

**FINAL PROJECT REPORT**  
**WTFRC Project #AE-05-503A**

**WSU Project #13C-3643-4190**

**Project Title:** Codling moth management with pheromones: key unanswered questions

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**Total Project Funding:**

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries <sup>1</sup>	13,0205	112,353	108,305
Benefits <sup>2</sup>	38,081	39,455	37,073
Wages <sup>3</sup>	15,000	19,500	37,000*
Benefits	1,800	1,950	4,075
Equipment	3,000	0	0
Supplies <sup>4</sup>	7,012	11,700	10,700
Travel <sup>5</sup>	8,100	10,500	9,000
<b>WSU total</b>	<b>83,586</b>	<b>81,393</b>	<b>91,629</b>
<b>MSU total</b>	<b>48,730</b>	<b>53,756</b>	<b>54,915</b>
<b>USDA total</b>	<b>35,082</b>	<b>39,609</b>	<b>40,689</b>
<b>Ag Canada total</b>	<b>35,800</b>	<b>20,700</b>	<b>21,420</b>
Miscellaneous	0	0	0
<b>Total</b>	<b>203,198</b>	<b>195,458</b>	<b>206,153</b>

<sup>1</sup> For WSU part only - salary (1 mo.) for Senior Scientific Assistant; salary (11 mo.) for Research Assoc.

<sup>2</sup> Benefits for Senior Scientific Assistant; 34% for Research Associate.

<sup>3</sup> Hourly help to assist with setting up experimental apparatus, collection and analysis of data.

\* Increase of \$20,000 in WSU portion of grant is for Vince Hebert's program to collaborate in assessing release and purity of different pheromone products. Also reflects some reduced allocation to other PIs.

<sup>4</sup> Supplies will include lures, traps, flagging materials, cell phone charges and fuel.

<sup>5</sup> Travel to experimental plots; pays for one car for 6 months.

**Project objectives:**

1. Determine the active space of different pheromone sources (females, lures, dispensers, flakes, fibers, etc.) under MD and non-MD situations. (WSU, MSU)
2. Determine where in the tree CM females call. (WSU, USDA, CA)
3. Determine the aggregation of CM in MD and non-MD orchards. (WSU, CA)
4. Determine the impact of pheromone purity, addition of minor pheromone components, and plant volatiles on attraction of CM in MD orchards. (USDA, MSU, CA)
5. Determine the spatial arrangement of pheromone sources that maximizes MD. (WSU, MSU)
6. Define the effect of host plant volatiles on CM pheromone biology. (USDA, CA)
7. Characterize responses of CM from different geographical areas to pheromones and plant volatiles as baselines for future assessments of resistance. (USDA, WSU, MSU)
8. Utilize the information in objectives 1-6 to optimize pheromone delivery technologies for CM control and monitoring. (WSU, MSU)

**Project summary:**

This project brought together a team of scientists who have been conducting research on tree fruit IPM and behavioral controls of pest for decades. Two post-doctoral students, Lukasz Stelinski and Matthew Grieshop, joined the research team in 2005. Both young scientists contributed to the team and both are now employed as full time scientists at major universities. The research team working on this project met on several occasions over the last three years via conference call and in person in Michigan and Wenatchee to plan and coordinate research. Ideas generated and progress towards objectives was a direct result of the team synergy. This project for the first time developed baseline data on behavior and electrophysiology that can be used to evaluate the development of CM resistance to pheromones. The location of resting CM adults in trees was more evenly distributed than previously thought. The active space of a calling female, female mimics, and other attractive sources, was more clearly identified and generally smaller than expected. The attraction of pheromone components is better understood and confirms that minor components do not seem to add to the behavioral response of males. A combination of kairomones was shown to be more attractive to CM than pear ester. High numbers of mini-dispensers (wax drops, flakes or fibers) distributed throughout the tree canopy had a strong effect on the ability of CM males to located attractive sources. The relationship between the number of pheromone dispensers per area and the release rate per dispensers appears to be the key to making mating disruption more robust. The addition of kairomones to pheromone dispensers does not appear to enhance mating disruption.

**Recommendations:**

True team projects are expensive to fund but the team assembled to work on this project believe the interaction, synergy and progress towards objectives was greater than would have occurred if individuals were working alone. Lessons learned about CM responses to pheromone and kairomones suggest that research focus should be directed at:

- Defining a pheromone delivery system that optimizes mating reduction in CM;
- Develop effective killing stations for kairomones and pheromones or combinations;
- Determine if combinations of pheromone delivery technologies make a more robust MD system than any technology alone or combinations of MD and lure-&-kill stations;
- Evaluate new kairomones as monitoring systems for CM; and
- Investigate the utility of technologies developed for CM as management tools for leafrollers.

**Significant findings – 2007**

1. a) Confirming past results, this year's results suggested the *active space of the female* changes with the season, but is *approximately 10m*.

- b) Testing of MD and monitoring lures must be done across both generations and under different horticultural planting designs in order to make robust recommendations.
  - c) Virgin females and 10 mg codlemone lures were similarly attractive to wild BC (30.1 vs. 31.7%) and lab-reared BC males (22.6 vs. 17.6%) but more wild males were recaptured.
2. a) Significant number of moths were found on the ground in screened tents; 21% of females and 34% of males 16 hours after release, and 52% of females and 24% of males after 64 hours.
    - b) Using a vacuum method to collect wild CM adults showed that there was a relatively even distribution of male and female moths throughout the tree canopy.
    - c) The outcome of an Isomate C Plus treatment was greatly affected by the location of dispensers. Substantial mating high in the canopy occurred when dispensers were placed low, while the greatest mating low in the canopy occurred when dispensers were placed high.
  3. a) Laboratory moths released in an orchard were recovered (36% males and 28% females) on ground tarps after a permethrin+PBO as a knockdown agent was applied by airblast-sprayer. Both wild and laboratory moth captures were fairly uniformly distributed across the three rows. In addition to adult moths, several CM larva and a single OBLR were recovered over the course of the experiment.
    - b) Fewest moths were captured where both traps and dispensers were placed high (4m) in the tree. However, mating of tethered virgin females was lowest where dispensers were placed at a combination of heights, 2m and 4m.
  4. a) In all trapping experiments, traps with acetic acid plus pear ester captured many more male and female codling moths than unbaited traps or traps baited with the individual compounds. Overall, about twice as many males and twice as many females were captured with acetic acid, compared to pear ester, but in two tests this ratio was 5 to 1.
    - b) The increased complexity of the female blend (lab females 2006) and wild females (2007) did not appear to significantly affect landing rates in laboratory flight tunnel assays. It therefore seems unlikely lures for MD or monitoring could be improved by adding additional components from female pheromone glands.
  5. a) In the first generation CM recapture was suppressed most by the 10-flake dispenser at a rate of 1,500 per acre. There was a gradual reduction in moth recapture when the number of single flakes per acre increased from 500 to 15,000.
    - b) In the second generation, CM recapture was suppressed most by single flake dispensers at a rate of 15,000 per acre.
  6. None of the plant volatiles, *ethyl (E,Z)-2,4-decadienoate*, *linalool*, *E-3-hexen-1-ol*, and *farnesol*, tested when added at any ratio with either amount of codlemone, increased male response over codlemone alone in this lab setting.
  7. a) In comparative flight tunnel study trials the response of BC and WA moths were statistically indistinguishable and similar across years (2006 and 2007).
    - b) Wild moths were significantly less responsive to codlemone or the codlemone + PE combination lure than the lab SIR moths when tested under standardized laboratory conditions.
    - c) Laboratory moths are useful for assessing general behavioral responses to various chemical lures in the lab, but can't be compared with wild moths in an absolute sense.
    - d) Based on electrophysiological studies there does not appear to be any consistent measurable difference between CM populations in their physiological sensitivity to pheromone or pear ester. Any behavioral differences observed in the field must be the result of differential sensitivity to environmental cues or dispersal propensity, e.g. following diapause compared with non-diapause lab individuals.
  8. a) When Hercon micro-flakes were applied to trees at rates of approximately 42, 126 and 420 per tree they disrupted CM at the rate of 83%, 81%, 97% compared to the control. In the same test Isomate C-plus at 400 dispensers per acre disrupted CM attraction to traps by 94%.

- b) A new method of pheromone application was combined with a new pheromone carrier formulation that had a significantly lower moth capture than Isomate C+. The test formulation at 6000 dispensers per acre required approximately 25% less time to apply than the 400 Isomate ropes.

## **2007 Methods and Results**

### **Objective 1 – Active Space:**

#### **Virgin Females - Release Recapture Distances - Grid Releases & Row Recapture**

**Methods:** In 2007, the distance over which wild males emerging from diapause “respond” to calling virgin wild females was tested in the same high-density apple block used in 2005 and 2006. Fifty to 100, two-day-old wild males were released at distances of 5, 10, 20, 40 and 80 m downwind of ten, virgin-female-baited sticky traps. Three releases were made in late spring on May 28, June 4 and June 11 and four releases in summer on August 14, 17, 21 and 24. Catches were recorded daily for three days, but because most moths were recaptured on the first night thus all data presented are limited to catches during the first 24 h only.

**Spring Releases.** During three spring releases the mean ( $\pm$ SE) percentage of female-baited traps that caught at least one released wild male within the first 24 h was  $78 \pm 11.1\%$  (7-8 females). Within this same 24 h interval males were caught from all release distances, but generally mean recapture rates declined with distance (5m =  $14 \pm 4\%$ , 10m =  $22.1 \pm 3.5\%$ , 20m =  $9.1 \pm 3.2\%$ , 40m =  $5.3 \pm 2.3$  and 80m =  $4 \pm 1.5\%$ ). As shown previously, maximum catch was from males released 10 m downwind rather than the nearest release distance. This suggests that there is an optimum response distance for a given pheromone source, probably dictated by the horizontal and vertical dimensions of a plume at given downwind distances.

**Summer Releases.** Males were caught from all release distances within the first 24 h, *but this did not occur in all releases*. Overall, during summer 2007 the mean ( $\pm$ SE) percentage recapture rates within the first 24 h declined with distance, similar to the pattern seen in 2005 & 2006 (5m =  $9 \pm 4\%$ , 10m =  $13.7 \pm 3.2\%$ , 20m =  $8.5 \pm 3.2\%$ , 40m =  $2.5 \pm 0.3$  and 80m =  $3 \pm 2.3\%$ ). Again, maximum recapture was from males released 10 m downwind rather than the nearest release distance. The recapture rates were generally lower in summer 2007 compared with spring 2007, again reflecting the same patterns as seen in 2005 & 2006.

**Explanatory Hypotheses:** Lack of male recapture from greater distances in most summer releases, and lower overall recapture rates in summer compared with spring in three successive years, can be explained by several hypotheses:

- Hyp. 1) a female plume penetrates a less dense spring canopy easier and over greater distance than it penetrates a more dense summer canopy,
- Hyp. 2) tree canopy is denser in summer therefore summer males are physically impeded and move shorter distances within a summer canopy than they do a spring canopy, or
- Hyp. 3) it is a combination of these two factors.

**Practical Significance:** 1) These studies suggest the *active space of the female* changes with the season, but is *approximately 10 m*, and 2) the optimal pheromone monitoring lure or disruption dispenser may be different in different seasons, both likely due to crop canopy effects. Hence, testing of MD and monitoring lures must be done across both generations and under different horticultural planting designs in order to make robust recommendations.

#### **Virgin Females vs. 10 mg Pheromone lures - Central Point Release & Circular Recapture:**

**Methods:** In 2007, we repeated mark-release-recapture experiments using a single point release of moths and a circular trapping design in an attempt to find a pheromone lure that most adequately mimics a virgin female in terms of its active space, and to determine if the displacement of released males was more or less directional in the presence of different pheromone sources. Marked wild and

lab-reared BC moths were released simultaneously from a central release point within a small high-density Gala planting. Traps baited with either a virgin female or a 10 mg codlemone lure and deployed in circular patterns around the release point at distances of 10, 20 or 30 m. Releases of 400 - 500, two-day-old males were made May 31 (female traps) and June 14 (codlemone lures). Catches were recorded for three days but only the first 24 h recapture rates are reported.

**Results.** In circular recapture experiments virgin females and 10 mg codlemone lures were similarly attractive in recapturing released wild BC males (30.1 vs. 31.7%), and lab-reared BC males (22.6 vs. 17.6%) but more wild males were recaptured.

**Objective 2 – CM Location in the Canopy:**

**Methods:** Two experiments were conducted in 2007. A mark recapture study was conducted in an effort to determine the location of both adult male and female codling moth during daylight hours. Four large tents were erected over single apple trees, extending out over the drive row grass. Releases of moths marked with luminescent powder were made over seventeen dates. On each occasion, twenty male and twenty female moths were released between 18:00-19:00 hours and recaptured using a vacuum method at 16 hours, 40 hours, and 64 hours post release, between the hours 10:00 to 14:00.

**Results and Discussion:** Male and female moths were more evenly distributed within the canopy than had previously been reported with equal numbers found in the mid and upper canopy. Moths were also found low in the canopy. In addition, significant numbers of moths were found on the ground in screened tents. The mean temperature in the two microclimates did not vary over a 3-month period, but mean relative humidity was higher in the grass by 13.3% compared to the tree. The higher availability of moisture in the grass could provide an explanation for why moths are found in this habitat during hot, dry summer days.

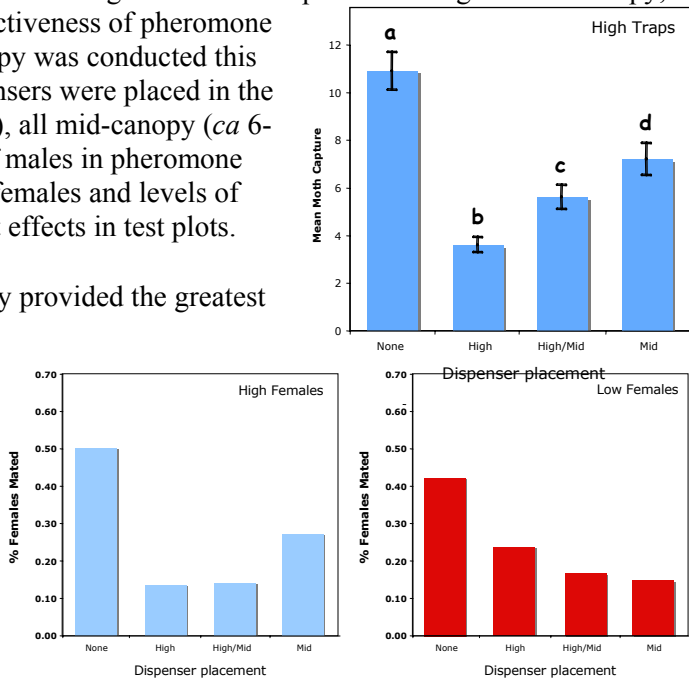
**Methods:** As a practical follow-up to our finding that moths are present throughout the canopy, an experiment aimed at evaluating the effectiveness of pheromone applied at different heights in the tree canopy was conducted this year at MSU's TNRC. Isomate 'rope' dispensers were placed in the canopy in three ways: all high (upper third), all mid-canopy (ca 6-8 ft), or half high and half mid. Capture of males in pheromone traps baited with lures, mating of tethered females and levels of fruit injury were used to evaluate treatment effects in test plots.

**Results and Discussion:**

- Dispenser placement high in the canopy provided the greatest trap-shutdown;
- Dispenser placement low in the canopy provided the weakest effect;
- All treatments provided equal shutdown of traps placed low in the canopy (data not shown).

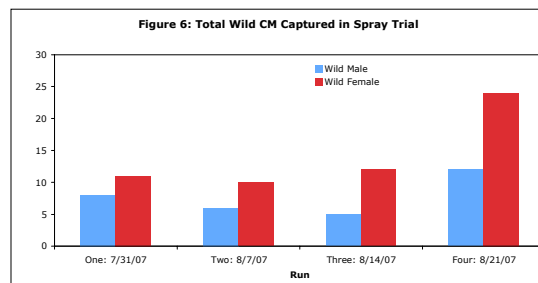
**The effect of dispenser placement on CM mating high or low in the canopy.**

- Greatest mating high in the canopy occurred when dispensers were placed low;
- Greatest mating low in the canopy occurred when dispensers were placed high.



**-Objective 3 – Aggregation of CM in Orchards.**

**Methods:** An experiment was conducted to evaluate a method of assessing CM adult



distributions in orchards. Four weekly applications of Pyranyl (Pyrethrum + PBO) on three adjacent 200' sections of apple trees. Ground tarps were placed along either side of the treated tree rows. Applications were made beginning at 8:30 AM on July 31, and 7, 14, and 21 Aug. Immediately prior to each application twenty male and female moths were distributed throughout each of the three 200' tree row stretches. Within 10 minutes of moth placement, Pyranyl was applied using an air blast sprayer at 100 GPA. After 15 minutes the tarps were examined for CM. Moths were collected and brought back to the laboratory and examined for sex and source (marked lab vs. unmarked wild).

**Results and Discussion:** A weekly average of 7.75 wild males and 14.25 wild females were recovered over the course of the four applications (Figs 6 and 7, at right). Laboratory males and females were recovered at the average rate of 36% and 28%, respectively. Both wild and laboratory moth captures were fairly uniformly distributed across the three rows. The spatial distribution of wild moths was pretty uniform over this relatively small study area. In addition to adult moths several CM larva and a single OBLR were recaptured over the course of the experiment. The pyrethrum sprays could be used to directly sample large contiguous orchard areas. However, initial setup costs are high though spraying and sampling costs are fairly low.

#### **Objective 4 – Pheromone purity, components and plant volatiles.**

CM probably uses volatile chemicals from apple and pear fruit in host-finding. These compounds may be useful for monitoring CM or for lure and kill strategies for CM population management. The aim of these studies was to discover kairomones that are chemical attractants for CM under field conditions.

#### **Methods:**

**Study 1.** Combinations of apple and pear odor compounds were evaluated in wing and bucket type traps. Most compounds were formulated in rubber septa. These experiments were conducted in commercial apple orchards.

**Study 2.** A series of trapping experiments in commercial apple orchards evaluated the attractiveness of apple and pear fruit placed within traps as bait. This included immature apples, infested apples, ripe apples, and ripe pears.

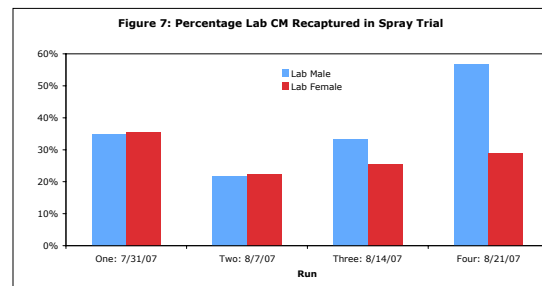
**Study 3.** The combination of acetic acid (AA) and pear ester (PE) was found to be promising from results of studies 1 & 2, and was further tested in multiple trapping tests. The synergy of the two compounds, when used together as a trap bait, was demonstrated in a series of field experiments that compared the two component blend, each chemical separately, and an unbaited trap. The experiment was conducted near Yakima and Wenatchee, Washington, in Medford, Oregon, in Michigan, in New Zealand, and in Hungary. These tests involved both flights of CM.

**Study 4.** A series of trapping tests compared different amounts and ratios of AA and PE in traps, when the chemicals were in separate dispensers (bottles and septa), or were mixed in the same dispenser (bottle). The amounts of acetic AA and PE released over time were determined empirically in the laboratory using three methods (gravimetric, SPME, air collections).

#### **Results and Discussion:**

**Study 1.** The combination of AA and PE was attractive in the field, luring significantly more males and females into traps, compared to unbaited traps. This lure was further evaluated to determine if the compounds are synergistic, and if the blend is superior to pear ester in attracting female CM.

**Study 2.** Both male and female CM moths were consistently captured in apple orchards in traps baited with immature and ripe apples, as well as infested apples and ripe pears. The CM response to ripe pear fruit suggests that moths are attracted to fruit in part to find sugar, and that AA as a sugar



fermentation product might enhance moth response to PE (Table 1). These findings also suggest avenues to pursue to seek another attractant based on apple odorants, since apples do not appear to produce PE.

Table 1. # of male and female CM captured/ week baited with apples and pears. Yakima Co. apple orchards.

Sex	Control	Green Apple	Infested green apple	Ripe apple	Ripe pear
<b>Test 1.</b>					
Females	0.0 ± 0.0a	NT	NT	1.9 ± 0.5b	NT
Males	0.0 ± 0.0a	NT	NT	2.0 ± 0.4b	NT
<b>Test 2.</b>					
Females	0.0 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.1ab	0.3 ± 0.3b	NT
Males	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.1a	0.6 ± 0.3b	NT
<b>Test 3.</b>					
Females	0.0 ± 0.0a	NT	NT	0.9 ± 0.4b	4.5 ± 1.0c
Males	0.0 ± 0.0a	NT	NT	0.6 ± 0.3b	7.0 ± 2.0c

Means within a row followed by the same letter are not significantly different at  $P < 0.05$ ; ANOVA followed by Tukey's test. NT is not tested.

**Study 3.** In all trapping experiments, traps with AA plus PE captured many more male and female CM than unbaited traps or traps baited with the individual compounds. Overall, about twice as many males and twice as many females were captured with AA, compared to PE, but in two tests this ratio was 5 to 1 (Table 2, below).

Table 2. # of male and female codling moths captured per trap-check in traps with acetic acid and pear ester.

Test and Conditions	Control	Acetic Acid	Pear Ester	Combination
1. Yakima, Aug., 2006				
Females	0.0 + 0.0a	0.4 + 0.2a	0.2 + 0.1a	2.2 + 0.7b
Males	0.0 + 0.0a	0.5 + 0.3a	0.3 + 0.2a	2.3 + 0.7b
2. New Zealand, Nov. 2006				
Females	0.0 + 0.0a	0.2 + 0.0a	6.0 + 1.2b	16.9 + 2.2c
Males	0.0 + 0.0a	0.9 + 0.3a	15.5 + 3.2b	32.6 + 3.3c
3. Yakima, 2007 season				
Females	0.0 + 0.0a	0.5 + 0.2b	2.1 + 0.5c	3.6 + 0.6d
Males	0.0 + 0.0a	0.5 + 0.1b	3.4 + 0.6c	6.2 + 0.9d
4. Wenatchee, 2007 season				
Females	0.0 + 0.0a	0.2 + 0.1b	1.7 + 0.3c	2.8 + 0.4d
Males	0.7 + 0.3a	1.6 + 0.4b	2.7 + 0.5c	4.3 + 0.7d
5. Hungary, 2007				
Females	0.0 + 0.0a	0.3 + 0.1	0.7 + 0.2	2.1 + 0.3
Males	0.0 + 0.0a	0.1 + 0.0	0.1 + 0.1	0.3 + 0.1

Means within a row followed by the same letter are not significantly different at  $P < 0.05$ . ANOVA followed by Tukey's test.

**Study 4.** With AA dispensed from a bottle and PE dispensed from a septum, greatest captures of CM in traps were with AA in bottles with 5 mm diameter holes in the lid, and septa with a 500 mg load of PE. With this lure, CM catches were similar with UniTraps, Delta traps and Wing traps. When AA and PE were mixed in a bottle with a 3 mm hole in the lid, greatest numbers of CM were captured with 5 to 20% PE in AA. Preliminary laboratory results indicate a stable release of these two compounds from the mixture for at least 2 wks.

#### Wild Female Gland Extract vs. Codlemone in Clean Air

**Methods:** The role of minor pheromone components found in female pheromone glands as attractants for male codling moth was evaluated in flight-tunnel choice tests. In 2007, we tested synthetic codlemone of the highest purity (99.5%) delivered at a female equivalent rate (10 pg / min) from a microsprayer while paired (10 cm source separation) against a *pheromone gland extract from the wild BC moths* delivered at an equivalent codlemone rate.

**Results:** The BC laboratory strain (n = 100 moths flown) and wild BC moths (75 moths flown) landed on each source with equal frequency, indicating wild females and lab females produce an equally attractive source. Overall response among lab males was higher than wild males. The increased complexity of the female blend (lab females 2006) and wild females (2007) did not appear to significantly affect landing rates in laboratory flight tunnel assays. It seems unlikely lures for MD or monitoring could be improved by adding additional components from female pheromone glands. Improved MD will likely come about by modifying the pheromone dispenser release rate.

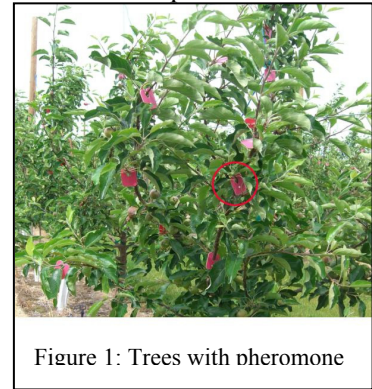
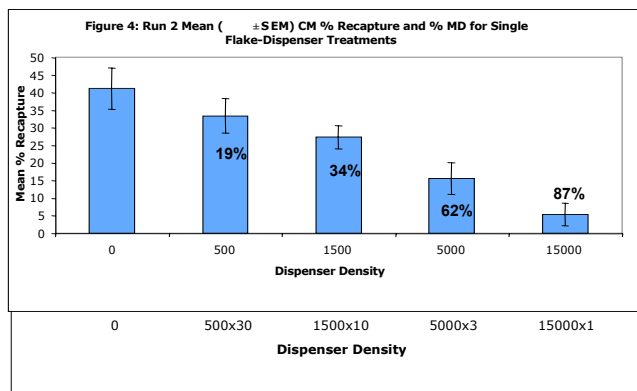
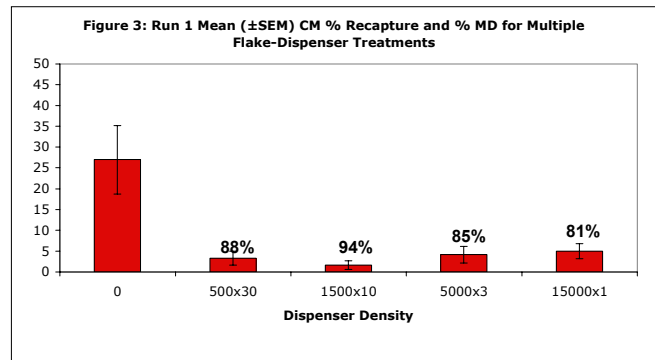
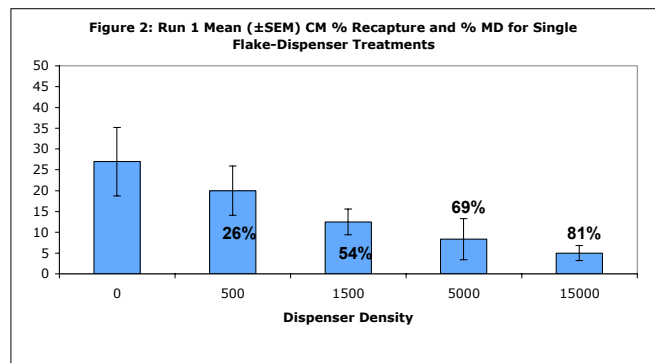


Figure 1: Trees with pheromone

**Objective 5 – Spatial arrangement of competing pheromone sources.**

**Methods:** Mark Release and Recapture (MRR) of laboratory reared CM was used to assess eight different hand applied mating disruption treatments utilizing Hercon Flakes. The eight treatments consisted of: 1) an untreated control, 2) 500 single flakes per acre, 3) 1500 single flakes per acre, 4) 5000 single flakes per acre, 5) 15000 single flakes per acre, 6) 500 30-flake clusters per acre, 7) 1500 10-flake cluster per acre, and 8) 5000 3-flake cluster per acre (Figs. 1-3, at right). Treatments 1-5 represented an increase in both the number of pheromone point sources as well as overall active ingredient, while treatments 5-8 represented an increase in pheromone point sources while keeping the amount of active ingredient per area constant. Marked moths were released in the center row of 0.2 acre plots. Two pheromone traps were placed in each plot, within 10m of release point. Marked moths were released during the first (May 28-July 2) and second (July 20-August 12) CM generation. A subset of the dispensers used in the experiment were collected each week and sent to the WSU-FEQL laboratory for volatile collection analysis.

**Results and Discussion:** Volatile collections from field-aged samples indicated that the release rate of flakes used in this study was greatly reduced after 2-3 weeks, therefore, only the first 2 weeks of data are presented. During the first CM generation





recapture declined with increasing number of point sources when single flake clusters were considered (Fig. 2), however this pattern was not apparent when the total amount of active ingredient was controlled for (Fig. 3). In fact the 1500 10-flake treatment provided numerically superior reduction in moth activity. In the second CM generation the 15000 single-flake treatment provided the most suppression of moth activity (Figs. 4 and 5, at right).

*Practical Significance:* The treatment providing the most consistent disruption of moth activity was the 15000 single-flake treatment. This lends support to the hypothesis that maximizing low release point sources will enhance mating disruption. However, during the first run total amount of active ingredient seemed to be the determining factor for mating disruption. As moths used during both runs came from the same non-diapausing colony it is possible that some environmental factor may be mediating mating disruption between the first and second CM generation. Potential environmental factors include: temperature, wind conditions, canopy architecture/development, or a combination of factors.

### ***Objective 6 – Effect of host plant volatiles on CM pheromone biology.***

#### **Flight tunnel tests of pheromone + plant volatiles - No-Choice tests – clean air**

**Methods:** In 2007, we completed studies started in 2006, where several previously identified plant volatiles (kairomones) known to attract male codling moth to varying degrees were tested as potential synergists of codlemone. Experiments were conducted in a flight tunnel using BC laboratory-reared moths. Plant volatiles (*ethyl (E,Z)-2,4-decadienoate, a linalool, E-3-hexen-1-ol, and farnesol*) added to these septa in serial amounts ranging from 0 - 10,000 mg to produce lures with the following codlemone + kairomone ratios (mg:mg).

**Results:** In 2007, studies confirmed when using *no-choice tests*, that none of the plant volatiles tested, when added at any ratio with either amount of codlemone, increased male response over codlemone alone in this lab setting. These particular kairomones do not appear to hold promise as improved monitoring tools for CM.

### ***Objective 7 – Baseline characterization of behavior and electrophysiology.***

**Methods:** In 2007, wild CM larvae collected in tree bands from Washington (WA) and British Columbia (BC) in winter 2006, were shipped to Summerland for 2007 -behavioral tests needed to increase sample sizes for those done in 2006. We compared the relative responses of each population to codlemone, pear ester and a natural female pheromone gland extract. Adults from all populations were emerged from diapause larvae and flown (two-day-old) in a flight tunnel.

**Results and Discussion:** In these flight-tunnel tests, the responses of males from different populations to a 10 mg codlemone lure were numerically different ranging from 42 – 80.4% source contact. The SIR laboratory strain of moths was most responsive (80.4±5.7% in 2007), while wild moths from BC (58.6±5.7%, 2007) and WA (59.2±6.4%, 2007) had near identical intermediate responses and wild MI moths in 2006 showed the lowest and most variable responses to this codlemone lure (42.4±15.6%).

The above procedure was repeated to compare the response of males from different populations to a codlemone + pear ester combination lure (10 mg + 100 mg, respectively). The BC laboratory strain of moths was most responsive to this combination lure (75.8±4.4%), with wild moths from BC (53.9±4.7%) and WA (62.1±10.8%) having similar intermediate responses and wild MI moths (2006) showed the lowest responses to this combination lure (27.1±7.4%).

*Practical Significance:* The responses of BC and WA moths were statistically indistinguishable and similar across years. Wild moths were significantly less responsive to codlemone or the codlemone + PE combination lure than the lab moths when tested under standardized laboratory conditions. Laboratory moths are useful for assessing general behavioral responses to various chemical lures in the lab, but can't be compared with wild moths in an absolute sense.

## Male + Female EAG's

**Methods:** Physiological responses of over wintered wild male and female CM from different populations (BC and WA) to codlemone and pear ester delivered individually were measured using electroantennogram techniques (EAG's). Excised antennae from two-day-old moths were challenged with serial dilution series of codlemone and pear ester. The normalized responses of antennae from each population were compared to each other and those of the USDA and SIR laboratory strains. Responses of male and female antennae were compared but analyzed separately.

**Males.** In 2007, like 2006, there were no significant differences among the wild populations (BC vs. WA) in the EAG responses of males to either codlemone, or pear ester, at any dose tested (n = 30 males / population / chemical). The dose-response regression lines for each chemical were also similar among the wild populations, all increased linearly as a function of log-dose. As expected, male antennae from all wild populations were significantly more responsive to codlemone (ca. 100H) than to pear ester. EAG responses of the BC laboratory strain were significantly more responsive to the three highest concentrations of each stimulus than were any of the wild populations. These differences may reflect a more uniform quality of the non-diapause laboratory-reared insects compared with wild moths emerging from several months in diapause storage.

**Females.** In 2007, like 2006, females from all wild and laboratory populations exhibited similarly low responses to codlemone and no dose-response relationships were evident in any population over the range of codlemone doses tested. Generally, females within each population had greater antennal response to pear ester than did males from the same population. As expected, females from all populations exhibited significant dose-response relationships to pear ester. Unlike 2006, wild female CM populations from BC and WA exhibited similar EAG responses to pear ester. The response of wild females from BC and WA was also similar to the responses of the BC lab females.

**Practical Significance:** There does not appear to be any consistent measurable difference between CM populations in their physiological sensitivity to pheromone or pear ester. Any behavioral differences observed in the field must be the result of differential sensitivity to environmental cues or dispersal propensity, e.g. following diapause compared with non-diapause lab individuals.

## Objective 8 – Development and optimization of pheromone delivery technology.

**Methods:** A six-acre apple block (243 trees/acre) was used in the trial. Five 90' by 90' subplots, each consisting of five tree rows were evenly spaced throughout the block and randomly assigned one of five treatments: 0 pheromone, one, three or ten passes with the flake applicator, or Isomate C-plus at 400 dispensers/acre (dpa). Flakes were applied at the rate of 1.0 lb per acre. A deposition test indicated that with each pass approximately 42 flakes were deposited per tree. Marked CM moths were released in the center of each plot once per week for four weeks and traps, two per plot baited with 0.1 mg lure, were checked twice a week and lures were changed weekly. Wild moth captures were recorded in addition to the marked moths. Pheromone treatments were applied on July 23, 2007 with marked CM released on July 25, August 1, 8, 15, and 22.

**Results and Discussion:** The 1, 3, 10 pass, and Isomate C-plus treatments disrupted CM at the rate of 83%, 81%, 97%, 94% compared to the control (Fig. 1, at right). A slightly different trend was observed for wild moths with 72%, 92%, 90%, and 97% disruption compared to the control for the 1, 3, 10, and Isomate C-plus treatments (Fig. 2, at right). During weeks four and five, an increasing number of CM were recaptured in all three-flake treatments but not in the control or Isomate treatment.

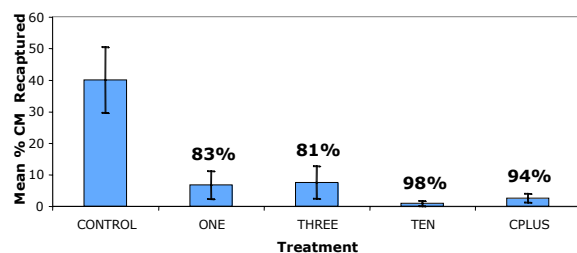


Figure 1: 2007 Mean ( $\pm$ SEM) Percentage Marked Codling Moth Recapture and Percentage Disruption

*Practical Significance:* Results for the 3 and 10 pass flake treatments were similar to the single row treatment experiment conducted in 2006, suggesting that geometric shape of the treated area *did not have* an impact on the flake treatment but had a much greater effect on the Isomate C-plus treatment. This further suggests that MD due to large numbers of relatively low release rate devices, such as the Hercon flake, may be possible in relatively small orchards with higher edge to area ratios. However, it required 10 passes of flakes (ca. 420 per tree) to match the disruption effect provided by Isomate C-plus in rectangular orchard plots. Further research into the spatial scale and application area shapes at which either high density, low release rate (i.e. Hercon Micro-disrupt) and low density, high release rate (i.e. Isomate C plus) pheromone dispensers operate might aid growers in choosing the appropriate technology for their orchard.

**Methods:** A new method of pheromone application was combined with a new pheromone carrier formulation and tested against Isomate dispensers for control of CM. Tests were performed over the summer of 2007 in various locations throughout southern Michigan. Pheromone dispensers were produced by melting paraffin wax, adding various additives and pheromone. Isomate C+ was applied at 400 dispensers per acre, totaling 73 grams of codlemone per acre. The test formulation was applied at 6,000 dispensers per acre. Three treatments of codlemone concentrations were compared in this test formulation to total amounts of 50, 150 and 500 grams per acre. Five replicates of each treatment and a negative control were compared throughout the second flight of CM.

**Results:** All pheromone treatments significantly reduced trap catch when compared to the negative control. The test formulation treatment with 150 grams of codlemone per acre had a significantly lower moth capture than Isomate C+. The test formulation at 6,000 dispensers per acre required approximately 25% less time to apply than the 400 Isomate ropes.

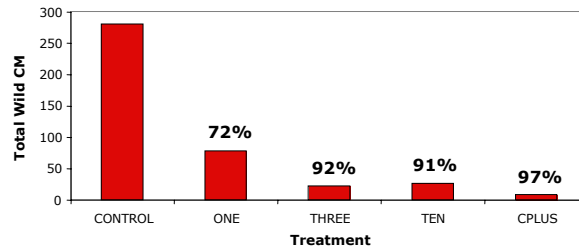


Figure 2: 2007 Wild CM Capture and Percentage Disruption