PHEROMONE-BASED CODLING MOTH AND NAVAL ORANGEWORM MANAGEMENT IN WALNUTS

J. Grant, C. Pickel, D. Light, S. Goldman Smith, and J. Lowrimore

ABSTRACT

Tests to evaluate and compare four medium density passive (“meso”) pheromone dispensers were conducted in five commercial walnut orchards in San Joaquin and Stanislaus County. Successful trap capture and mating suppression was achieved in these trials, but a corresponding reduction in nut damage at harvest was not.

Two long-term aerosol dispenser codling moth mating disruption trials in Glenn and Butte Counties were continued in 2012 with mixed results. Trials conducted in collaboration with Steve Welter and Frances Cave to evaluate the effectiveness of “reduced rate” codling moth aerosol dispensers and with Charles Burks in support of his work to further refine mating disruption for navel orangeworm in walnuts showed promise for future expansion and validation.

OBJECTIVES

1. Continue puffer sites in Glenn and Butte Counties as demonstrations.
2. Validate the techniques for large-scale pheromone mating disruption in walnuts, with an emphasis on managing navel orangeworm (NOW) as well as codling moth (CM) with pheromone puffers.
3. Field-test modified “reduced rate” codling moth aerosol dispensers that contain one-half the pheromone dose of standard dispensers currently in use.

SIGNIFICANT FINDINGS

- Tests of currently registered and experimental “meso” pheromone mating disruption dispensers in multiple locations documented the effectiveness of these products for suppressing codling moth trap captures and mating in walnuts. With continued refinement, testing, and validation, these products will provide an effective mating disruption option for orchards considered too small for successful use of aerosol dispensers.

- Two long-term Sacramento Valley codling moth pheromone mating disruption demonstration trials were concluded. These and other trials concluded earlier have documented strengths and exposed potential weaknesses in pheromone-based programs. They have also played an important role in educating Sacramento and San Joaquin Valley growers and pest control advisors about this technology and promoting confidence in its use.

- Expanded testing of “reduced rate” aerosol devices showed promise for effective suppression of codling moth at lower potential cost than current programs.
Monitoring of navel orangeworm at a Butte county site helped validate and strengthen efforts by Dr. Charles Burks to refine mating disruption techniques for this pest in walnuts.

PROCEDURES

Evaluation of medium-density meso emitters for controlling codling moth

Recent work by Stephen Welter and others has demonstrated that passive medium density (“meso”) pheromone dispensers show promise for reducing codling moth damage and mating in walnuts, and that this technology can be used in walnut orchards considered too small for successful use of low-density aerosol devices like puffers and misters.

During the 2012 season, we conducted tests to evaluate and compare four meso devices in five commercial walnut orchards in San Joaquin and Stanislaus County (Table 1). Test orchards were 26 to 40 acres in size and each was divided into five plots of roughly equal size (5 to 8 acres). All test orchards had a history of moderate to high codling moth population pressure. This work was partially funded by manufacturers of the products tested.

Table 1. Descriptive information and meso dispenser placement for test orchards.

<table>
<thead>
<tr>
<th>Site</th>
<th>Cultivar</th>
<th>Orchard size</th>
<th>Orchard spacing</th>
<th>Dispenser placement, one dispenser in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lockeford</td>
<td>Serr</td>
<td>40 acres</td>
<td>30’ X 30’</td>
<td>Every other tree in every other row for 4 rows, skip every 5th row</td>
</tr>
<tr>
<td>Stockton</td>
<td>Vina</td>
<td>35 acres</td>
<td>27’ X 23.5’</td>
<td>E/O tree in E/O row plus every fifth tree in alternate rows</td>
</tr>
<tr>
<td>Escalon</td>
<td>Vina</td>
<td>35 acres</td>
<td>30’ X 30’</td>
<td>Every other tree in every other row for 4 rows, skip every 5th row</td>
</tr>
<tr>
<td>Empire</td>
<td>Howard</td>
<td>30 acres</td>
<td>24’ X 13’</td>
<td>E/O tree every 4th row, alternating with E/O tree in every 5th row</td>
</tr>
<tr>
<td>Tulare</td>
<td>Tulare</td>
<td>26 acres</td>
<td>24’ X 14.5’</td>
<td>E/O tree in every 4th row</td>
</tr>
</tbody>
</table>

One plot at each site was designated as a no-pheromone “grower standard” and one of four mating disruption products were assigned to each of the other plots:

1. Pacific Biocontrol Isomate®-CM Ring. *Contains codling moth pheromone only*
2. Trece CIDETRAK® CM Meso (TRE 0553/12). *Contains CM pheromone only*
3. Trece CIDETRAK® CM-DA Combo Meso Low Rate (TRE 0648/12). *Contains CM pheromone and DA kairomone*
4. Trece CIDETRAK® CM-DA Combo Meso High Rate (TRE 0552/12). *Contains CM pheromone and DA kairomone*

All dispensers were applied in a rectangular grid pattern as shown in Table 1 to achieve a density of 20 dispensers per acre. Pheromone dispensers were installed at or shortly before biofix of the overwintered generation by laborers, either from the ground with long poles or from pruning towers using long poles depending on tree height and preference of the orchard operator. Dispensers were hung in the upper 20% of the tree canopy at all sites.
Codling moth (CM) flight activity was monitored throughout the season using standard plastic delta (orange Pherocon® VI) traps. Each plot had four traps: one baited with a standard “1X” CM Long-Life L2™ lure, hung 6’ to 8’ from the ground (1X-lure traps not hung in the Combo Meso plots); two with Pherocon CM-DA Combo™ lures; and one with both a CM-DA Combo and Pherocon AA lure. Traps were monitored weekly and lures replaced at the manufacturer’s suggested eight-week interval. Every two weeks, trap liners from the CM-DA Combo lure and Combo + AA lure-baited traps were replaced, sent to the lab, and the gender of captured moths determined under a stereomicroscope. Mating status of female moths was assessed by dissecting their mating pouches (bursa copulatrix) exposing the presence of spermatophores deposited by males in each mating.

At the end of the first and second generations, nut damage assessments were made by inspecting six hundred randomly selected nuts from all portions of the canopies for signs of codling moth damage. During commercial harvest at each site, six hundred nuts were collected at random after nuts had been shaken to the ground (and, in some cases windrowed), and these were cracked open and inspected for the presence of codling moth and navel orangeworm larvae and damage. Orchard operators and their pest control advisors were informed of the results of weekly trap monitoring results and in-season nut damage assessments, and were allowed to make supplemental insecticide treatments for codling moth and other pests as they deemed necessary. When treatments were made at each site, the same treatments were made to all the plots at the site. The number, type, and timing of supplemental insecticide applications – targeting codling moth or other pests with possible suppressive effects on codling moth - varied greatly among sites (Table 2). Many of these treatments likely impacted the trap captures and nut damage levels discussed below.

Table 2. 2012 Insecticide applications in test orchards. All treatments applied to all plots at each site.

<table>
<thead>
<tr>
<th></th>
<th>Codling moth</th>
<th>Walnut aphid</th>
<th>Walnut Husk Fly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lockeford</td>
<td>None</td>
<td>None</td>
<td>8/22 Lorsban+NuLure</td>
</tr>
<tr>
<td>Stockton</td>
<td>5/30 Belt</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>6/25 &amp; 7/5 Voliam Express, alternate rows</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/31 &amp; 8/15 Macho+Fanfare, alternate rows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escalon</td>
<td>6/5 Asana</td>
<td>5/7 Assail</td>
<td>7/30 Asana+Monterey Insect Bait</td>
</tr>
<tr>
<td></td>
<td>6/25 Altacor+Lambda Cy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8/16 Pennncap-M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empire Howard</td>
<td>7/20 Belt+Lambda Cy</td>
<td>5/30 Assail</td>
<td>9/7 Asana+Monterey Insect Bait</td>
</tr>
<tr>
<td>Empire Tulare</td>
<td>Same as Empire Howard</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reduced rate aerosol dispensers

Field trials conducted in 2012 compared Suterra CheckMate® Puffer® or Pacific Biocontrol Isomate® Mist dispensers releasing the standard amount of pheromone (“full rate”) in each emission to the same units releasing half this amount (“reduced rate”). These trails also included a third “grower standard” portion of the orchard where no pheromones were used. Trials were
conducted at four sites in Butte, Glenn and San Joaquin Counties in addition to trials conducted by Frances Cave and Stephen Welter in other locations. Individual treatment blocks within each site were 15 to 25 acres in size, with aerosol units hung in the upper 1/3 of the tree canopy and deployed in a square grid design at the rate of one unit per 1.5-2 acres. Puffers and misters were installed at or before the beginning of the first codling moth flight.

Codling moth activity was monitored using six Pherocon® VI traps in each treatment block, three baited with Pherocon CM-DA Combo™ lures and hung high in the canopy and three baited with a standard Suterra 1X Biolures and hung at 6-8 feet from the ground. Traps were monitored weekly and lures replaced at the manufacturer’s suggested eight-week interval.

In-season crop damage was assessed at the end of the first and second generation by inspecting 500-1,000 nuts per treatment block for codling moth damage. At commercial harvest at each site, random samples of 1,000 nuts per treatment block were collected from the ground after shaking and harvest damage determined by examination of each nut for codling moth and navel orangeworm damage.

Weekly trap captures were shared with growers and their PCA’s, as were data collected from in-season damage assessments. Growers were encouraged to spray all treatment blocks the same when insecticides were applied.

**Long-term area-wide puffers demonstrations**

Two long-term area-wide puffer demonstration trials initiated in previous years were continued in 2012: the 110-acre Glenn site begun in 2005 and the 205-acre Butte site, begun in 2007. Monitoring of CM and NOW for these sites was funded by USDA’s Area-Wide Navel Orangeworm project. The variety at both sites is Vina. Although both of these sites have been largely successful in reducing codling moth sprays and keeping harvest damage low, neither has achieved the degree of sustained population reduction over time observed at other trial sites.

Codling moth activity was monitored using Pherocon VI traps baited with CM-DA Combo lures and hung in the upper ½ of the tree canopy at a density of one trap per 15-25 acres. Traps baited with Suterra 1X Biolures and hung 6’ to 8’ from the ground were placed approximately every 50 acres to assess the degree of trap shutdown in the presence of pheromone. Traps were checked at least every other week and lures were replaced as suggested by the manufacturers. In-season nut damage was assessed at the end of the second flight in late July or early August. Approximately 500 nuts in the lower part of the canopy were examined per trapping location. At commercial harvest, 500 randomly selected nuts were collected from the ground after shaking near each trapping location. The Glenn site was harvested once. The Butte site was harvested twice and harvest samples were collected during both harvests. Nuts were examined and damage by codling moth and navel orangeworm was recorded. Trap capture and in-season damage observations were shared with the cooperating growers and their PCAs and supplemental insecticide treatments made as they deemed necessary (Table 3).
<table>
<thead>
<tr>
<th>Glenn puffers</th>
<th>Butte puffers</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 18 Altacor</td>
<td>May 8 Penncap M</td>
</tr>
<tr>
<td>June 5 Chlorpyrifos</td>
<td>July 27 Assail+NuLure, EOR</td>
</tr>
<tr>
<td>July 4 Altacor+Lambda Cy</td>
<td>Aug 9 Assail+NuLure, EOR</td>
</tr>
<tr>
<td>July 25 NuLure+Chlorpyrifos (east side only)</td>
<td>Aug 9 Reaper+Chlorpyrifos+NuLure, EOR</td>
</tr>
<tr>
<td>Aug 29 Ethephon+Lambda Cy</td>
<td>Aug 18 Reaper+Chlorpyrifos, EOR</td>
</tr>
<tr>
<td></td>
<td>Aug 31 Ethephon+Perm-up</td>
</tr>
<tr>
<td></td>
<td>Sept 10 Perm-up</td>
</tr>
</tbody>
</table>

At the Butte County site, navel orangeworm was monitored throughout the season in support of Dr. Charles Burks’ CWB-funded efforts to evaluate and improve mating disruption of this pest. Modified Suterra wing traps baited with unmated female navel orangeworm moths were used to monitor male moth flight and Trece navel orangeworm egg traps baited with an almond meal and crude almond oil mixture - placed at least two trees away from the female-baited traps - were used to monitor egg-laying. Female moths were replaced weekly and the egg trap bait was replaced at approximately four week intervals.

**RESULTS AND DISCUSSION**

**Evaluation of medium-density meso emitters for controlling codling moth**

*Moth trap captures.* Male codling moth captures in low hung traps baited with standard “1X” pheromone lures were completely suppressed by the Trece CM-DA High and Trece CM-DA Low products at all sites, and nearly so by the Ring and Trece CM product, compared to no-pheromone plots, which varied considerably (data not shown). Such suppression of 1X trap captures is considered an indicator of at least some level of mating disruption. Averaged across all sites, CM-DA Combo baited trap captures (of male and female moths combined) were reduced slightly more by the Trece products than PBC Rings, but captures in these traps were extremely variable. All the pheromone products suppressed trap captures significantly compared to no-pheromone plots, but there were no statistically significant differences among the pheromone products in this respect (Fig. 1). Traps baited with CM-DA Combo + AA lures tended to capture more moths at all sites than those baited with the Combo lure alone, though this difference was not statistically significant (data not shown), due again to high variability among traps and the small number of traps of each type (two and one, respectively) deployed in each plot.

*In-season and harvest damage.* Nut damage was assessed after the first and second generations in no-pheromone, Ring, and Trece CM plots only. At both evaluation times, there was generally less damage in plots where one of the mating disruption products was present than in no-pheromone plots, though statistically significant differences could not be distinguished (Figure 2).

Nut damage at harvest was also highly variable among sites and very low at the Lockeford, Stockton, and Empire Tulare sites (Fig. 3). The low levels of codling moth damage observed at the Stockton site was particularly surprising given the strong season-long moth captures at this site.
Some sites had considerable navel orangeworm damage at harvest. We attribute this primarily to early season predisposition by walnut blight and sunburn and, in some sites, harvest delays. It is likely that some of these nuts were infested first by codling moth. Given, however, the roughly similar (perhaps more independent) levels of navel orangeworm damage across experimental treatments (Fig. 4), the presence of this damage does not alter our conclusions about relative impacts of the products on harvest codling moth damage.

Effects of Treatments on the Mating of Females. There was a large variation between the test orchards in total numbers of codling moth males (Fig. 5) and females (Fig. 6) captured in CM-DA Combo lure- and Combo + AA lure-baited traps combined for the no-pheromone and the various pheromone treatments.

Also, there was an effect in the no-pheromone plots on the higher average number of male codling moths captured over the number in pheromone treatments in the five orchards tested (Fig. 7). However, the average number of females captured was not significantly different between the no-pheromone and the pheromone treatments.

The occurrence of mating and its frequency among captured females was generally similar but also varied between the no-pheromone and pheromone treatments as averaged over the five test orchards (Fig. 8). The proportion of females that remained virgins did not differ amongst the no-pheromone and pheromone treatments ($F_{4,20} = 0.935, P = 0.464$). As has been reported in most mating disruption studies, the level of single - once mated females dominated the observed mating status. In this study females that experienced only single-matings reached levels of around 60+ %, but did not differ significantly between the pheromone treatments and was lowest in the no-pheromone plots ($F_{4,20} = 0.716, P = 0.591$).

The key difference between the no-pheromone and pheromone treatments was the degree of multiple-matings (Fig. 8). Significant differences were found in the frequency of twice or double-mated females ($F_{4,20} = 5.170, P = 0.005$), with all pheromone treatments having significantly lower degrees of secondary matings than the no-pheromone plots. In addition, the CM-DA High rate treatment had a significantly lower frequency of double-mated females than the PBC Ring pheromone treatment (paired t-test: $t = 3.903, P = 0.017$). Similarly, all pheromone treatments had significantly lower degrees of triple-matings by the captured females than the no-pheromone plots ($F_{4,20} = 7.575, P < 0.001$). Such suppression of female mating is the expectation of mating disruption and the expression of differential efficacy of various products.

The suppression of trap captures and female mating observed in plots where mating disruption products were deployed is consistent with results of previous work with meso pheromone dispensers in walnuts. We are surprised and disappointed that harvest codling moth damage levels did not correspond better with these other indicators of effective mating suppression. Except for the Lockeford site, where puffers were used in 2009-11, test sites had not been under mating disruption previously, so perhaps with an additional year or two of use, harvest damage would better reflect the cumulative effects of suppression observed this season.
Reduced rate aerosol dispensers

Results from the four sites trials comparing reduced and full rate dispensers are presented - along with those from the other trials conducted by S. Welter and F. Cave - in a separate report in this volume.

Long-term area-wide puffers demonstrations

Annual total codling moth CM-DA trap captures varied greatly among years at both the Glenn (Figure 9) and Butte (Figure 10) sites but have not shown gradual population suppression observed at other trials. Both of these locations have untreated, no-pheromone walnut orchards adjacent on three sides, possibly allowing mated females to migrate in to the puffers block.

At both sites, harvest codling moth damage varied considerably from the previous year (Fig. 13 & 14). At the Glenn site, damage was much lower in 2012 than in previous years. In contrast, codling moth damage at the Butte site, averaged 4.3%, much greater than in 2011. Damage by navel orangeworm was low at both sites in 2012.

Results of the NOW monitoring conducted at the Butte County site - along with those from a southern San Joaquin Valley trial conducted by Charles Burks – are presented in a separate report in this volume.

The results from these and other large-scale puffer demonstration plots conducted over the past five to seven years indicate that aerosol dispensers can be very effective in reducing codling moth populations, damage, and the need for supplemental insecticide applications when deployed continuously in walnuts over multiple seasons. These outcomes, however, have not been achieved in all orchards where this technology has been implemented. The Glenn and Butte County trials will not be continued. These trials, along with others discontinued after the 2011 season, have served their purpose of documenting the strengths and potential weaknesses of aerosol dispenser-based programs and educating growers and PCAs on their successful implementation in walnuts. Future work should focus on developing a greater understanding of factors contributing to this less-than-optimal performance in some orchards, and should lead to more informed, confident, and successful use of this technology among walnut growers.
FIGURES

Figure 1. Total seasonal moth capture in CM-DA baited Pherocon VI wing traps hung high in tree canopies at five San Joaquin and Stanislaus County test sites.

*Mean separation by Fisher’s Protected LSD, P<0.05.

Figure 2. Average nut damage by codling moth assessed at the end of the first and second generations at five test sites.

*Mean separation by Fisher’s Protected LSD, P<0.05. ANOVA performed on arcsin√y transformed data.
Figure 3. Nut damage at harvest caused by codling moth (CM) and navel orangeworm (NOW) for pheromone and no-pheromone plots at each test site.

*Mean separation by Fisher’s Protected LSD, P<0.051. ANOVA performed on arcsin√y transformed data.

Figure 4. Average harvest codling moth (CM) and navel orangeworm (NOW) damage at five test sites for no-pheromone and pheromone treatments.
Figure 5. Total seasonal captures of codling moth males at each test site for pheromone and no-pheromone plots.

Figure 6. Total seasonal captures of codling moth females at each test site for pheromone and no-pheromone plots.
Figure 7. Average seasonal capture per test site of male and female codling moths for the pheromone and no-pheromone plots.

![Graph showing seasonal capture per test site of male and female codling moths](image)

Figure 8. Mating occurrence and frequency of female codling moths captured in no-pheromone and pheromone treatment plots as averaged over the five test orchards for the season. Mating status reported as non-mated (virgin), once mated (1-mated), twice mated (2-mated), and thrice mated (3-mated) females as proportion of total females captured in the CM-DA Combo lure- and Combo + AA lure-baited traps combined.

![Graph showing mating occurrence and frequency of female codling moths](image)

*Mean separation by Student-Newman-Keuls method, P<0.05.*
Figure 9. Total seasonal CM-DA trap captures at Glenn puffers, 2005-2012

Figure 10. Total seasonal CM-DA trap captures at Butte puffers, 2007-2012
Figure 11. Glenn Puffers Percent CM Damage at Harvest, 2005-2012

Figure 12. Butte Puffers Percent CM Damage at Harvest, 2007-2012