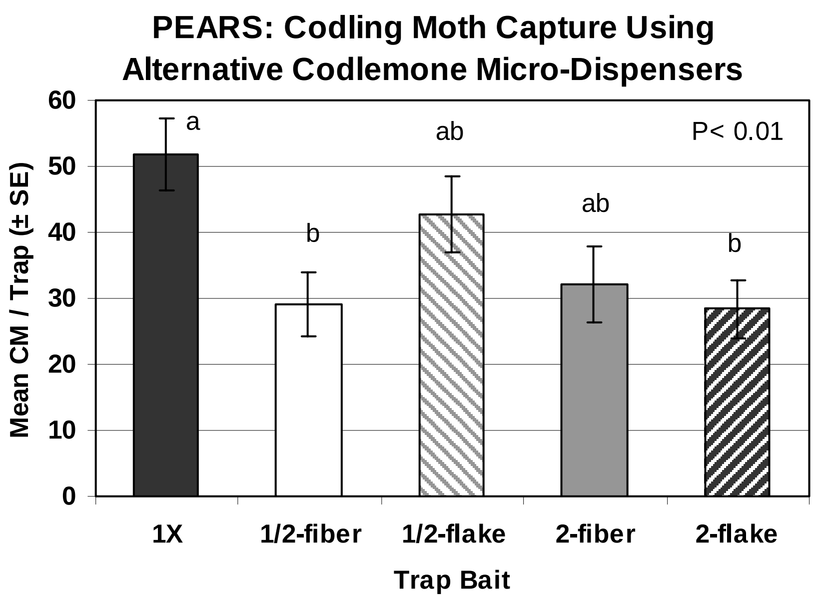

Figure 2. Pear trial 3. Codling moth capture in traps baited with codlemone in rates of 1/2 or 2 flakes or fibers or a standard lure in a conventional orchard.

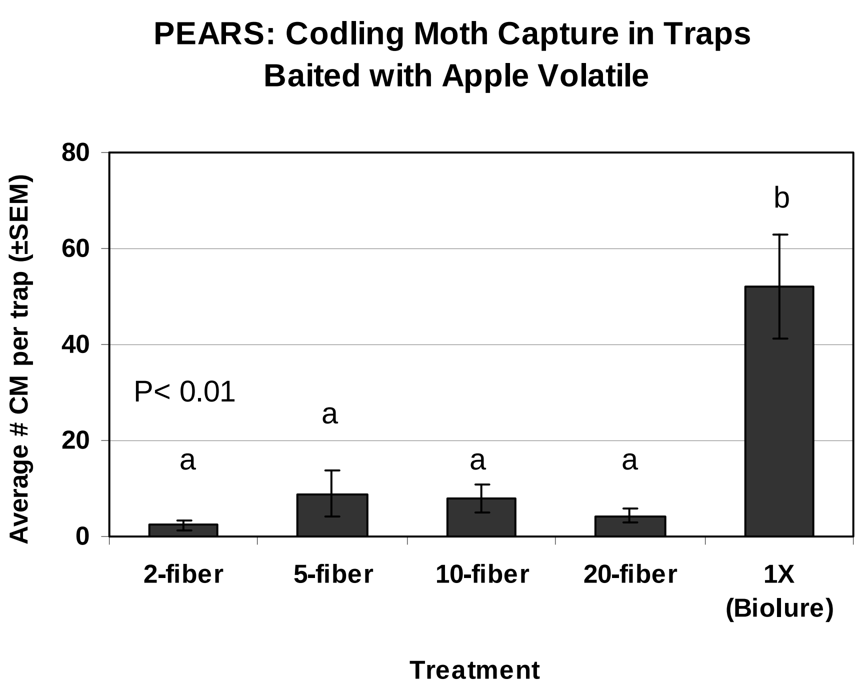

Figure 3. Pear trial 5. Codling moth capture in traps baited with apple volatile at the indicated number of fibers per trap or a standard lure in a conventional orchard.

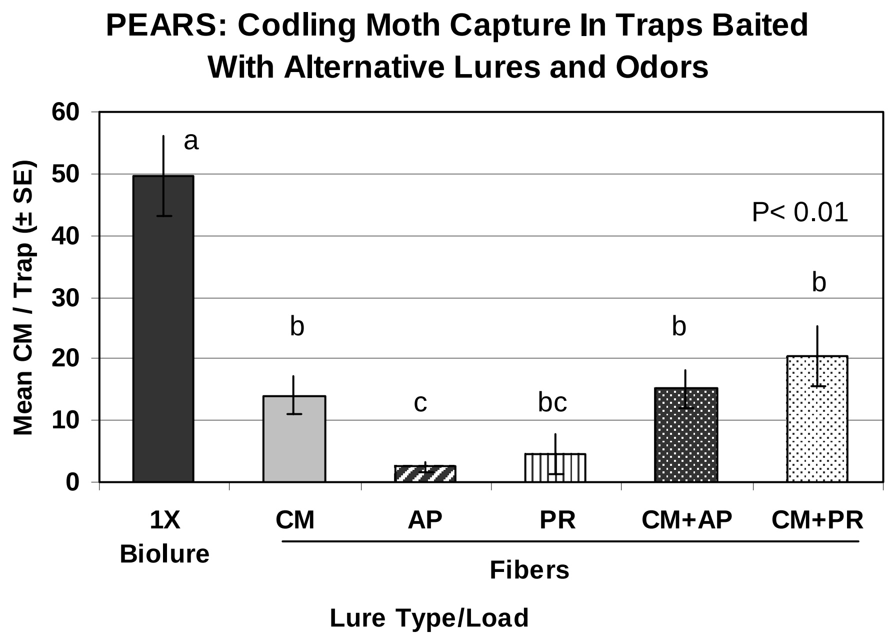

Figure 4. Pear trial 6. Codling moth capture in traps baited with codlemone, apple volatile (AP), pear ester (PR) or a combination at rates of 1 fiber of each volatile or a standard lure in a conventional orchard.

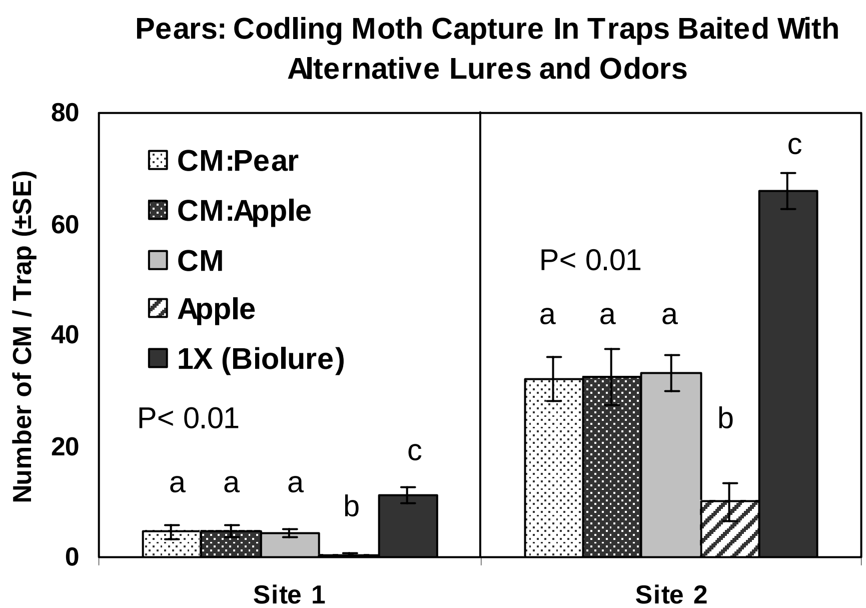

Figure 5. Pear Trial 7. Codling moth capture in traps baited with codlemone, apple volatile (AP), pear ester (PR) or a combination at rates of 2 fiber codlemone or 5 fibers volatile for the treatments indicated or a standard lure in a conventional orchard.

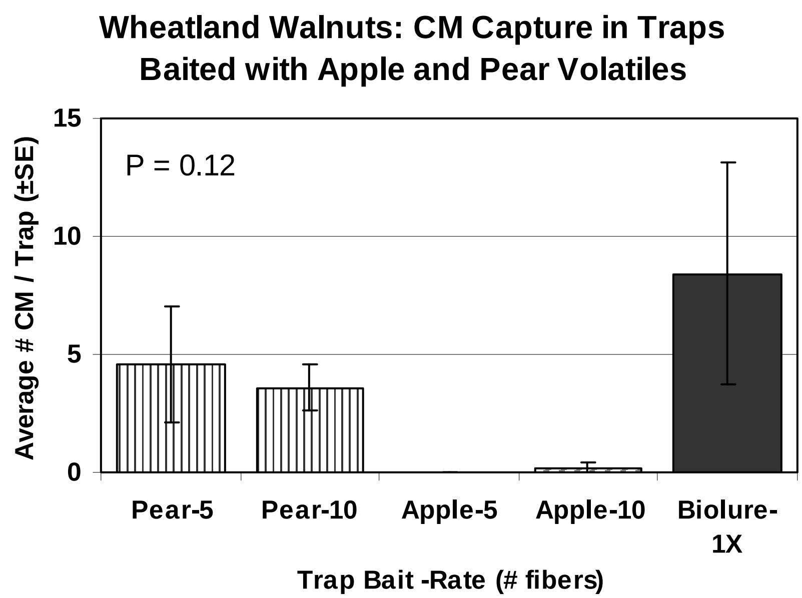

Figure 6. Trial 8 in conventional walnuts. Codling moth capture in traps baited with apple volatile (AP) or pear ester (PR) at rates indicated or a standard codlemone lure. Data shown is for the second trap reading only of this trial.

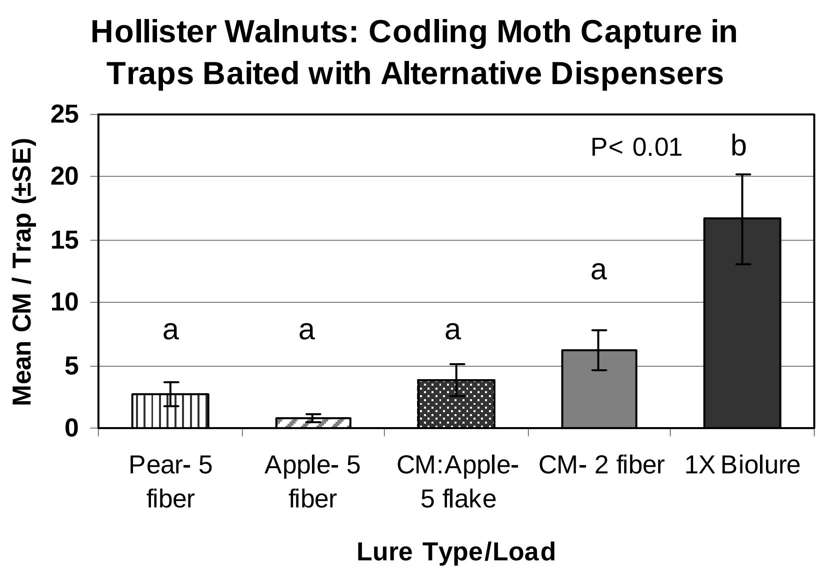

Figure 7. Trial 9 in conventional walnuts. Codling moth capture in traps baited with codlemone, apple volatile (AP), pear ester (PR) or a codlemone-apple volatile blend at rates and dispenser type indicated or a standard lure. Data from three weeks trap readings are summarized.

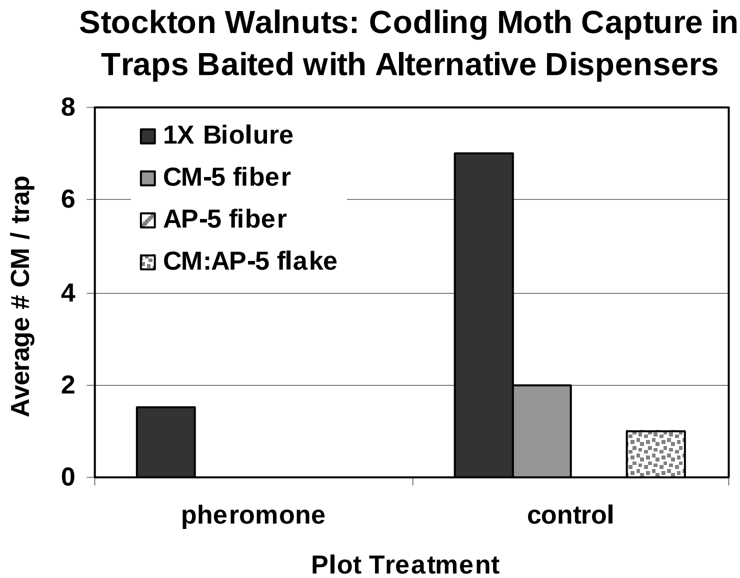

Figure 8. Trial 11 in conventional walnuts. Codling moth capture in traps baited with codlemone, apple volatile (AP), pear ester (PR) or a codlemone-apple volatile blend at rates and dispenser type indicated or a standard lure.

Meso-emitters – Pheromone mating disruption

These trials re-explored the possibility of using a reduced number of pheromone emitters per acre if release rates could be adjusted to higher levels. Original studies demonstrated that improved trap suppression and sometimes damage suppression was correlated with increased number of point sources per acre. The threshold at which increasing value was seen was relatively vague, but > 100 units per acre were the start of the decline in return per added dispenser. These data suggested that approaches such as the puffer (high dose) emitters would not be terribly successful if their modes of action are the same as the smaller point sources. Obviously, the long-term success of the areawide efforts in Lake County has proven otherwise using ca. 1-2 puffers per acre in comparison to the 400 ties per acre in the traditional Isomate program. Similarly, positive research results have been reported by A. Knight in Washington with his MOPs program in which many hand-applied dispensers were aggregated into a single unit. The number of MOPs applied per acre was 1.6-3.2 units per acre. There seems to be a gap between our understanding of how pheromone mating disruption works and the number of emitters required per acre. And finally, the microcapsules of the sprayable pheromone formulations in walnut orchards have proven very successful in suppressing traps using low rates of pheromone per acre (e.g. 10 gm per acre). Therefore, a meso-emitter, which is just an emitter with an intermediate release rate ("meso"), was considered and evaluated using intermediate numbers of applicators (>10) per acre.

In a series of "proof of concept" studies, a prototype "meso-emitter" was produced using a paraffin emulsion matrix, "SPLAT", containing codling moth pheromone. The rate of emission should be a function of the area exposed to the atmosphere, whereas the duration of the unit should be a function of the volume of the unit as a reservoir for pheromone. A different formulation of paraffin emulsion with codlemone had previously been studied such that its efficacy and longevity were at least partially understood. The amount of pheromone used in the preliminary work is based on a load rate of 10 gm ai per acre rather than an absolute release rate per dispenser. This resulted in only 12 dispensers per acre being needed in our preliminary studies. Efforts over the winter will focus on understanding release rates from a meso-emitter so that future field trials may be designed around release rather than load rates.

One of the advantages of this type of approach is the passive nature of the release mechanism. While the puffers have proven to be quite successful, their usage has proven more limited in pome fruit, whereas in walnuts the first 2 areawide projects are based on a modified puffer program. One reason for more limited adoption has been the wariness by some growers of the mechanical nature of the puffer, which has recently undergone a re-design to address problems identified by researchers, the company, and the private sector. The concept of a "meso" emission device is readily transferable to other products already developed, e.g. the Suterra membrane dispenser, hollow filled fibers (Isomate), or Hercon laminates. While logistic difficulties in re-tooling may present a barrier, no conceptual barrier exists. For example, the Suterra membrane dispenser can be altered to increase the size of the membrane or the membrane type can be changed to allow for a greater release rate. The size of the unit and hence its reservoir capacity could also be increased to last an entire season. In short, the advantages of this approach, if successful, would be as follows:

1) the passive release nature of the emitter eliminates the risk of sporadic mechanical failure associated with higher dose emitters
2) the reduced number of dispensers per acre (ca. 10-20 per acre) reduces application costs and increases application speed
3) the low pheromone load rate per acre offers the potential to reduce overall material cost
4) modification of existing technologies with their current knowledge base avoids problems such as how to stabilize the pheromone, possible accumulation on the surface of the product, or environmental effects on release rates

Given that this approach had never been tested, the initial evaluation has focused on the ability of this application to shut down a standard 1X monitor trap rather than damage suppression. History has already shown that programs can effectively shut down traps, yet still fail to shut down damage. Conversely, if a program fails to shut down codling moth traps, then it obviously is not passed onto the next stage of testing.

Materials and Methods

The meso-emitter was constructed using SPLAT Cydia 30M-1 (ISCA Technologies, Riverside, CA) which is a paraffin emulsion formulated with 1.5% ai codlemone. Hemi-spherical halves of the apple maggot trap were filled with approximately 55.6 grams of SPLAT and allowed to dry on the exposed surface before placement in the field (ca.1 to 5 days). A wire frame was placed over the base of each apple maggot trap and embedded ca. 1 cm below the surface of the matrix. The wire frame was attached to a wire that was used to hook over a branch in the orchard. Emitters were dispensed at a rate of 12 per acre (10 gm ai codlemone per acre) into 2-acre plots by hanging at approximately mid-canopy (pears) or at approximately 14-15 feet (walnuts). The emitters were placed at regular intervals to form a uniform grid in the 2-acre plots. Treatment and control plots were monitored by traps (Pherocon VI) baited with 1X Biolure and (e)-beta-farnesene (apple volatile) loaded into Scentry fibers as described below.

Pears. The SPLAT trial was conducted in an 80 acre Bartlett pear orchard using a conventional (spray) control program near Walnut Grove. This plot was initially set with one 2-acre treatment plot and two control plots. In the first trial the three plots were monitored by three traps (Pherocon VI) baited with a 1X Biolure; traps were read once after 3 days. The second round at this site utilized the same plots, with monitor traps changed to four traps per plot; two traps baited with a 1X Biolure and two baited with (e)-beta-farnesene (two fibers per trap) were read once after 5 days. A third set up in these sites was as the preceding trial but five traps were placed per site; two traps baited with a 1X Biolure and three traps baited with apple volatile (2 fibers per trap) were read after 5 days. The fourth setup was as the preceding trial with the exception that the load of apple volatile was increased to 5 fibers and traps were read after 3 days. Trap position was re-randomized between each of the above trials and trap readings. For the fifth trial, a second SPLAT treatment plot was placed in this orchard using newly constructed emitters such that two treatment plots and two controls ran concurrently. However, the initial plot had emitters that were now 14 days old, compared to 2 days old. Each plot was monitored with two traps baited with 1X Biolure and three traps baited with (e)-beta-farnesene (five fibers per trap). Traps were read once after 3 days.

Walnuts. Two replicates of a SPLAT trial were sited in conventional or unmanaged walnut orchards in the Hollister area. In both sites, codling moth was monitored in each SPLAT treatment and control plot by two traps baited with 1X Biolure and three traps baited with (e)-beta-farnesene (5 fibers per trap). Traps were read weekly for three weeks in one site, and one week in the second site. Age of the meso-emitters was 5-days in the first site (which ran for three weeks) and 17 days in the second site (which ran for one week). A similar protocol was followed for the walnut orchards with 12 SPLAT emitters per acre placed into the orchards at ca. 12-15 feet into the canopy within a uniform grid.

Results

The meso-emitters suppressed trap counts on average by 94% in pears across trials. Trap counts in the 1X pheromone traps in the control area were relatively high in the pear orchards with average counts ranging from 31.8 - to 84.3 moths per trapping period. The trapping period was typically 3-5 days per trial. Thus, the program was a good test of the approach under high density populations of codling moth. An occasional trap along the margins did catch codling moth, but presumably this occurred because the small plot size of only 2 acres for the treated area made edge effects more prominent. This will need to be confirmed next year in case this assumption is not true. Even with 400 ties per acre, edge effects have been previously noted as significant in other passive pheromone systems.

Trap suppression for the first 2 trials was 96.5 and 100%, at a time when moth flights were strong with 45.7 and 84.3 moths per trap per period in the control sites (Fig. 1 and 2). Strong flights were recorded in both non-pheromone treated plots (Control E and W). Pheromone traps were less suppressed for trials 3 and 4 when 81.1 and 82.9% suppression was observed (Fig. 3 and 4). The pattern of effective suppression was again documented with the inclusion of the 2nd pear plot within the same orchard for the 5th trial and we observed 100% suppression of traps (Fig. 5). The 100% suppression occurred despite an average count in the non-treated areas of 33 moths per trap in 3 nights.

The results were similar in the 2 walnut orchards over the test periods (Fig. 6 and 8). Trap flights in Site 1 ranged from 26.5 to 34.5 moths per week in the non-treated areas (Fig. 7). Traps in the pheromone treated areas were again suppressed from 96%, 74%, and 96% over the course of the three weeks. For Site 2, treated with 17-day-old emitters, the results were similar but pressures were lower with an average of 9 moths caught in the non-treated area and 0 (or 100% suppression) were found in the pheromone treated area. Thus, the patterns are similar across crop types. Site 1 was an orchard with limited management such that the canopy of the orchard was relative Spartan. Black line virus had also eliminated many trees such that re-growth by black walnut from the rootstock was relatively common. The effects of the orchard structure on the pheromone dispersion might have allowed some capture of codling moth in the treated areas during the second week. The results with the apple lures were approximately the same as in pears with limited counts in the traps baited with lures containing the apple volatile.

The effects of aging by the meso-emitters cannot be determined with these data. However, dispenser duration should not be the most important consideration since emitter longevity should be the most easily manipulated variable since pheromone load can be adjusted relative to desired emission rate. At 12 emitters per acre, the time and man-power required for the acre application was minimal compared to the traditional application at 200-400 ties per acre.

In the pear site SPLAT trials, counts from traps baited with the apple volatile were small compared to traditional pheromone lures, with average counts in the untreated areas of 11.5, 1.65, and 0.7, 1.15, and 0.15 over the course of the last 4 trials. Several variables were changing over the course of the trials with pear maturation rapidly increasing given that the trials utilizing plant volatiles were conducted late in the season. The highest trap capture occurred in Trial 2 and the numbers declined with each successive trial. A similar pattern has been observed for the pear ester in some orchards presumably due to a changing background in volatile emissions. Clearly, the apple volatile was effective at luring moths into the traps, but the rate and effect of season on lure efficiency needs to be determined.

One outcome that was not anticipated with the fact that the traps baited with the apple volatile did not catch any codling moths in the pheromone treated plots. While synergistic effects of plant volatiles have been noted in the literature, the apparent antagonistic effects have not been reported. If this effect is repeated, either a different plant volatile (e.g. another apple volatile or the pear ester) will need to be considered.

One factor that has not been considered is the lack of stability of the apple volatile in the fiber or flake. While lures were typically changed between trials, the longest any lure was kept in as a treatment was 2 weeks. Questions of volatile stability will need to be addressed next year.

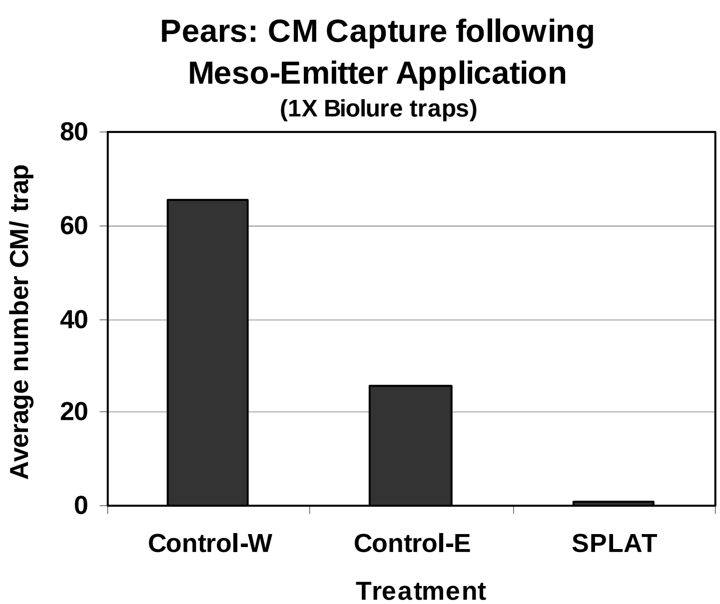

Figure 1. Meso-emitter trial 1 in pears. Codling moth capture in traps baited with 1X Biolures.

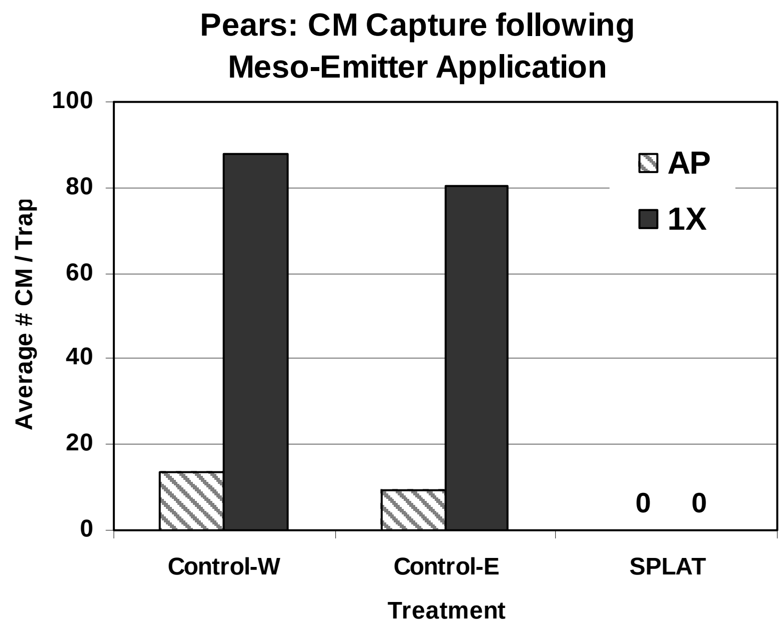

Figure 2. Meso emitter trial 2 in pears. Codling moth capture in traps baited with 1X Biolure or apple volatile (2 fibers per trap).

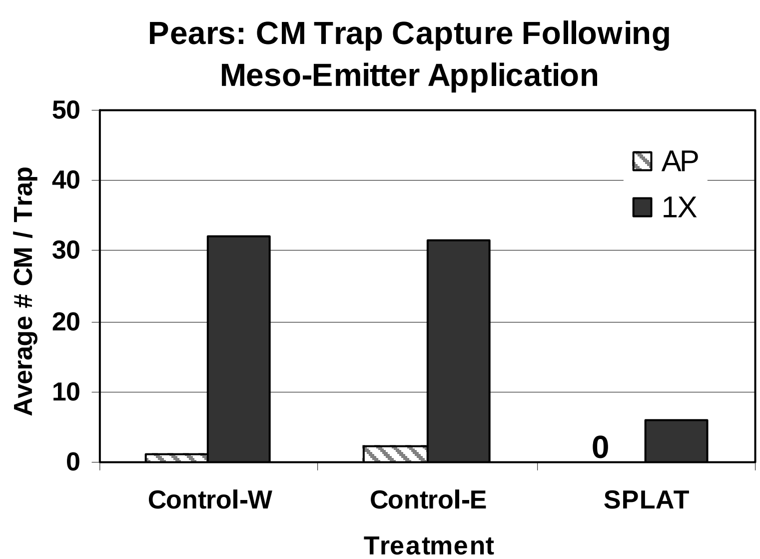

Figure 3. Meso-emitter trial 3 in pears. Average number of codling moth captured in traps baited with 1X Biolure or apple volatile (2 fibers per trap).

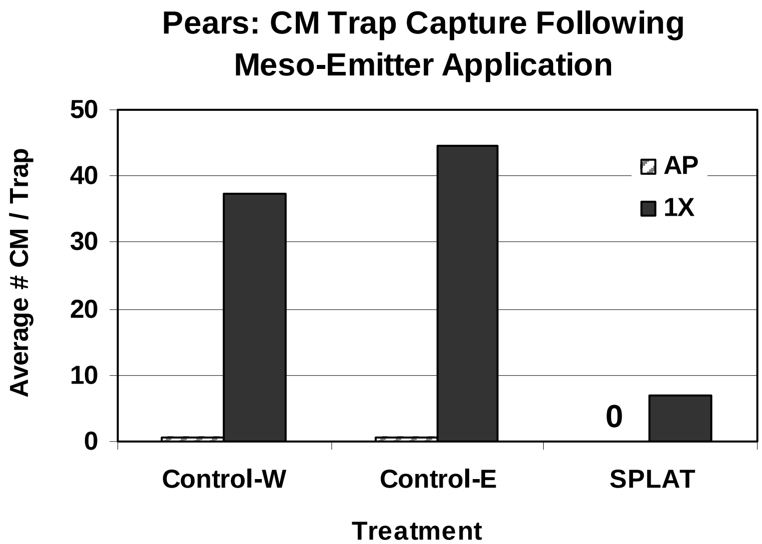

Figure 4. Meso-emitter trial 4 in pears. Average number of codling moth captured in traps baited with 1X Biolure or apple volatile (5 fibers per trap)

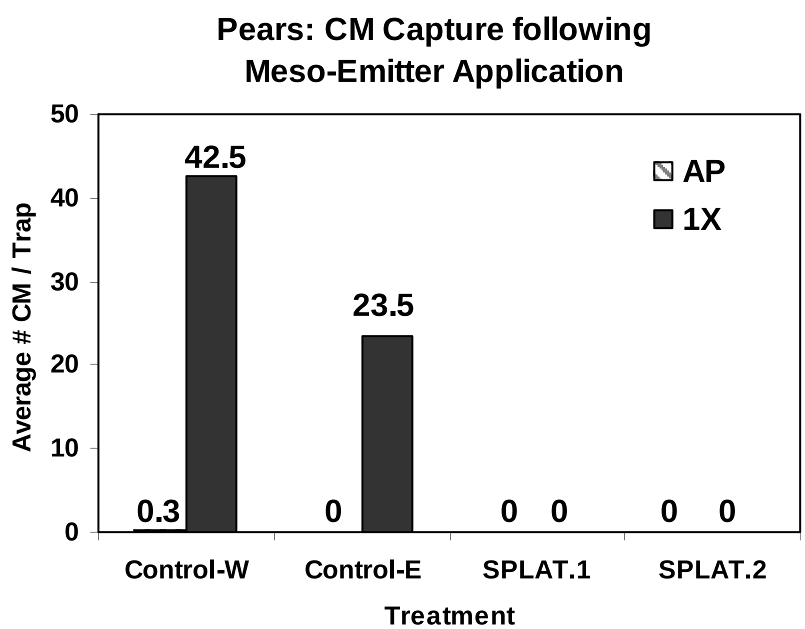

Figure 5. Meso-emitter trial 5 in pears. Average number of codling moth captured in traps baited with 1X Biolure or apple volatile (5 fibers per trap) in two SPLAT and two control plots.

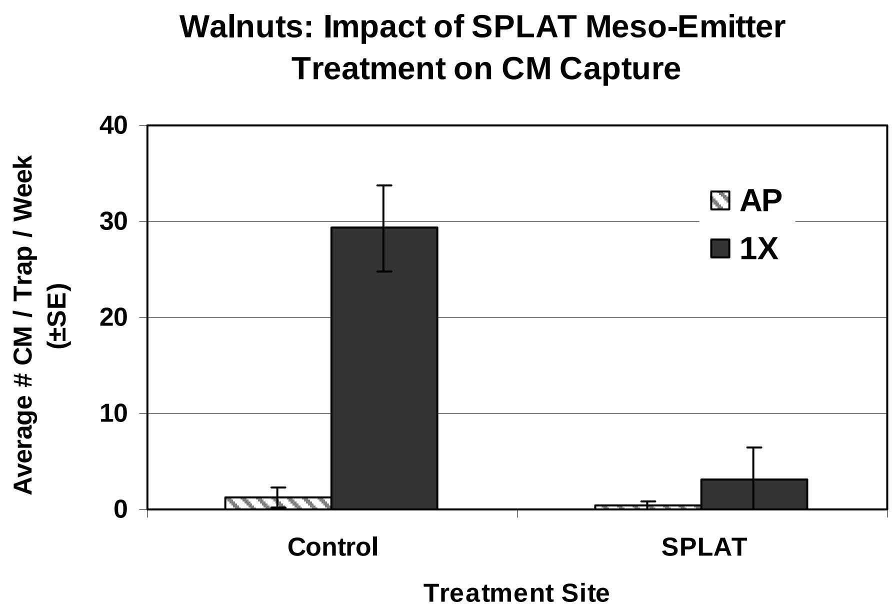

Figure 6. Average number of codling moth captured per week over three weeks in traps baited with 1X Biolure or apple volatile (5 fibers per trap) in one SPLAT and control plot.

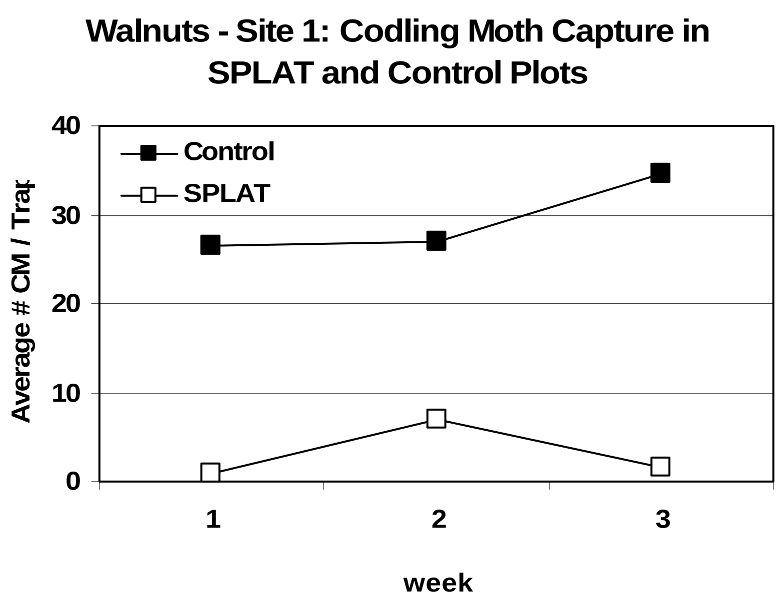

Figure 7. Average number of codling moth captured over three weeks in traps baited with 1X Biolure or apple volatile (5 fibers per trap) in one SPLAT and control plot.

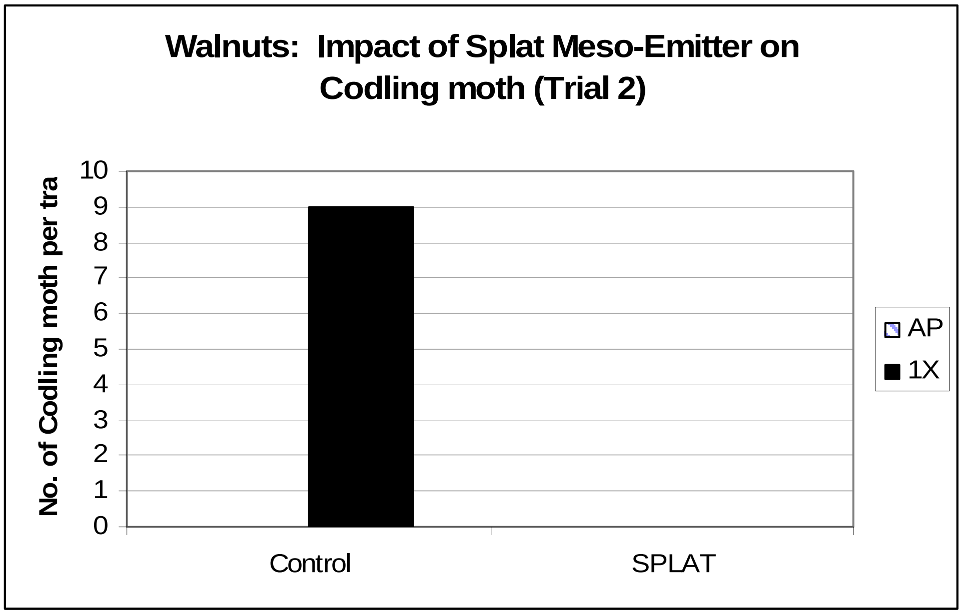

Figure 8. Average number of codling moth captured in Trail 2 using 1X Biolure or apple volatile (5 fibers per trap) in one SPLAT and control plot.

Intrepid and Rimon Insecticides: Baseline resistance levels

Newer insecticides continue to be developed for codling moth as regulations for existing materials, e.g. azinphosmethyl or methyl parathion, make their use more restricted. Similarly, resistance to organophosphates has increased slowly in many orchards throughout the state as shown in field bioassays over time.

Two newer materials that have shown promise in efficacy trials are Intrepid (Methoxyfenoxide) and Rimon/Diamond (Novaluron) for control of codling moth. Rimon EC is not registered in pears in CA at this time primarily because of phytotoxicity. However, novaluron may be registered using a different formulation, Diamond (see label for future registration information). (R. van Steenwyk, pers. com, 11/29/05)
Baseline data for these materials needs to be generated before widespread usage has taken place and prior to any direct selection for resistance. Similarly, orchards with organophosphate resistance have also shown resistance to a wide range of insecticide classes presumably due to multiple detoxification pathways. Determination of baseline resistance levels allows us to track how resistance levels are changing if issues with efficacy should arise.

Therefore, assays for the toxicity of two newer compounds, Intrepid and Novaluron, were developed for an egg bioassay and a larval bioassay using 2 laboratory colonies. While no toxicity was expected from the egg bioassays, the possibility of unexpected results was evaluated. The two colonies were collected from either an organic apple orchard which has not been treated for at least 20 years with an organophosphate (AJ colony) or from an orchard with documented OP resistance (FR colony). Historically, OP resistance has proven very difficult to maintain in the laboratory given the high metabolic costs of resistance such that susceptible females simply out reproduce the OP resistant females. So, the intent of the study was to challenge the materials with populations with very different histories of exposure to organophosphates rather than to colonies with specific resistance levels.

Resistance levels to organophosphate were significantly different between the colonies, but at very low levels. The resistance level in the organic apple was higher than levels traditionally found in our "susceptible colonies" from the early 1990's. Similar to experiences with an organic pear orchard in the Sacramento delta, the general rise in resistance levels on a regional scale seems to increase the background level of populations in the organic orchard as well. Secondly, despite having been shown to have OP resistance in an earlier field bioassay, the level in the FR colony appears to have reverted to some degree as expected. What was not clear was what would be the effect of the historical resistance on the results for the 2 colonies given that low non-significant levels of resistance (e.g. as low as 2X) have been correlated with significantly higher levels of resistance to other insect growth regulators (Moffitt et al. 1988).

Materials and Methods

Resistance Assays. Resistance assays were conducted for two insect growth regulators. Methoxyfenoxide acts by inducing an incomplete and premature molt in larvae. The primary activity of novaluron interferes with cuticle formation in larvae following a molt. Both compounds must be ingested to function by their primary mode of action. We conducted both larval and egg toxicity trials for each compound using recently established lab colonies of codling moth. The FR colony was collected in July 2004 from a highly resistant population in apples near Gridley, CA. The susceptible AJ colony was established from diapausing pre-pupae collected October 2004 from tree bands in an organic apple orchard near Philo, CA. The last laboratory assays of these 2 colonies for azinphosmethyl resistance indicated LC_{50}s for the historically OP resistant colony (FR) and the organic apple orchard (AJ) of 0.74 and 0.43 mg/ml, respectively. The level of susceptibility for the FR was ca. 2 fold greater than typical historical bioassays for susceptible lab colonies. The lethal concentration ratios were significantly higher for the FR colony at 1.7 fold.

Larval assays. Codling moth rearing cups were treated with a dose series of methoxyfenozide (Intrepid 2F, Rohm and Haas, Philadelphia, PA) or novaluron (Rimon 0.83 EC, Crompton Manufacturing Company, Inc., Middlebury, CT). We reared codling moth in one ounce cups containing an agar-wheatgerm based diet. In this trial we flooded the diet surface with 0.1 ml of material and then let the solution dry before placing a newly hatched larva into each cup. Cups were then held at 80°F and larvae evaluated for mortality after 5 and 14 days. Mortality data was analyzed by probit analysis (POLO, LeOra Software, Berkeley, CA) and LC50 values compared by lethal concentration ratio (LCR) to determine significance of observed differences.

Egg assays. Wax paper sheets were sprayed using a Crown Spra-Tool® with approximate field rates of Intrepid 2F (16 fl oz/acre) or Rimon (32 fl oz/acre). Virgin pairs of codling moth were then place in small tubular oviposition cages, ca 2 inches diameter x 4 inches long, lined with the treated egg sheets. Adults were provided a sugar water source and held until eggs were laid. Once eggs were deposited, the cage was disassembled and up to 20 eggs marked for evaluation. Egg sheets were individually bagged and held at 80°F for 6 days before scoring for hatch. For each treatment (Intrepid, novaluron, water control), twenty cages were set up for the AJ colony (60 total) and fifteen cages were set up for the FR colony (45 total).

Results

Baselines for Intrepid (Methoxyfenoxide) and Rimon (novaluron) were developed successfully for the 2 colonies using the larval bioassay. The five day bioassay proved the most efficient (Fig. 1) with higher variability observed after 14 days, thus preventing the development of a statistically valid probit line for the historically OP resistant colony (FR) (Fig. 2, Table 2). A significant difference between the 2 colonies was observed after 5 days for Intrepid with an 18.1 fold increase in resistance level (Fig. 1, Table 1). No difference in susceptibility to novaluruon (Rimon/Diamond) was observed between the 2 colonies for either time frame that mortality was evaluated (Table 1 and 2, Figures 3 and 4). These data from the 2 colonies suggest that variation already exists between orchards relative to their level of susceptibility to Intrepid. No such variation was observed in the novaluron trials.

These data should not be used to extrapolate to field performance. First, the nature of the bioassay is different from a field application which makes direct comparisons very difficult if not impossible. Secondly, the differences between the 2 colonies was greater at dilution rates much lower than field application rates with the larger differences starting to be observed at 1/10 of the field dilution rate.

In the egg bioassay, not all caged pairs were successful at producing eggs. The FR colony was especially poor at egg deposition. Thus, sample sizes varied as follows: the number of eggsheets used to evaluate the AJ colony response to Intrepid, novaluron, and water was 15, 14 and 17, respectively. The number of FR eggsheets available was 3, 7 and 6, respectively. The effect on the egg bioassay was largely as predicted with limited effect of either compound on egg hatch rates (Fig 5), but the Intrepid did have a small, but significant effect on egg mortality ($P = 0.04$). . No colony*treatment interactions were significantly different from each other or from the control ($P > 0.05$).

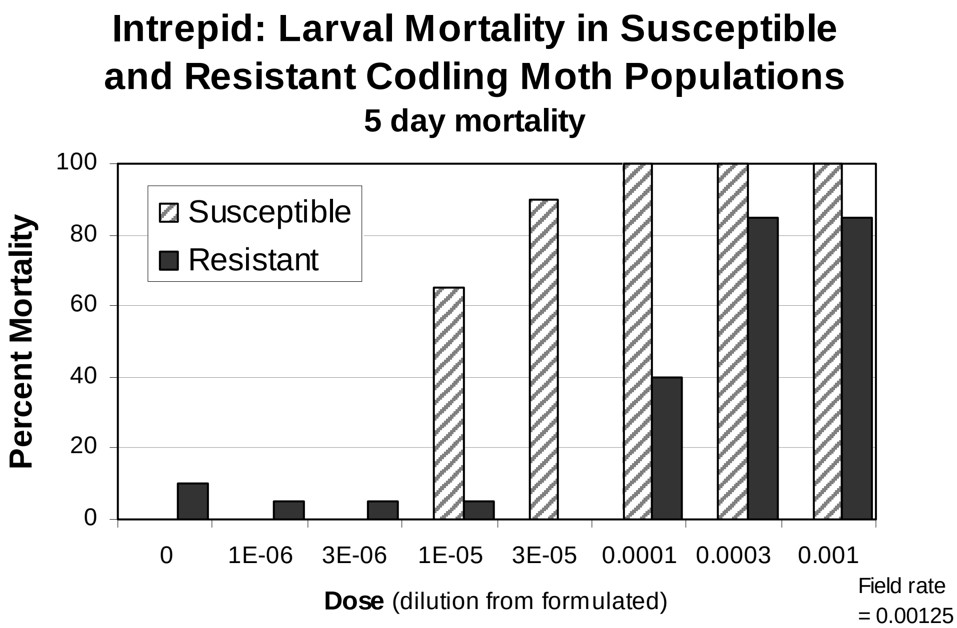

Figure 1. Effect of intrepid on larval mortality after 5 days of exposure for 2 colonies of codling moth with different exposure histories.

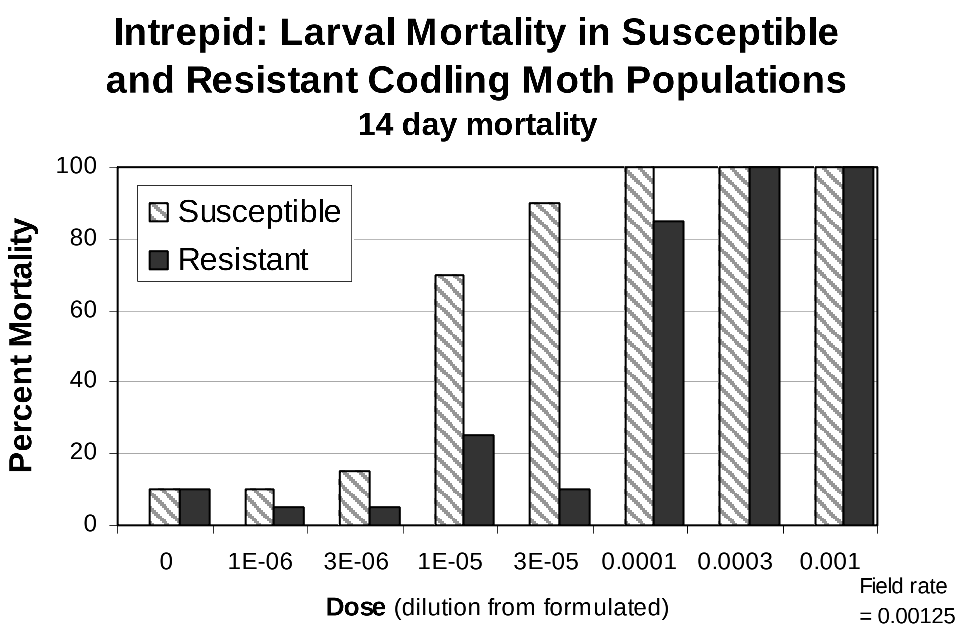

Figure 2. Effect of intrepid on larval mortality after 14 days of exposure for 2 colonies of codling moth with different exposure histories.

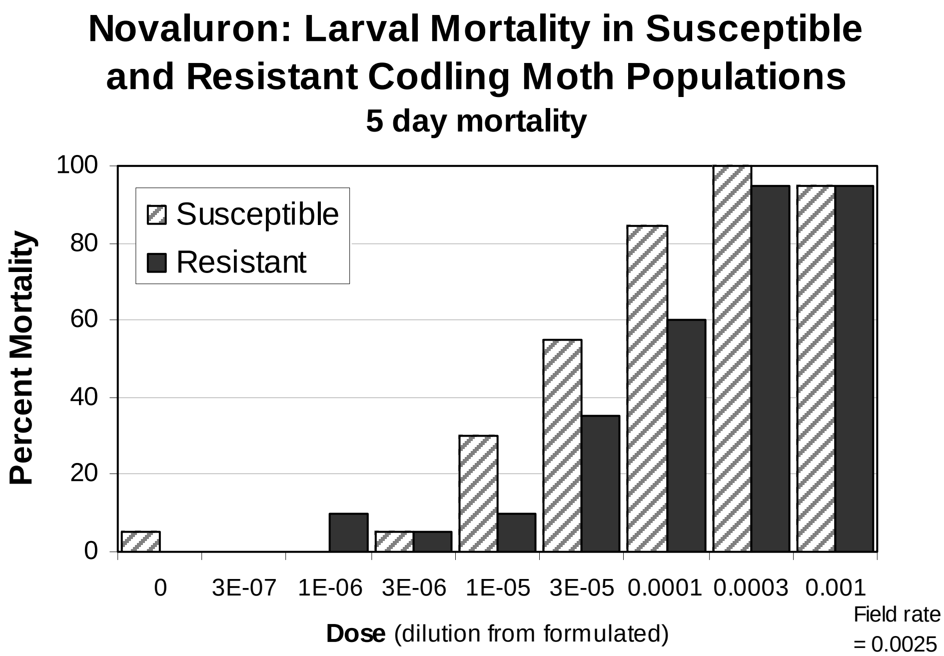

Figure 3. Effect of novaluron on larval mortality after 5 days of exposure for 2 colonies of codling moth with different exposure histories.

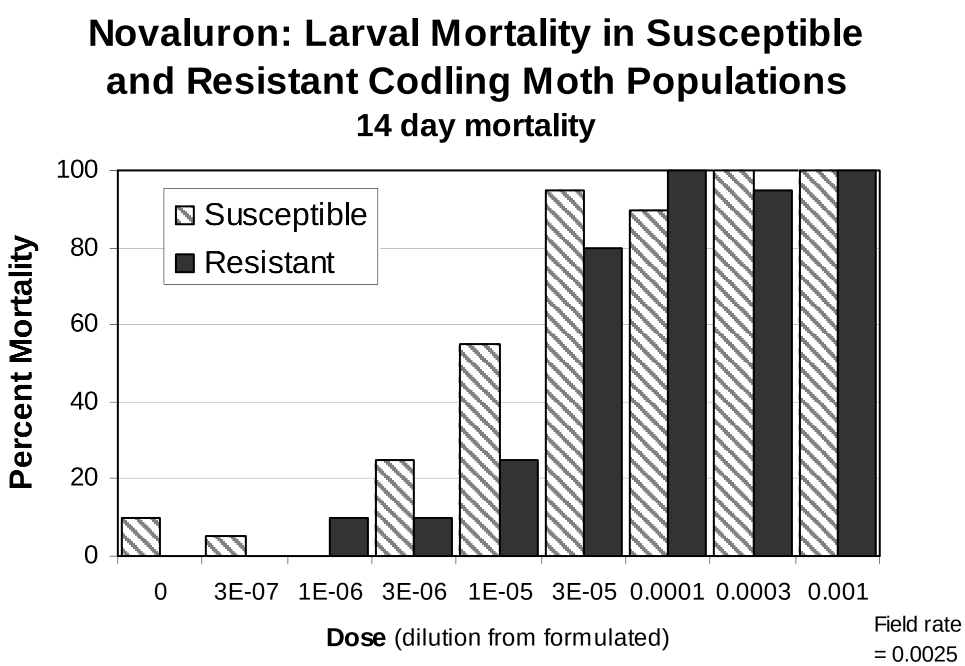

Figure 4. Effect of novaluron on larval mortality after 14 days of exposure for 2 colonies of codling moth with different exposure histories.

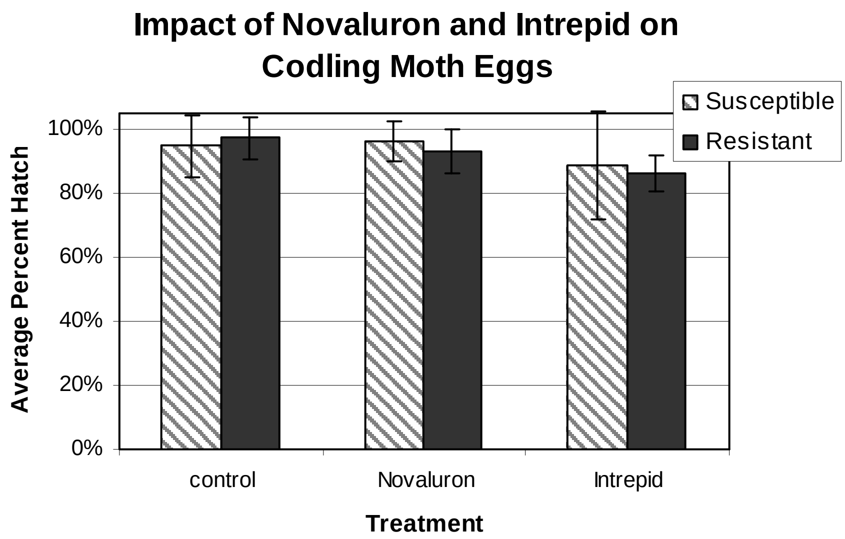

Figure 5. Impact of Novaluron and Intrepid on codling moth egg hatch. No significant differences observed at P> 0.05.

Table 1. Five-day mortality assessment for susceptible and resistant lab populations of codling moth. - LD50 and LCR values and confidence limits.

	LD50	95% CL	LCR (95% CL)
Intrepid			
Susceptible (AJ)	0.00001	0.00001 – 0.00001	18.1 *
Resistant (FR)	0.00017	0.00009 – 0.00032	(10.8 – 30.3)
Novaluron			
Susceptible (AJ)	0.00003	0.00001 – 0.00006	1.71 ns
Resistant (FR)	0.00005	0.00002 – 0.00012	(0.87 – 3.37)

* = significant at 95% limits; ns = not significant

Table 2. Fourteen-day mortality assessment for susceptible and resistant lab populations of codling moth. - LD50 and LCR values and confidence limits.

	LD50	95% CL	LCR (95% CL)
Intrepid			
Susceptible (AJ)	0.00001	0.00000 – 0.00001	--
Resistant (FR)	--	--	
Novaluron			
Susceptible (AJ)	0.00001	0.00001 – 0.00002	1.43 ns
Resistant (FR)	0.00001	0.00001 – 0.00003	(0.77 – 2.6)

* = significant at 95% limits; ns = not significant

Night Behavioral Field Work

Initial efforts to determine if the plant volatiles would result in direct contact with the fibers or flakes containing multiple plant volatiles proved disappointing. Using 3 sets of observers, an array of locations were established in 3 trees each. Each observer wore a LED headlamp system with adjustable red light levels. Therefore, the lowest level of red light which still allowed for the observer to see the platform was used. Each location was marked and a single odor source placed at the location. The following odors were evaluated a) apple alone b) pear alone c) apple plus pheromone or d) pheromone alone). The orchard was being used in other trials such that high trap counts (>50 per week) were being recorded in traditional pheromone traps. The locations were monitored continuously from 9:00 PM to 1:00 AM.

While male moths were detected flying in the area of the locations, their flights proved inconsistent and relatively directionless in appearance. This was somewhat surprising in that traps in the same orchard baited with the apple/pheromone odor in the Scentry fiber were capturing moths during the same evening. Each location was monitored either for a fixed amount of time (e.g. 10 minutes) or each location was included in a repeated “sweep” of multiple locations for a fixed amount of time in order to maximize the likelihood of finding a moth orienting to the odor source.

Despite seeing multiple males as the night progressed in the areas of the traps, no orientation to a specific odor source was noted. Given the variability and low numbers of actual males flying to the odor sources, the decision was made to focus our efforts on the wind tunnel operation during the winter months.

Work in Progress

The work to complete a real-time EAG system has almost been completed. A system using a set of four computer controlled values has been successful built and programmed. Four airstreams can be mixed in different ratios using the existing software (MatLab) and presented in different timings as desired to a live antenna. The electrical responses of the antenna to the odor source(s) are sent to the computer and recorded for analysis. This system is intended to pursue the effects of odor combinations as lures or disruptants including pheromone or plant volatiles. In addition, the role different odor backgrounds can be addressed using a small Teflon chamber to hold plant materials (e.g. leaves, walnuts or pears of different ages) such that the airstreams can present a “background” material in addition to the test volatile compounds.

The construction of a metal framed and glass walled wind-tunnel has been completed with the airflow system to be completed this winter. Data from the EAG and field studies will be used to direct the behavior experiments looking at moth attraction to odor sources of different types or combinations in the wind tunnel.