FINAL PROJECT REPORT: PR-12-101

Project Title: Efforts to disrupt winterform re-entry using repellents or attractants

PI: David Horton
Organization: USDA-ARS
Telephone: 509-454-5639
Email: david.horton@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City: Wapato
State/Zip: WA 98951

Cooperators: ISCA Technologies, Riverside, California
Jocelyn Millar, University of California, Riverside

Other funding sources: None

Total Project Funding: $38,000

Budget History:

Item	Year 1: 2012	Year 2: 2013
Salaries	$14,615	$14,615
Benefits	$ 4,385	$ 4,385
Total	$19,000	$19,000

OBJECTIVES

Develop approaches to disrupt colonization of orchards by post-diapause winterforms (*repellents*) and to delay mating of colonists following arrival (*pheromone*).

- Determine whether SPLAT products developed by ISCA and shown to repel other psyllids can be used to disrupt re-entry by returning winterform psylla.
- Determine whether saturation of atmosphere with 13-MeC27 pheromone slows mating by returning colonists.

SIGNIFICANT FINDINGS

Repellents

- SPLAT-DMDS (disulfide compound) failed to repel winterform pear psylla in a series of small and large cage studies
- SPLAT-DMDS failed to affect psyllid colonization of pear trees and egglaying in a large field trial
- New SPLAT product (proprietary; developed from volatiles extracted from a plant essential oil) shown to repel both winterform and summerform psylla in olfactometer trials. However, product was not effective under field conditions.
- Pear ester shown to repel both winterform and summerform pear psylla in olfactometer trials. Product was not effective under field conditions.

Pheromone (13-MeC27)

- The GC-MS trace identifying the 13-MeC27 compound was found to include two peaks hidden by the 13-MeC27 peak.
  - Those peaks were identified, and the compounds were synthesized (Jocelyn Millar, UCR). Olfactometer trials showed that one of the compounds (11-MeC27) is attractive to male psylla.
- Small cage studies suggested that saturation of cages with 13-MeC27 slowed rate that females were mated, suggesting the compound interfered with mate location.
- Efforts were initiated to develop a sprayable formulation of the pheromone compatible with grower practices. The low volatility of the compound prompted concerns about use of septa with which to saturate an orchard for disruption trials (i.e., need for excessive number of point sources).
  - The compound is fully soluble in oil, and was found to retain its activity when mixed in 1% horticultural oil (+ water): olfactometer trials, arrestment trials, and field trials confirmed activity.

METHODS

Objectives were addressed using a series of cage studies, olfactometer assays, and field tests. Additional detail provided below in **Results & Discussion**.

RESULTS & DISCUSSION

1. SPLAT-DMDS: effects on colonization (cage study). Pear seedlings (1.5 foot tall) were placed at opposite ends of a screened cage (6 ft long x 2 ft x 2 ft). The repellent was placed at one end of the cage, at the base of seedlings. Winterform psylla (100 mixed sex) were released in the center of the cage, and location was determined 24 hours later. ***Results.*** A significant preference was noted for one end of the cage, irrespective of treatment, due to a slight light gradient (Figure 1, top two bars).

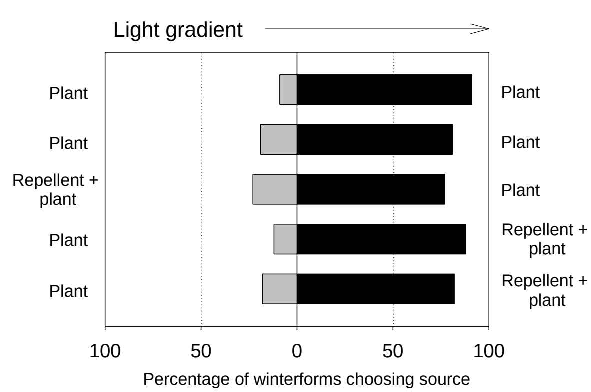

Figure 1. Large cage study with SPLAT-DMDS

Having the repellent at that end of the cage did not overcome that light effect (Figure 1, bottom two bars). This study provided no evidence that the compound is repellent to psylla. No phytotoxicity was seen associated with the compound.

2. SPLAT-DMDS: effects on departure (cage study). Winterform psylla were allowed to settle on pear seedlings at one end of the cage. A second set of seedlings were placed at the opposite end of the cage, and the repellent was applied to the bottom of the original set of plants, just below the feeding psylla. All plants were examined for psylla at 2 hours and at 24 hours. Control trials (no repellent) were run in parallel. ***Results.*** There was no evidence that the repellent prompted movement off of the treated plants (Figure 2). Psylla were observed feeding within several inches of the compound.

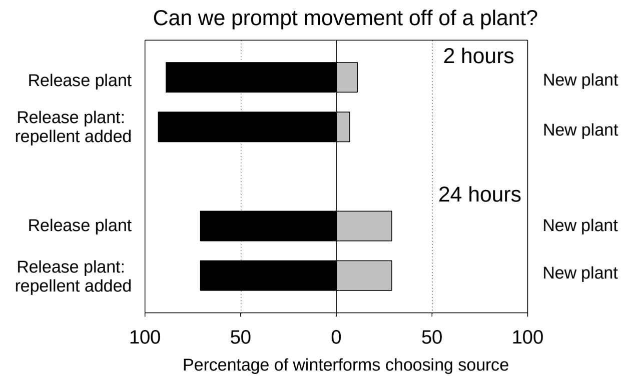

Figure 2. Cage study to examine whether SPLAT-DMDS prompts movement off of plants.

3. SPLAT-DMDS: field trial. A field trial with the SPLAT-DMDS formulation was done in a small (48 tree; 4 rows x 12 trees) orchard located at the Moxee farm to determine whether the compound interfered with winterform colonization of trees during the re-entry period (March/April). Three trees

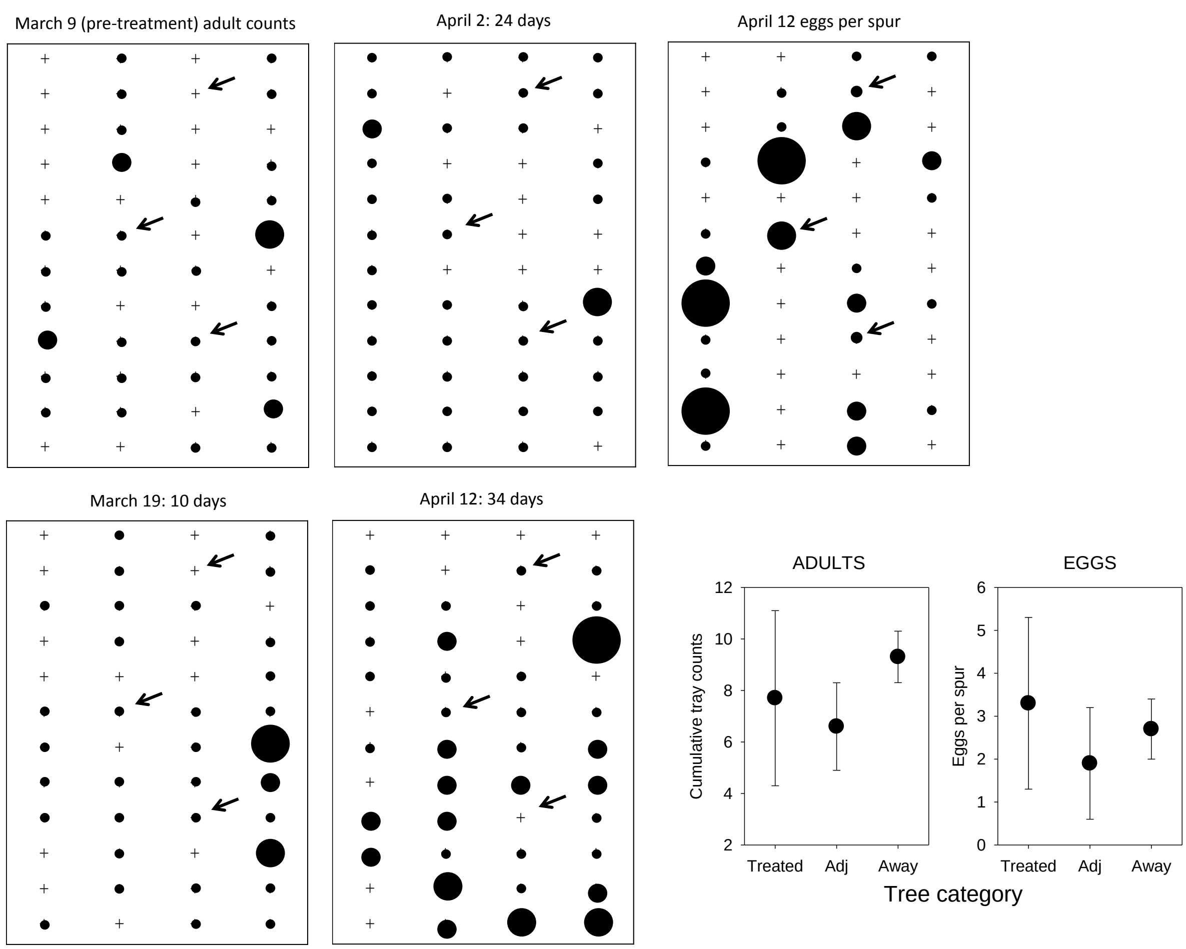

Figure 3. Field trial (48-tree orchard). Arrows: location of SPLAT-DMDS. Size of circle proportional to numbers. Four left-most panels: adults. Graphs in bottom right show count data summarized by whether tree was treated, adjacent to a treated tree, or away from treated tree. + indicates no psylla.

in the center two rows were chosen to receive the compound (Fig. 3 arrows). The compound was applied where lower-most limb attached to the trunk. All 48 trees were then sampled at intervals to determine adult numbers and egg numbers. ***Results.*** There was no evidence that the repellent slowed colonization of the three treatment trees (Figure 3; trees marked with arrows) or neighboring trees, nor did it affect egglaying (size of the circle in each figure is proportional to numbers). The graph at the bottom right compares cumulative numbers of adults and eggs on the treatment trees, the trees immediately adjacent to the treatment trees, and trees away from the treatment trees. There was no evidence that the SPLAT product affected distribution of adults or eggs.

4. SPLAT-New product: olfactometer trials. Olfactometer trials were done with a new SPLAT product (proprietary) shown to repel potato psyllid. The compound was extracted from volatiles emitted by a plant essential oil. The olfactometer trial compared pear leaves vs pear leaves + SPLAT product. ***Results.*** The product was shown to repel both winterform and summerform psylla (Figure 4). A field trial in 2013 was conducted of identical design to that used for the DMDS trial in 2012. Weekly samples were again taken but failed to show any repellency of the product (weekly data not shown; summary data shown in Figure 5).

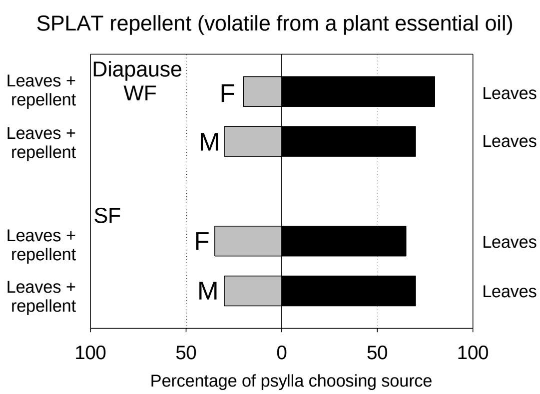

Figure 4. Olfactometer test of new SPLAT psyllid repellent (proprietary).

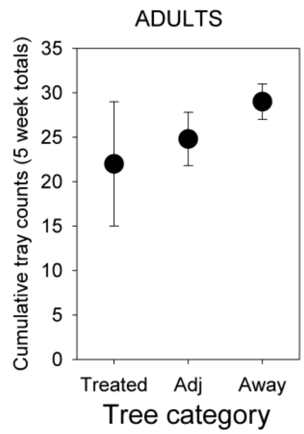

Figure 5. Tray counts on trees treated with SPLAT product, trees adjacent to treated trees, and trees distant from treated trees.

5. Pear ester: olfactometer trials. Olfactometer trials were done with pear ester in efforts to find a new attractant for pear psylla. ***Results.*** Pear ester was actually repellent to both summerform and winterform psylla in olfactometer trials (Figure 6), rather than attractive; this was possibly a concentration effect. A field trial failed to demonstrate activity in the field (data not shown).

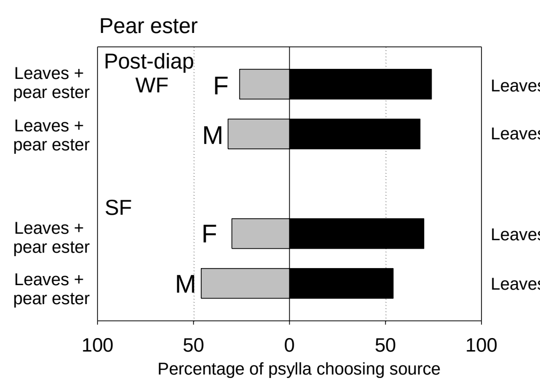

Figure 6. Repellency of pear ester to winterform and summerform psylla in olfactometer trials.

6. Pheromone, new compound: olfactometer trials. Two compounds "hidden" in GC-MS profiles by the 13-MeC27 peak were discovered by J. Millar, identified (9-MeC27, 11-MeC27), and then synthesized. Synthetic formulations were evaluated for attractiveness to summerform males. ***Results.*** The 11-MeC27 product was attractive to male summerforms (Figure 7).

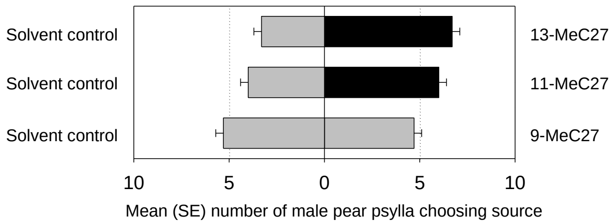

Figure 7. Olfactometer trial showing attractiveness of 11-MeC27 to male summerforms.

7. Pheromone, effects on mate-finding of male winterforms. Small cage studies were conducted to determine whether saturation of a cage with 13-MeC27 interferes with how rapidly males find and mate previously unmated female winterforms. Unmated psyllids were collected from a pear orchard in late winter before mating had begun in the field. Cages (with and without pheromone) received a mix of pear seedlings and pear cuttings. I added 50 females to each cage, and allowed them 24 hours to settle. After 24 hours, 50 males were added to each cage. Females were removed at 24 hours and 48 hours following addition of males, and dissected to determine spermatophore numbers (an index of mating frequency). ***Results.*** Saturation of cages with 13-MeC27 led to a statistical drop in number of spermatophores passed (compare black bars [=disruption cages] with gray bars [=control cages] in Figure 8). Results are consistent with the hypothesis that saturation of small cages with 13-MeC27 slowed rates at which males were able to locate females for mating.

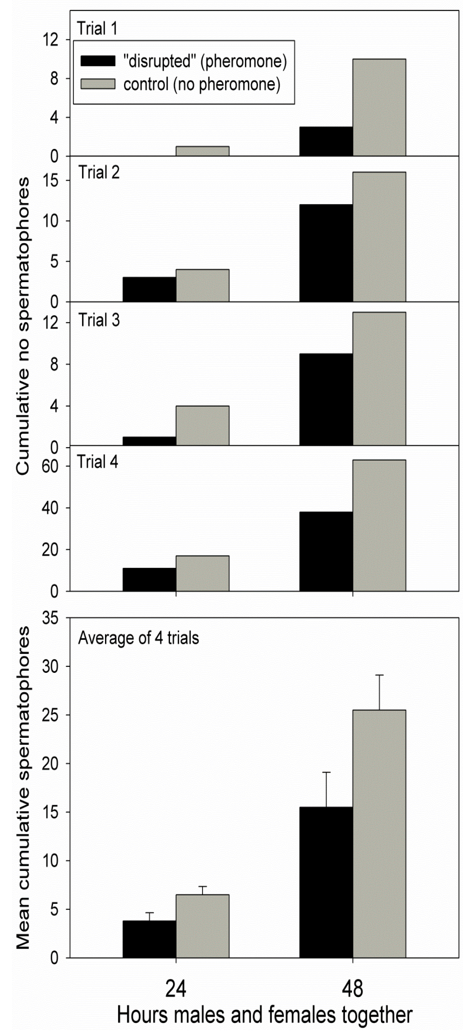

Figure 8. . Spermatophores per 25 females in "disruption" cages and pheromone-free cages**.**

8. Oil as carrier of 13-MeC27. Due to low volatility of 13-MeC27, an individual septa would presumably provide a signal over only a limited area. Saturation of an orchard with the active volatile would thus require many point sources. Pear growers apply oil (1-2% in water) in late winter to control mites and other soft-bodied arthropods. Because 13-MeC27 is a hydrocarbon, it is fully soluble in oil. I examined in a series of assays whether 13-MeC27 in oil was biologically active. ***(A). Olfactometer trial***. I examined response of male winterform psyllids to oil-treated shoots vs oil+pheromone-treated shoots. Shoots were treated with 1% oil or 1% oil+pheromone, with rates chosen to provide a signal of about 50 female equivalents on the oil+pheromone side of the olfactometer. Two trials, each with 100 males (10 runs x 10 males per run) were conducted. ***Results***. In both trials, a statistically significant number of males chose the pheromone side of the olfactometer (Figure 9). ***(B). Physical contact assay.*** I next examined whether treatment of shoots with oil+pheromone led to increased time males spend in physical contact with the shoot. Shoots treated with 1% oil or 1% oil+pheromone (concentration of pheromone as in the olfactometer trial) were paired in small circular arenas (15 cm in diameter). Shoots were placed with cut ends in water, 5 cm apart. Five winterform males were added to each chamber, and location of each male was recorded at 10 minute intervals over a 2 hour assay period. The assay included 20 replications of 5 males each. ***Results.*** Mean cumulative number of male psyllids in contact with the pheromone-treated shoot was significantly larger than number of males in contact with the oil-only shoot (Figure 10).

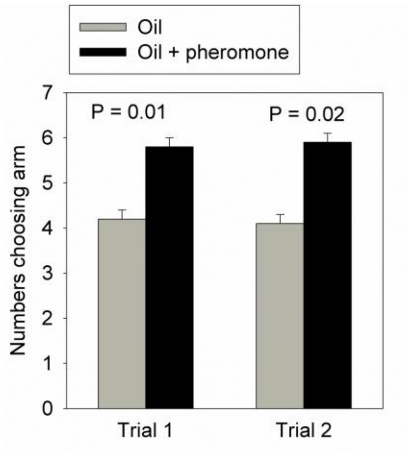

Figure 9. Results of olfactometer trial with oil as carrier.

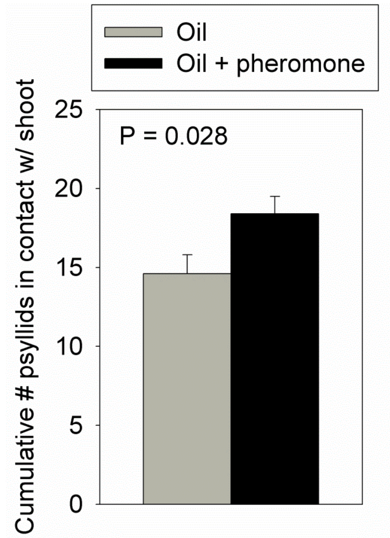

Figure 10. Arrestant properties of pheromone with oil as carrier.


Figure 11. Mesh cylinder (with tangle-foot) encircling treated shoot.

(C). Field trials with oil+pheromone formulation. Finally, field trials were done twice late in the winterform generation (April 2013). Paired shoots were sprayed with 10 ml of 1% oil in water or 1% oil+pheromone (n=10 pairs). Volume of pheromone per shoot was enough to provide a signal equivalent to about 50 females. Shoots were each about 18 inches in length. Each shoot was then encircled with a mesh cylinder that had been coated with tangletrap (Figure 11). Traps were left in the field for 3 days. ***Results.*** In the early-April trial, I found a substantial and highly significant increase in numbers of male psyllids on pheromone-treated shoots compared to oil-only shoots (by almost 20 psylla per trap; Figure 12: top panel). Females showed no preferences. Psyllid numbers declined substantially by the second trial in mid-April (Figure 12: bottom panel), as this was extremely late in the generation, and males were only marginally more abundant in the pheromone treatment. ***Conclusions from all assays with oil as carrier.*** Assays showed that 13-MeC27 retained its biological activity when dispensed in 1% horticultural oil, suggesting that it may be possible to develop a sprayable formulation of the attractant.

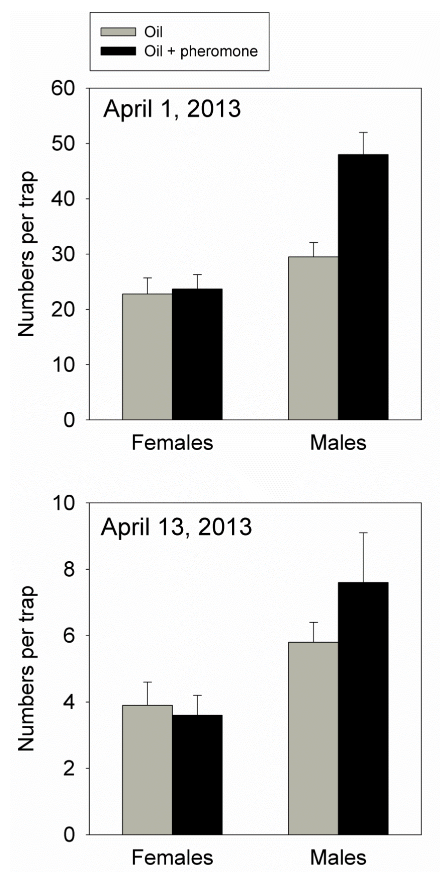

Figure 12. Winterform attraction in field to pheromone-treated shoots using oil as carrier.

EXECUTIVE SUMMARY

Management of pear psylla requires some level of control of the post-wintering winterform generation. Pear psylla at that time of year may be vulnerable to certain control approaches, due to characteristics of the psyllid's life history. These traits include (a) the requirement, following wintering, to rapidly locate a host for feeding (to replenish reserves expended in winter) and egglaying; and, (b) the life-history fact that pear psylla females overwinter in an unmated condition. (a) Strategies that could be used to slow or delay colonization of pear orchards through the use of repellents would lead to lowered densities of egglayers, added stress on psyllids to replenish expended reserves, and reduced fitness of egglayers. (b) Strategies that could be used to delay mating would lead to delays in egglaying combined with the production of early-season infertile eggs.

Repellents. Two compounds known to be repellent to other psyllid species were formulated into SPLAT release media by ISCA. Neither product exhibited field activity against returning winterforms, despite evidence for repellence in olfactometer trials.

Pheromone disruption. Small cage trials suggested that saturation of cages with the 13-MeC27 sex attractant led to lowered rates of mating. However, the very low volatility of the pheromone would make it difficult to obtain a similar level of saturation under orchard conditions in the absence of logistically infeasible numbers of point sources. Discussions with J. Millar (UCR) led to the suggestion that the attractant could be applied as part of the grower's typical dormant and delayed dormant oil sprays, given that the attractant (a hydrocarbon) would be completely soluble in horticultural oil. Laboratory and field trials suggested that the compound retains its biological activity in 1% oil (+ water).

Future directions. *Repellents*. I will be examining (with ISCA) a sprayable formulation of a potato psyllid repellent, rather than as a SPLAT-dispensed formulation. *Pheromone*. A field trial will be done Feb.-April 2014 to examine whether the sprayable formulation of 13-MeC27 affects mating rates of winterform psylla under orchard conditions.