FINAL PROJECT REPORT

Project Title: Evaluating plant volatiles for augmenting biological control

PI: Vincent P. Jones
Organization: WSU-TFREC
Telephone: 509-663-8181 x291
Email: vpjones@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Jay Brunner, TFREC

Other funding sources

Jones VP and C O'Leary. Spatial and temporal dynamics of attracting green lacewings to synthetic lures in apple orchards for pest suppression.
Agency Name: WSU-BioAg Grant
Amt. awarded: $37,866

WTFRC Collaborative Expenses: None

Total Project Funding: Year 1: 54,573 Year 2: 55,493

Budget History:

Item	2015	2016
Salaries[1]	26,826	29,030
Benefits[2]	2,492	2,406
Wages	18,720	18,720
Benefits[3]	1,835	449
Equipment	0	0
Supplies[4]	2,500	2,600
Travel[5]	2,200	2,288
Miscellaneous	0	0
Plot Fees	0	0
Total	54,573	55,493

Footnotes:
[1] New PhD student
[2] 4.8%
[3] 2.4%
[4] includes lab and field supplies
[5] w/in state travel

Objectives:

1. Determine the area of attraction of lures for the lacewings *C. nigricornis* and *C. carnea*.
2. Evaluate the effect of lure placement on population growth of woolly apple aphid.
3. Determine if increased lacewing egg deposition occurs within the active area of the lures and if this results in greater larval lacewing densities.

Significant Findings:

- Activity of lacewings is primarily concentrated around dusk and dawn.
- Distance of attraction from both the squalene and the AMP lures was <10' for both species of lacewings. This suggests large-scale disruption of natural enemy searching will not cause pest outbreaks in other areas of the orchard and that the lures can be used to manipulate lacewing adults on a small scale.
- Video observations showed lacewing activity around squalene lures was 18-fold higher than around control lures and 2.2 fold higher around AMP lures than control lures.
- Lacewings attracted to the squalene lures only spent an average of 8.8 min on lures, so that normal behaviors associated with mating, feeding, and oviposition would not likely be significantly affected and biological control would not be interfered with.
- Use of lures appears to reduce the buildup of WAA populations in the fall after the summer population crashes related to high temperatures.

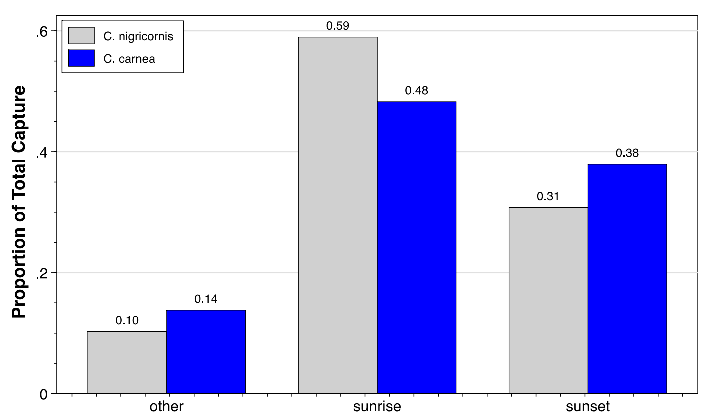
Results and Discussion

Objective 1. Determine the area of attraction of lures for the lacewings C. nigricornis and C. carnea.

Methods:

Determine the area of attraction of lures for the lacewings *C. nigricornis* and *C. carnea*.
We used high-resolution video cameras at distances of 0, 5, 10 and 150 feet from a tree where either a squalene or an AMP lure (Acetic Acid, Methyl Salicylate, and 2-Phenylethanol) were placed. The camera at the 150-feet distance was considered to be a control that indicated the general activity level in the orchard. Studies were performed from late June to late August 2015, with the lures being left in the field for a 4-day period and recording each day. PVC frames held the cameras 8 feet above ground level, pointing vertically down at an 8 in. x 8 in. platform held at 4 feet off the ground. The platform, made of wood, was covered in gray construction paper and a one-sided 8 in. x 8 in. clear sticky trap. Lures were attached on the top side of the platform using binder clips. The cameras were set to record for 10 hours total over each day; the hours were adjusted throughout the summer in order to record the hour immediately before and after sunrise and sunset. The other 6 hours of recording were evenly distributed throughout the rest of the day and night. During the non-recording days, lures were removed and the entire recording set up was shifted so that residual volatiles would not interfere with the next set of recordings.

Fig. 1. Proportion of total observations of *C. nigricornis* or *C. carnea* at different times of the day.



Results:

Daily Activity Patterns:

Video analysis of the time of day showed that peak activity of *both C.*

nigricornis and *C. carnea* occurred around sunrise, with the second peak around sunset. Flight activity at other times of the day or night was very limited, with only 10-15% of the total flights observed (Fig. 1).

Distance of attraction:

C. nigricornis was most abundant on the video on the lured tree, with the activity five or 10 feet way not significantly different from the activity recorded with the 150-foot control camera (Fig. 2). We were surprised to see *C. carnea* in the vicinity of the squalene lure (squalene traps almost exclusively catch *C. nigricornis*), but the distance drop-off was similar to *C. nigricornis* with the numbers significantly higher on the lured tree, but no significant difference between the longer distances. It is possible that squalene affects *C. carnea* much like it does female *C. nigricornis* which are attracted near squalene lures, but will not enter traps.

The AMP lure had a slightly slower drop-off with distance, with both *C. carnea* on trees five feet away from the lure still showing activity about half of that on the tree containing the lure (Fig. 3). However, trees 10 feet from the lure showed no more attraction than the general orchard seen 150 feet away. *C. nigricornis* is also attracted to the AMP lure (although not as strongly as to squalene), but there were no significant distance effects likely because only 17 lacewings total were captured.

Fig. 2. Percent of total activity observed at each distance from a squalene lure. Bars for each species with the same letter are not significantly different at $p = 0.05$.

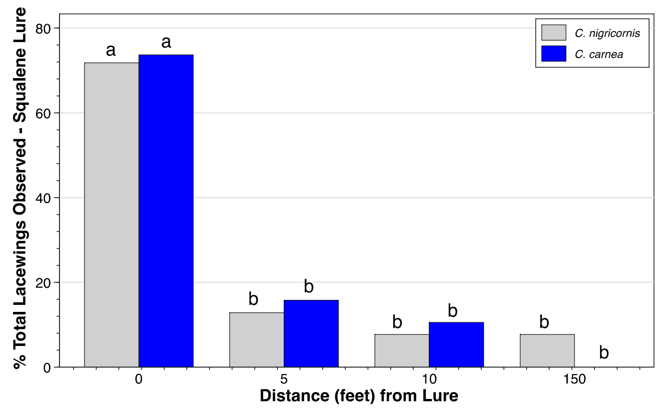
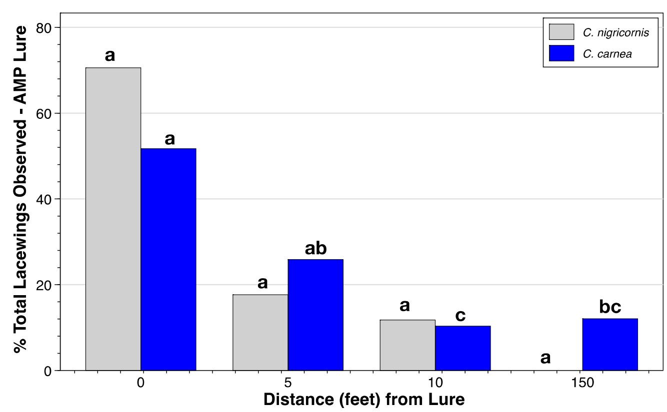


Fig. 3. Percent of total activity observed at each distance from an AMP lure. Bars for each species with the same letter are not significantly different at $p = 0.05$.



These results show that the lure activity is very restricted spatially. These results are similar to work with other types of plant volatile lures that have been performed in corn and soybean and independent results performed by Dr. Tom Unruh at USDA-ARS in 2016. These results strongly support the idea that we can use the lures to aggregate populations of natural enemies in areas of high pest density without disrupting the overall spatial distribution of within the orchard.

Objective 2. Evaluate the effect of lure placement on population growth of woolly apple aphid.

Methods:

In 2016, we used video recording to evaluate activity of lacewings in trees with either an AMP lure, a blank (water filled) control lure, or a squalene lure. We set up two replicates of trees spaced 4 rows apart; within a row, six consecutive trees were considered a treatment (AMP, Control, or Squalene) with a single lure put between tree 3 and 4 in the row; all treatments were separated by >60 feet. Because some lures are suspected of inducing the production of plant volatiles in trees that are attractive to natural enemies, we moved the lures each time cameras were moved between replicates; this kept the trees from being exposed continuously to the volatiles from the lures, which would minimize plant induction of volatiles. Monitoring began 10 June and ran through 23 Sept. Video recordings started 28 June and ran through 23 Sept. During that time, we moved the cameras from replicate 1 to replicate 2 or vice-versa at weekly intervals.

Cameras were focused on a platform with a lure and recorded for 8 hours a day, with most of the coverage overnight. Cameras recorded from 6-8 pm, 12-1 am, 3-5 am, 12-1 pm, 4-5 pm, and 8-10 pm. When any lacewing came into the field of view, its behavior was classified into seven different categories: (1) crosses the video screen without stopping, (2) seen on a branch, leaf or trellis adjacent to the platform, (3) on the platform but not on the lure, (4) circles lure, (5) actually on the lure, or (6) interacts with another lacewing generally on the lure. For each category, the number of individuals and the length of time to complete the behavior was calculated. We summarized the data by plot as well as a grouped (both plot data).

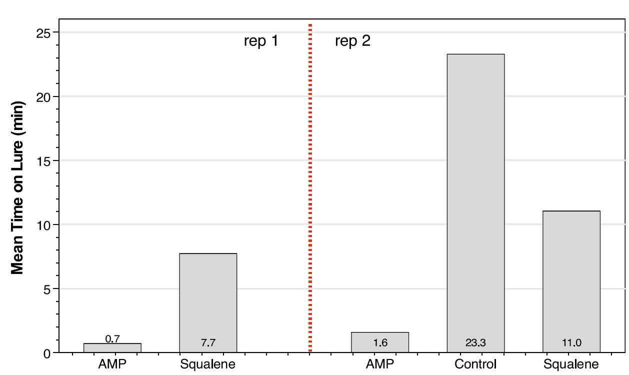
Results:
The data clearly showed that in both replicates, the squalene lure had the greatest activity of any kind adjacent to the lures (Table 1). Over both replicates, 85% of all activity was seen in the squalene treatment versus 10% and 5% for the AMP and Control lures, respectively. In the squalene treatments, 49% of the activity was a lacewing "landing on the lure" and 25% "landing on the platform adjacent to the lure." We also found some instances of interaction between different lacewing individuals, but only on the squalene lure; most of these were short-lived and resulted in one of the individuals leaving the lure.

Table 1. Number of times different behaviors were observed in field video observation in 2016.

	Treatment			
Behavior	**AMP**	**Control**	**Squalene**	**Total**
Crosses screen w/o stopping	96	52	145	293
On leaf, branch or trellis	26	9	106	141
On platform	24	5	336	365
Circles lure	5	0	42	47
On lure	4	3	620	627
GLW-GLW interaction	0	0	35	35
Total Observed	**155**	**69**	**1284**	**1508**

The data showed a completely different pattern for the AMP and Control treatments where the most common behavior was "moving across the screen without stopping" (63% and 72%, respectively), followed by "resting on a leaf, branch or trellis" and "resting on the platform" (Table 1). The relatively low lacewing activity associated with the AMP lure is likely caused by much lower population levels of *C. plorabunda* which is the primary lacewing attracted to AMP; trapping in areas close by (but not in the plots) showed the trap catch of *C. nigricornis* (highly attracted to squalene) was 4-fold higher than that of *C. plorabunda*.

Fig. 4. Mean time lacewings spent on lures for different lure types in the two different replicates.



This data confirms observations made in previous years using unbaited sticky traps, where we found that trees with lures had a roughly 8-10-fold increase in lacewing activity over untreated areas.

A major concern of using the lures has always been whether the lacewings might spend too much time on the lure at the expense of mating, laying eggs or feeding in its vicinity. On average, video analysis showed that lacewings spent 7.7-11 minutes on the squalene lures (Fig. 4), with some spending as much as 2 hours; however, 75% of individuals spent less than 12.2 minutes and 90% spent less than 21 minutes on the lure (summarized over the two replicates). These data suggest that the lures are not so disruptive that biological control would be negatively affected.

Unfortunately, the experimental protocol in 2016 – designed to minimize the induction of plant volatiles – proved to be ineffective for evaluating biological control of WAA associated with the lures. The plots were too small and re-randomized several times, and the lures were never in place for more than 7-10 days in any plot. However, results in 2015 detailed below show that the longer term use of lures has the potential to slow population growth and provide additional control. We plan to perform additional studies this coming year on a larger scale in commercial orchards to evaluate suppression of WAA population levels and will provide a report on the results.

Suppression of WAA populations on lured trees (2015):

Methods:
We set up 24 trees in a modern fruiting wall orchard that were separated from each other by 200 feet and each randomly assigned one of the three treatments: a squalene lure placed on the tree, an AMP lure, or no lure at all. We evaluated WAA population levels at weekly intervals by evaluating 10 randomly selected shoots in each of the 24 trees and determining the average percentage infestation. The evaluations were made over a six-week period.

Results:
We found that the initial set up of the experiment did not account for the extraordinarily warm conditions. We had initially hoped to sample for a week or two, then put out the lures and see how the population changed after the lures were placed. However, the warm temperatures completely flat-lined the population so that from 6 July to 21 July 2015 no infested shoots were found. Fortuitously, this allowed us to follow the population rebounding after the temperatures started to drop over the period from 27 July to 20 August. Overall, the percentage infested shoots increased from 0% (21 July) to nearly 50% in the control by 20 August, with the lure-treated trees having significantly lower infestation levels compared to the control trees (Fig. 5).

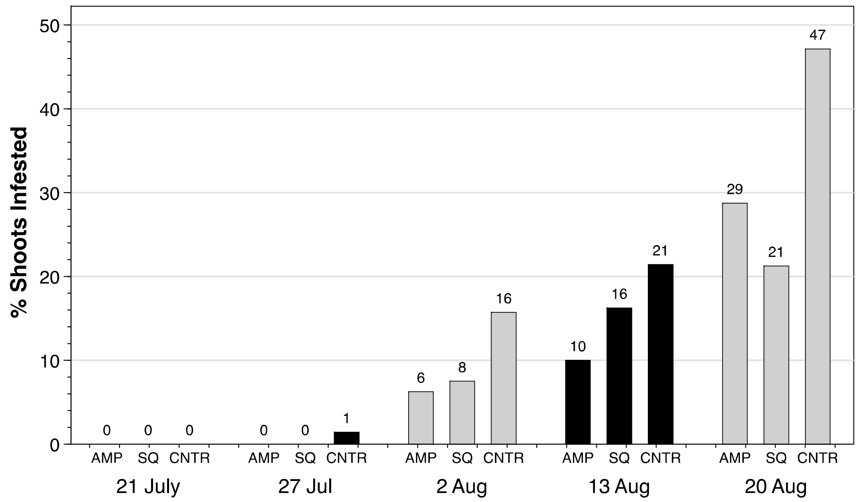
Objective 3. Determine if increased lacewing egg deposition occurs within the active area of the lures and if this results in greater larval lacewing densities.

Methods:
Monitoring of lacewing egg deposition in 2015 was performed in the same plots as reported in Objective 2 (*Suppression of WAA populations on lured trees*). Each week, lacewing eggs were counted using a 3-minute visual count on the central tree and the two adjacent trees within the row. The evaluations were made over a six-week period.

Results:

The number of eggs present on the trees was similar in all three treatments when averaged over the six-week period. ANOVA showed there were no significant differences between the different treatments, even though in 4 of 5 samples the AMP lure had higher numbers of egg masses found. Although there is an expectation that there would be more eggs laid on lured trees, the higher levels of infestation on control trees would tend to induce natural plant volatile emission which could result in higher egg-laying on those trees.

Fig. 5. % Shoot infestation with WAA over time.



Executive Summary:

This project developed the basic information on lacewing behavior around the tested natural enemy lures needed to understand if the lures might interfere with biological control. The results clearly show that the range of attraction of the lures is short (<10 feet), they increase activity 8-18-fold in their immediate vicinity, and do not cause lacewings to spend inordinate amount of time just sitting on the lures. In addition, studies in 2015 showed that lures applied early in the summer helped reduce the population buildup of WAA after populations rebounded when temperatures dropped in mid-late August.

After testing the lures for enhancing biological control on a small scale in this grant, we intend to start some studies this next year on a larger scale in commercial orchards and will provide a new progress report that gives results next year. Overall, we were able to show that the use of the lures (especially squalene) could be a viable option to help reduce WAA in orchard hot spots. Our concerns about negatively affecting lacewing behavior were not confirmed. The results that suggest no practical interference with biological control, so that use of the lures in areas should be able to re-distribute natural enemies within an orchard block and reduce WAA population levels. That we did not find the expected increase in lacewing egg deposition around the lures was most likely due to the relatively small scale of our experiment, which makes it hard to see significant differences. The larger-scale studies we will start this next year should answer this question, while at the same time allowing us to get a better measure of biological control for WAA over a much larger scale.