FINAL PROJECT REPORT

Project Title: Kairomones for monitoring and control of native and invasive moths

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Other funding sources

Agency Name: Agriculture and Agri-Food Canada**,** Science and Technology Branch, Annual Call for Research, Development and Technology Transfer Proposals
Amt. requested: $90,000 USD total over three years (2016-2019)
Notes:

Total Project Request: **Year 1**: $55,000 **Year 2:** $59,000 **Year 3:** $65,000 **Year 4**: $0

Budget 1
Organization Name: ARS, USDA
Contract Administrator: Chuck Myers
Telephone: (510) 559-5769
Email address: chuck.myers@ars.usda.gov

Item	2015	2016	2017	2018
Salaries			38,000	0
Wages	17,500	20,000	22,000	0
Benefits	1,500	1,500	2,000	0
Supplies	6,000	7,500	8,000	0
Travel (local to research plots)	1,000	3,000	3,000	0
Miscellaneous			3,000	0
Plot Fees	3,000	3,000	3,000	0
Total	29,000	35,000	79,000	0

Footnotes: [a] $20,000 that was approved for 2016 ($59,000) was used in 2017 to support a visiting Italian scientist ($38,000 total for salaries).

Budget 2
Organization Name: Agriculture & Agri-Food Canada
Contract Administrator: Karen St. Martin, and Goewin Demmon
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Item	2015	2016	2017	2018[a]
Wages[1]	18,000	0	0	0
Benefits	2,500	0	0	0
Supplies	4,000	4,000	6,000	0
Travel[2]	1,000	0	0	0
Miscellaneous	500	0	0	0
Total	26,000	4,000	6,000	0

OBJECTIVES:

1. Optimization of one or more new kairomone attractants for North American tortricid pests, the work will include chemical analysis, electrophysiology work, and field testing of dosages and chemically-related compounds in lures and various traps.
2. Evaluate the attractiveness of new kairomones in apple and pear orchards situated within the major fruit growing districts of Washington State and British Columbia. This will include studying active space, trap design, trapping grid optimization, and development of long-lasting lures
3. Establish the correlation for each species of moth catches (both males and females) using new kairomones with larval densities and fruit injuries in the spring and summer.
4. Conduct small plot studies examining the potential of using new kairomones as a female moth removal tactic to manage these pests in apple and pear orchards.

SIGNIFICANT FINDINGS

- Volatile analyses of leafroller and budmoth infested apple shoots identified six specific compounds released only following herbivore damage.
- 2-phenylethanol (PET) and phenylacetonitrile (PAN) when used with acetic acid (AA) co-lures were identified from as being the most attractive to a large number of tortricid pests in North America, Europe, and New Zealand.
- A ternary blend and several binary ratios of three of these compounds were found not to significantly increase moth catches compared with the single most attractive compounds.
- Traps baited with the respective kairomone lures were highly effective in monitoring pest species in orchards but caught < 50% as many moths as sex pheromone-baited traps.

- Effective lures (10 mg red septa and long-lasting proprietary closed membrane cups) loaded with either PET + AA or PAN + AA were developed in collaboration with Trécé Inc (Adair, OK). All lures were found to be highly effective.
- PET+AA is more attractive for leafrollers and PAN+AA is more attractive to eye-spotted bud moth (ESBM). PET/PAN+AA was effective for both moth groups.
- Volatile captures by Valentino Giacomuzzi (Free University of Bolzen, Italy) of apple volatiles from foliage untreated or subjected to OBLR larval feeding were analyzed by Jim Mattheis (ARS, Wenatchee, WA) and provided a complete description of the array of important volatiles to be considered as attractants.
- No further discoveries of new attractive plant volatiles were made in extensive field trials, however, several compounds were found to reduce moth catch.
- Orchards treated with sex pheromone dispensers for OBLR were effectively monitored all season with PET+AA-baited traps.
- We showed that the new LR lures can be used in the same trap with lures for codling moth to monitor both pests with a single trap.
- Bucket traps using a solution of propylene glycol to retain moths were found to be the most effective low-maintenance trap for use in mass trapping.

- The project-developed a new longer-lasting AA lure that can be used over the entire season.
- A new combination host-plant volatile (HPV) lure (TRE1379) was developed that contains equal amounts of PET and PAN and has a broader activity for the various tortricid leafroller and budmoth pests that can exist in Washington and around the world. We demonstrated that this combination lure does not lose any efficacy for any of the tortricid pest species studied when compared to either of the two components used alone.
- We learned that combining the sex pheromone and either plant volatile in a trap is antagonistic and reduces captures of male leafrollers. However, including the acetic acid co-

lure cancels out this negative effect. Nevertheless, this suggests that the HPV+AA lure should be used alone instead of in combination with sex pheromone to monitor leafrollers, unlike what we previously found with pear ester and codlemone for codling moth.

- We refuted published reports that apple seedlings infested with leafroller larvae are more attractive than clean trees to adult leafrollers. Conversely, we demonstrated that several volatiles co-released with the attractants by infested apple seedlings are repellant to adult leafrollers.
- We showed that female moths captured on liners can lure males into traps and that with the oblique-banded leafroller this effect is more pronounced on hot melt pressure-sensitive adhesives compared with the standard Tangletrap adhesive. With codling moth this effect also occurs but the two adhesives do not differ.
- We demonstrated that adult feeding (water or honey water) by leafrollers is important for moths to allow full mating and egg laying to occur if the moths are unable to mate for 2 or more nights. Relative humidity is also an important environmental factor affecting moth longevity and realized fecundity.
- Using a proboscis-extension bioassay we have shown that the new attractants elicit a feeding response by both sexes of leafroller adults. This suggests that the volatiles may signal the presence of a food source for the moths.
- Characterization of apple volatiles and testing of their potential for leafroller attraction led to the discovery of a new attractant for codling moth
- Mass trapping studies were conducted in seven apple plots during 2017 for leafrollers and codling moth. Bucket traps baited with bisexual lures for both pests were used at a density of 24 per acre. Population densities of both pests were significantly reduced between generations, except in 1-acre plots surrounded by unmanaged and infested orchards.
- Studies with this range of tortricids demonstrated that the use of pear ester can be added to allow traps to monitor both codling moth and leafrollers in single traps.

- The combination PET+PAN kairomone lure was evaluated for light brown apple moth with help from Dr. Lucia Varela with UC Extension. Unlike in published reports the PET+AA outperformed PAN+AA for this species. This promotes the eventual commercial development of a single kairomone lure for world-wide leafroller monitoring.

RESULTS AND DISCUSSION

This project has been highly successful as eight peer-reviewed scientific articles have been published and several more are in preparation. The project was strongly supported by R&D from Trécé scientists to develop effective, long-lasting lures for PET, PAN, and AA. Various studies were conducted over the course of the study to determine optimal emission rates from lures. At present, a worldwide patent has been filed by New Zealand researchers for these lures. We have no information on the success of this patent or when commercial lures will be available to growers.

The results from each of the first three years of the project have already been presented in previous reports. In 2018, a no-cost extension was granted and several studies continued. In Washington we evaluated again the use of the TRE1379 lure that is loaded with both PET and PAN for our key leafrollers in western North America. In addition, studies were continued in Europe (Hungary and Sweden). Lures were shipped to Dr. Lucia Varela with UC Extension who evaluated them for the exotic pest, light brown apple moth in her area. She found that PET+AA was more effective than PAN+AA which contradicts the initial results from New Zealand. However, again the combination PET+PAN lure appears to provide a similar degree of effectiveness and we would expect that this is favorable for commercial development. At present, only the eye-spotted budmoth is more attracted to PAN+AA than PET+AA and the combination lure can likely still be used for this species.

Additional studies were conducted in 2018 to analyze apple volatiles at different times of the season and to evaluate whether leafroller adults are attracted to potted trees with actively-feeding leafroller larvae. Apple foliage in the field suffers a range of factors that cause micro abrasions on the foliage which then triggers the plant to release key volatiles. Our data does not support previous NZ claims that PET or PAN are con-specific attractants. Injured leaves actually release a large number of compounds and we have shown that some of these are repellent for leafroller adults. Instead, our studies show that only with pristine greenhouse plants do you see any attraction of adults to plants with larval feeding. The more sophisticated analytical techniques used at the ARS Laboratory in Wenatchee allowed us to see that acetic acid is released by plants regardless of leafroller feeding, likely due to microorganisms existing in the phyllosphere of plants and certain species synergized by the exudates from micro wounds. Thus, we have discredited many of the initial claims made by the New Zealand researchers and this will be useful to other scientists following our work.

Several studies were completed in Canada during 2018. Electrophysiological (EAGs) studies were performed on male and female, OBLR and PLR adults to understand the basis for a behavioural interaction (decreased catches) when sex pheromone and kairomone lures were combined in traps. Both OBLR and PLR males exhibited significantly stronger EAG responses when exposed to the sex pheromone component Z11-14:OAc and the aromatic 2-PET simultaneously, than when exposed to either compound alone. Both OBLR and PLR females showed significantly weaker EAG response when exposed to Z11-14:OAc and 2-PET simultaneously, than when exposed 2-PET alone. These EAG differences could explain smaller leafroller trap catches when these compounds are combined in traps. Proboscis Extension Reflex (PER) trials were performed on male and female, OBLR and PLR moths, to better understand the behavioural response to the range of aromatics identified in this study. Both sexes and species exhibited their strongest PER responses to 2-PET, followed by Z3-Hexenyl-benzoate > Benzyl alcohol > Indole > PAN. All other compounds failed to elicit a significant response. In contrast to its importance as an attractant in traps, acetic acid elicited weak PER responses and only when it was diluted to a concentration of 0.01%. The combination of AA + 2-PET elicited PER responses equivalent to PET alone. The PER responses of moths that had had their antennae removed were as strong as those with intact antennae. This indicates that olfactory receptors for these aromatics are present on body parts other than antennae, possibly the maxillary palps. Neither male nor female OBLR and PLR adults exhibited a PER upon exposure to the sex pheromone component Z11-14:OAc and simultaneous exposure to Z11-14:OAc and 2-PET did not significantly reduce the PER response.

Trapping studies in Canada in 2018 showed that leafroller and ESBM aromatic kairomones, 2-PET and PAN, respectively, had no significant affect on the response of adult Apple Clearwing Wing moths to their sex pheromone or kairomones. Multicomponent kairomones consisting of AA-PE-PET-PAN and ethyl butyrate, were used for season long monitoring of six apple pest species simultaneously. Only *Hedya nubiferana*, an invasive bud moth in Canada, showed any significant reduction in catch relative to catches with its own kairomone AA-PE.

During 2018 we developed a prototype device, ***Grey Ghost*** for eventual use in attract and kill of leafrollers (Fig. 1). This was developed by cutting small pieces of the EPA-registered fabric (ZeroFly screen, Vestergaard-Frandsen) impregnated with 0.4% deltamethrin and fashioning a hanger and placing the device in the canopy of the tree. These nets have recently been tested with the brown marmorated stink bug, *Halyomorpha halys*, in the eastern U.S. as much larger ghosts placed around orchards. In our laboratory, we are running various tests to examine whether moths contact the deltamethrin-treated netting, how long they contact it for, and whether they die after contact. Moths are not repelled and will land on the fabric, and bouts of exposure can last from 1-40 s. Moths

exposed for < 10 s will mostly die by 24 h. Sublethal exposure for the moths that survive shows a significant reduction in mating and egg laying. We established three 1-acre plots treated with 24 Grey Ghosts each at our USDA research farm. We compared moth catches of Pandemis and obliquebanded leafrollers in the center of these three plots plus paired untreated plots. Unfortunately, very few leafrollers were caught during 2018 perhaps because of the extensive 2017 mass trapping studies conducted in the same blocks in 2017. Therefore, this approach still needs to be evaluated. Similar trials conducted with codling moth suggested this ghostly approach might be effective and studies are continuing with that key pest.

Fig. 1. The Grey Ghost baited with lures for leafrollers and placed in the canopy of an apple tree, 2018.

Studies were conducted in 2018 to assess whether traps could be baited with the 4-way K for codling moth and with the PET/PAN+AA lure for leafrollers to promote the development of electronic smart traps. Unfortunately, the flight of leafrollers was very low in the orchard where the trial was conducted, and it remains unclear whether this approach is compatible.

EXECUTIVE SUMMARY

The volatile profile from apple foliage was carefully characterized through this project using the ARS facility (Dr. James Mattheis) in Wenatchee's expertise and a scientist (Dr. Valentino Giacomuzzi) in Italy. A survey of the key volatiles presented alone and in combination with acetic acid were evaluated for Pandemis and obliquebanded leafroller in a series of trials. We established that two volatiles, 2-phenylethanol and phenylacetonitrile when used in combination with acetic acid are attractive to both sexes of at least fifteen leafroller species (data supplied by Dr. Marco Tasin in Sweden and Dr. Julia Jósvai in Hungary) including all the important pest species in the western North America. The project was strongly supported by R&D from Trécé scientists to develop effective, long-lasting lures for all three volatiles. Various studies were conducted over the course of the study to determine optimal emission rates from lures. At present, a worldwide patent has been filed by New Zealand researchers (Drs. Ashraf El-Sayed and Maxwell Suckling) for use of these lures. We have no information on the success of this patent or when commercial lures will be available to growers. There remains considerable interest to have these lures made available to monitor orchards treated with sex pheromones in Washington State. Working in orchards with sizeable populations of leafrollers was a major limiting factor impacting this project. Our limited data suggests that these lures would have potential if used for lure and kill technologies, but more field validation following our project is needed.