FINAL PROJECT REPORT
WTFRC Project Number: CP12-106A

Project Title: Optimizing attract and kill to enhance control of apple pests

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Cooperators: Peter McGhee, PhD student, Michigan State University

Other funding Sources: *None*

WTFRC Collaborative expenses: *None*

Total Project Funding: **Year 1:** $60,000

Budget 1:
Organization: WSU-TFREC **Contract Administrator:** Carrie Johnston; Kevin Larson
Telephone: 509-335-4564; 663-8181 X221 **Email:** carriej@wsu.edu; kevin_larson@wsu.edu

Item	**2012**
Salaries [1] Technical assistant	18,334
Benefits [2] Technical assistant	6,398
Wages (temporary labor) [3]	3,520
Benefits [3]	610
Equipment	0
Supplies [4]	638
Travel [5]	500
Plot Fees	0
Miscellaneous	0
Total	30,000

Footnotes:
[1] Technical Assistance TBN (0.5 FTE for 8 months).
[2] Technical Assistance TBN (34.9%).
[3] Temporary labor ($11/h, 40h/wk, 8 wks); benefits at 17.3%.
[4] Includes monitoring supplies, rearing materials for colony, sterile moths.
[5] Within State Travel.

Budget 2:
Organization: Michigan State Univ. **Contract Administrator:** Emily Flanner
Telephone: 517-355-5040,x256 F **Email:** flanner@cga.msu.edu

Item	**2012**
Salaries	14,000
Benefits (38%)	5,320
Wages	0
Benefits (7.5%)	0
Equipment	0
Supplies	680
Travel	0
Miscellaneous	0
Total	20,000

Budget 3:
Organization: USDA-ARS, Wapato **Contract Administrator:** Jim Harris
Telephone: (509) 454-6565 **Email:** James.harris2@ars.usda.gov

Item	**2012**
Salaries	0
Benefits	0
Wages	7,500
Benefits (10% of labor)	750
Equipment	0
Supplies	1,750
Travel	0
Miscellaneous	0
Total	10,000

Project objectives:

1. Develop commercially viable attract-and-kill technologies by optimizing moth attraction, moth contact, and the within-orchard spatial distribution of technologies.

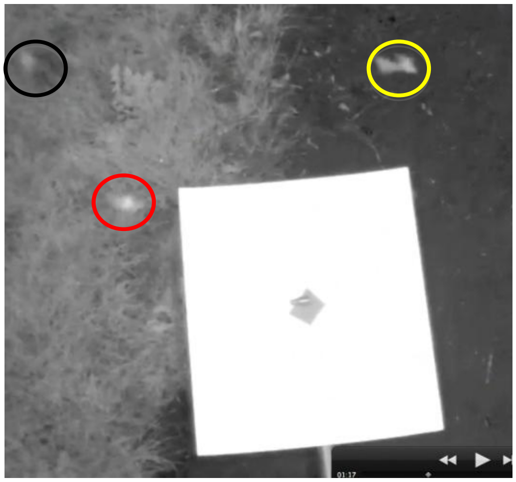
Significant Findings:

1. Purchase of a new video recording system provided much more detailed images and flexibility with managing images over the previous system.
2. Attempts to construct a trap that mimicked the complexity of foliage and provided multiple landing areas did not prove successful.
3. As part of the effort to construct a complex trapping surface we discovered that dry sticky liners were about 10% as efficient at capturing codling moth (CM) as a standard polybutene liner.
4. In a wind tunnel OBLR moths were attracted to and made contact with the pheromone source that was associated with a flat platform more frequently than to a pheromone lure alone.
5. The increase of OBLR pheromone load into grey rubber lures increased capture of male moths up to 10 mg, for both a three- and four-component pheromone blend.
6. In wind tunnel studies the commercial product SPLAT with the lowest CM pheromone concentration attracted the most CM and accounted for the most contacts with the source.
7. When moths contacted the SPLAT containing 3% cypermethrin 70-80% were knocked down, that is were unable to continue directed flight activity.
8. Field studies showed that there was strong support for interaction between N-butyl sulfide (NBS) and acetic (AA) in capture of CM.
9. When NBS and AA were combined with pear ester (PE) the capture of CM was higher that NBS+AA or AA+PE.

Results and Discussion:

Fig. 1. Switch and hard-drive recorder with fan inside shelter and camera mounted above platform with attractant source.

A key aspect of developing an attract-and-kill (A&K) technology for either codling moth (CM) or leafrollers is to determine the impact of design on moth behavior, especially making contact with the technology so that intoxication occurs. In the past we have evaluated different types of A&K designs that were aimed at optimizing attraction to and contact with, or capture in, the technologies. With CM we found that a high pheromone release rate from lures attracted moths to the source but inhibited entry into a trap and/or contact with the attractant source. We have used simple home security video cameras to record and analyze behaviors of moths, but these simple and cheap systems had several limitations associated with transferring videos into formats that could be easily analyzed and the resolution was limited, especially for night active moths. This year we invested in a video recording system (not funding by the commission grant) that provided high-resolution digital images of moth activity (Fig. 1). The cameras were of megapixel quality allowing for a wide angle of view but with the capability to zoom in on close range behaviors, i.e. contact with attractant sources. We recorded images from four stations focusing primarily on CM behavior, as leafroller populations in the study site, Sunrise Research Orchard, were too low to provide sufficient responses to attractants.



We initially recorded behaviors to platforms (non-sticky trap liners) with two attractant sources of different strength, a 1 mg or 0.1 mg lure (Fig. 1 – and image at right). In the image at right there are three moths attracted to the pheromone source associated with the platform. For each day we captured and saved sections of video from each camera that contained moth activity. Unfortunately as of the writing of this report we are still in process of analyzing data from these videos to classify behaviors. One observation that led us to test different A&K designs (see below) was that moths approaching a flat platform were observed to predominantly approach the pheromone source from beneath the platform and fly under it, thus loosing the plume of the attractant. Moths were seen repeatedly moving from side to side under the platform. A video will be shared at the final report on this project showing this behavior.

We evaluated four different A&K technology designs, each baited with a 0.1 mg lure. These models included a badminton racquet, a whiffle ball, a shuttle cox, and a wooden dowel (Fig. 2). The different designs would allow not just attraction to but hopefully contact with a toxic surface. The alternative A&K designs were an attempt to modify the approach of moths so that they would contact a surface as they were approaching the attractant source. Of course in an A&K design that surface would be coated with a toxicant.

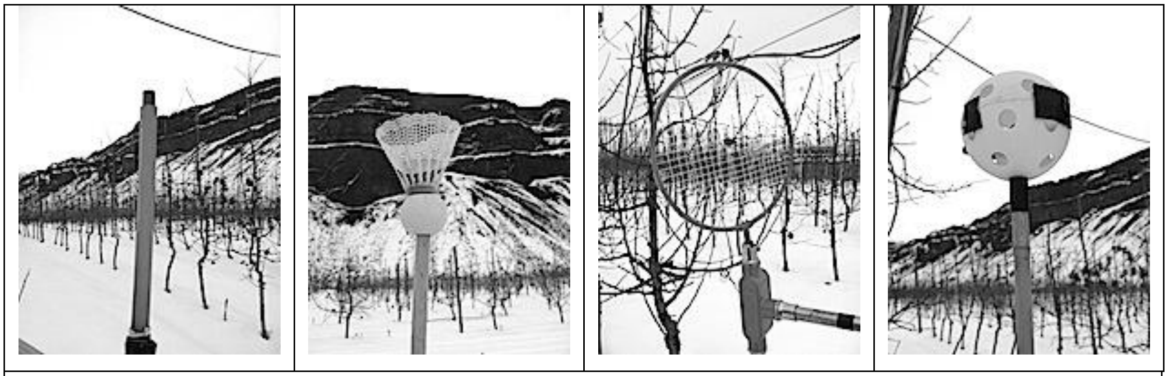


Fig. 2. Four devices associated with CM pheromone lures used to video record CM behaviors when approaching or contacting surfaces.

We also conducted some trials on trapping designs that were intended to mimic more complex structures and optimize moth attraction to and contact with various surfaces. We constructed two models, a multi-layered circular and panel trap (Fig. 3), each of which had sticky upper surfaces only, lower surfaces only, or both were sticky. We used dry sticky trap liners (bottoms) to construct these traps as it made it easier to put them together. We compared captures in the different trap designs with a standard delta trap baited with either a dry sticky liner or a polybutene liner, which is more typical of monitoring traps for CM and leafrollers. Traps were baited with a CM L2 pheromone lure. The multi-layered traps caught very few moths, but were captured most on the upper sticky surface, 86%, compared to the lower sticky surface. These data are confounded by the discovery that the dry sticky surface was not a good capture substrate for CM adults. Average moth captures in the delta traps with the dry sticky liners was 2.7 ± 1.5, about 10% of the capture in delta traps with the standard polybutene sticky liners, 22 ± 11.2, respectively. The multi-layered traps captured large numbers of non-target insects, especially leafhoppers and flies, indicating that the dry sticky surface was efficient in retaining some kinds of insects. Further studies should be conducted to determine if different dry sticky trap liners have limited capacity to capture CM and other pest moths, as some of these trap liners are sold for use in monitoring traps in orchards for moth pests.

Fig. 3. Trap designs tested in 2012 for capture of CM and leafrollers.

Obliquebanded leafroller (OBLR) males were flown in a wind tunnel to assess behavior of males exposed to pheromone lures (1 mg of a three-component blend). OBLR males were flown to a lure associated with either a vertical or horizontal card or to a lure only (Fig. 4). OBLR males made more upwind flights toward, 90%, and more source contacts, 80%, with a card present than when only the lure was present, 60% and 24%. In addition, OBLR males spent nearly twice the time searching around the lure when it was associated with a card. These data show that some structure, vertical or horizontal, in association with a pheromone attractant is important in enhancing OBLR searching time and source contact.

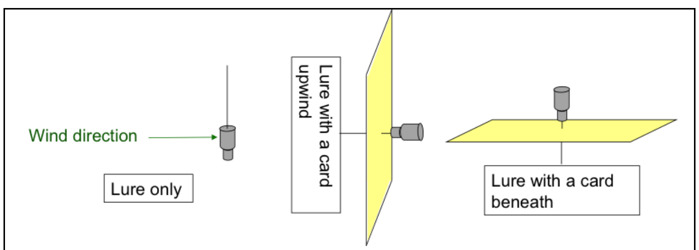


Fig. 4. Three design set-ups compared in wind tunnel studies attracting OBLR to pheromone lures.

As a follow up to the above study a piece of polyester fabric (10 x 28 cm) was treated with deltamethrin, a synthetic pyrethroid. A 1 mg pheromone lure was then associated with the fabric, either treated with deltamethrin of untreated. OBLR moths were flown in a wind tunnel towards the fabric, similar to the vertical card shown in Fig 4. Behavior (wing fanning, upwind flight, source contact, and no response) of 20 OBLR moths was recorded. We found there was no difference in any of the behavioral parameters measured for moths flown to the treated or untreated fabric with source contacts being between 60-70%. This showed that the pesticide was not a repellent to OBLR moths. In addition, moths that contacted the fabric in each treatment were recaptured and held in a small plastic container and mortality recorded. After 1h 100% of the moths contacting the deltamethrin treated fabric were knocked down (inability to manage controlled activity) and after 24h 100% of these moths were dead while only 8% of moths contacting the untreated fabric were dead. If these behaviors could be replicated under field conditions there is promise that a simple A&K product could be developed for OBLR.

Three- and four-component blends of chemicals previously reported as OBLR pheromones were loaded into grey rubber septa at rates of 0.01, 0.1, 1, 10 and 20 mg per lure. These lures, along with commercially available OBLR lures from Trécé Inc., were placed in pheromone traps and OBLR captures recorded. The number of OBLR males captured increased with increasing load rate up to 10 mg, with no difference in captures between 10 and 20 mg lures, and these captured more moths than the Trécé lures (Fig 5). There was a slightly higher capture of OBLR in the three-component pheromone blend, especially at lower load rates, than the four-component blend. These data show that increasing load rates can increase moth captures in pheromone traps, but does not mean that increased load rates will increase OBLR moth contact with a pheromone source associated with an A&K device.

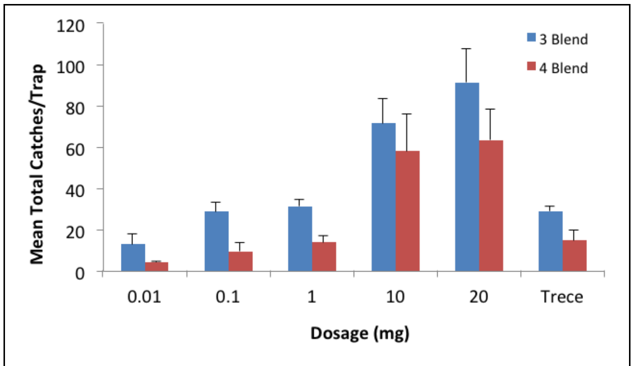


Fig. 5. Average number of OBLR males captured in traps baited with different loads of two pheromone blends.

Male CM were flown in a wind tunnel to different SPLAT™ formulations containing varying concentrations of CM pheromone plus 3% cypermethrin, a synthetic pyrethroid. Moths were also flown to a lure loaded with 0.1 mg of CM pheromone. SPLAT formulations and the CM lure were aged for 7, 14 and 28 days prior to testing in the wind tunnel. The size of the SPLAT dollop was held constant at 0.1 ml for each treatment. Source contact was highest to the SPLAT formulations containing the two lowest CM pheromone concentration and equal to the 0.1 mg lure. There was no difference in moth behaviors to different aged SPLAT formulations or 0.1 mg lure.

The formulation of SPLAT containing the lowest concentration of CM pheromone was used in another wind tunnel study where moths that contacted the SPLAT, about 55%, were recaptured and evaluated for knock down. Seventy percent of the moths were knocked down on the day of recapture and this increased to 80% on the second day after contact.

Preliminary field trials with a SPLAT CM A&K formulation (with cypermethrin) and two SPLAT CM pheromone only (mating disruption) formulations showed an advantage of the A&K formulation over the pheromone only formulations.

Based on previous research four chemicals, benzyl ether, N-butyl sulfide (NBS), acetaldehyde, and acetic acid (AA) were evaluated for their co-attraction of male and female CM. There was no or little evidence for a positive interaction between benzyl ether and AA or acetaldehyde and AA, but there was strong support for interaction between NBS and AA (Fig. 6). Further studies showed no advantage of adding either acetaldehyde or benzyl ether to AA+NBS, nor did a combination of all four chemicals increase moth capture over AA+NBS. Increasing the release rate of NBS by changing the hole size in the release vial from 1 to 12 mm showed no difference in CM captures when the release rate of the AA lure was held

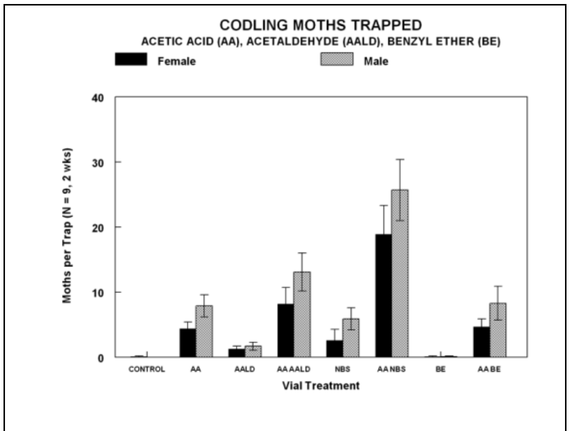


Fig. 6. Average number of CM captured in traps baited with different combinations of kairomone attractants.

constant. There was also no difference in CM captures when AA and NBS were released from separate, vials each with a 3 mm hole, or when the two chemicals were mixed in the same vial with the 3 mm hole.

The number of CM captured in traps baited with AA+NBS lures was lower than traps baited with AA+PE (pear ester), but captures of CM were higher still when all three chemicals were combined (Fig. 7).

Further studies evaluating a tube-type A&K design showed a significant increase in male but not female captures as tube length varied over the range of 5.5 to 10 cm. Changing the width of the tube significantly increased capture of both male and female CM over a range of 1.75 to 3.0 cm.

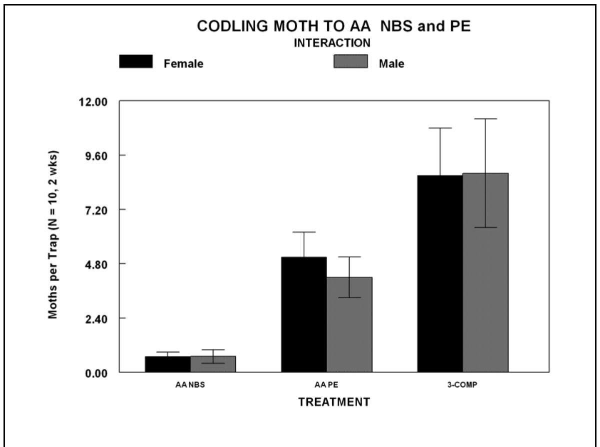


Fig. 7. Mean number of CM moths captured in traps baited with AA+NBS, AA+PE or AA+NBS+PE.

Executive Summary:

This project involved collaborative research of three institutions examining issues of codling moth and obliquebanded leafroller behavior as it relates specifically to the development and design of attract-and-kill technologies. At its core the development of an attract-and-kill technology must deal with attraction to and contact with a source or surface in order to have success. Attraction without source contact, as we have observed on many occasions, does not achieve the killing component the attract-and-kill concept and such technologies typically end up as a weak form of mating disruption. In this study we utilized a high quality field video camera system to capture behavior of codling moth to different sources as a basis for evaluating structures that enhance source contact. Various kinds of trapping systems were also evaluated for codling moth and obliquebanded leafroller and some key parameters were identified that would need to be incorporated into a trap-out attract-and-kill design. One unanticipated consequence of our research was the discovery that dry sticky trap liners were not efficient in capturing codling moth, though they did capture many other kinds of insects. This phenomenon needs further investigation as dry sticky trap liners are sold for use in monitoring traps for pest moths. Wind tunnel results showing differential behavior of obliquebanded leafroller moths with or without a flat surface associated with attraction to and contact with an attractant source demonstrate the need for some kind of structure in combination with a pheromone source to enhance an attract-and-kill design. The high knock down rate for obliquebanded leafroller moths flown to a pheromone lure associated with a fabric panel treated with deltamethrin was an encouraging step towards the development of an attract-and-kill device for this insect. Wind tunnel studies with codling moth that showed highest source contact to a SPLAT attract-and-kill formulation with the lowest pheromone concentration confirmed previous studies, however, the failure in the field of the SPLAT attract-and-kill formulation to increase suppression of codling moth male captures in pheromone monitoring traps over a SPLAT formulation with pheromone only was a repeat of previous experiences and suggested that moths are not contacting the attract-and-kill product in sufficient frequency to add value to a mating disruption effect. Pear ester has been the best kairomone found for attracting codling moth. Recent research has shown that combining acetic acid with pear ester increases attraction and capture of codling moth. The search for additional kairomones that attract codling moth have been frustrating, but the discovery that N-butyl sulfide combined with acetic acid is attractive to codling moth was encouraging. In addition, when N-butyl sulfide and acetic acid were combined with pear ester, codling moth capture in field traps was greater than in traps baited with pear ester and acetic acid. Results from this one-year project point to promising lines for further investigation in the development of attract-and-kill technologies for codling moth and leafrollers, but sources of funding other than the commission will be sought for this work.