NAVEL ORANGEWORM IN WALNUTS: MONITORING AND MANAGEMENT

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ABSTRACT

In 2014, navel orangeworm research examined the impact of an early insecticide treatment for navel orangeworm, and the association of pheromone trap counts with harvest damage.

Monitoring was extended to additional sites in Tulare and Stanislaus counties to examine the impact of an early treatment, timed to target eggs and neonates from the overwintering generation of navel orangeworm. These additional sites were also used to compare seasonal abundance and damage patterns to the sites monitored over the previous two growing seasons. Both the newly established sites and 2014 sites monitored in previous years were used to examine the association between pheromone trap counts and damage.

Seasonal abundance in the previously established Tulare County sites and in the newly established Stanislaus County insecticide test plots was similar to the trends seen in 2012 and 2013; i.e., higher navel orangeworm counts until the beginning of June, lower counts in June and July, and higher counts again starting in early August. The newly-established insecticide test plots in Tulare County had fewer males in the early season, but also had greater counts following August 1. Damage pattern analysis found that most insect damage occurred after husk-split, indicating navel orangeworm was the principal source of damage at the time of commercial harvest. This was consistent with the past two growing seasons, although there was greater evidence of codling moth damage in 2014 compared to these previous two growing seasons.

The early treatment resulted in a small nominal reduction of total males captured prior to August 1, and a modest reduction in harvest damage. These differences were not statistically significant. There was a moderate (albeit not statistically significant) association of pheromone trap counts in August with damage at first commercial shake in the newly-established Tulare County sites. However, no such association was detectable in the newly-established Stanislaus County sites or the previously-established Tulare County sites.

The results from research in 2014 suggest that one or more other factors may be of equal or greater importance with abundance in determining navel orangeworm damage at harvest. The period before and during the initiation of husk-split seems to be more important for monitoring with pheromone traps. Future navel orangeworm research should examine the relative efficacy and persistence of different husk-split insecticide treatment options, and the impact of ethephon on efficacy and persistence.

OBJECTIVES

1) Determine the impact of an earlier spring insecticide application on subsequent NOW trap.

2) Compare pheromone traps counts and subsequent damage.

SIGNIFICANT FINDINGS

- Data in 2014 confirm findings over the past two seasons that navel orangeworm is a primary cause of insect damage, although current-year data indicate that the contribution from codling moth varies from year to year.

- Trends in seasonal abundance were more uniform across the study sites in August, compared to earlier in the year. There was was modest and variable association of navel orangeworm pheromone trap counts in August with kernel damage at commercial harvest. The available data suggest that navel orangeworm pheromone data provide the greatest utility during this August period.

- The effect of an additional insecticide treatment targeting eggs and neonates from overwintering navel orangeworm had a modest impact on harvest damage. This effect was not statistically significant under the conditions of the 2014 study.

- These previous two findings suggest that, within the range of navel orangeworm abundance encountered in this study, one or more additional factors are of equal or greater importance in determining the proportion of walnuts damaged by navel orangeworm.

PROCEDURES

Sites and monitoring. Season-long monitoring with pheromone samples were used, in conjunction with sampling (see below), to determine the impact of an experimental additional insecticide treatment and to examine the association between pheromone trap counts and damage at harvest. All pheromone trapping was conducted using NOW Biolure (Suterra LLC, Bend OR) in an orange wing trap (Suterra LLC, Bend OR). Previous Walnut Board research has shown that NOW Biolure is more effective in the wing trap compared to other designs (Burks and Higbee 2015).

Monitoring was conducted in 2014 in ten Tulare County sites previously used for navel orangeworm research in 2014 (Burks et al. 2012, Burks et al. 2013). These orchards are described in tables in these two previous reports. Briefly, these sites ranged from 10-80 acres. The orchards were of various ages and heights, but predominantly more mature orchards containing susceptible varieties such as Serr and Vina. As with non-mating disruption sites in previous years, a single pheromone trap was used to monitor a 10 acre portion of each of these sites. In previous years, pheromone for this monitoring was provided by unmated females. Other research has shown that wing traps baited with unmated females and monitored at weekly intervals capture similar numbers of males compared to wing traps baited with NOW Biolure, apparently because initial greater effectiveness of the females is offset by shorter field life (Burks and Higbee 2015).

Samples were taken from each site at husk-split, and again at the time of first commercial harvest. Husk-split sites were taken using poles, with additional height obtained by working from a pick-up bed, ATV platform, or a 10-foot ladder. Harvest samples were taken from the orchard floor shortly after the trees were shaken as a part of commercial harvest. Samples of 100 walnuts were taken in or under 10 adjacent trees near the center of the monitoring block. Walnuts in Tulare County were held at -20°C until laboratory examination. Evaluation used guidelines from the UC IPM website (http://www.ipm.ucdavis.edu/PMG/C881/m881hppests.html). Insect infestation was assigned to one of three categories: codling moth, navel orangeworm, or unknown; as described in the previous report (Burks et al. 2013). Walnuts in Stanislaus County were held at ambient conditions and examined at soon as possible. NB. At the time of writing, 70% of the Tulare County walnut samples have been evaluated.

Monitoring and sampling at these sites were used to confirm trends observed over the previous two field seasons with respect to seasonal abundance and the primary importance of navel orangeworm (vs. codling moth) as a cause of damage, and also to examine the association of pheromone trap counts with damage. Additional sites were established for an experiment examining an early insecticide application, as described below.

Impact of an additional early insecticide application. A study of the effect of an early insecticide application was conducted using additional sites in Tulare and Stanislaus counties. The Stanislaus County sites were added because of observations of navel orangeworm damage in codling moth study sites in Stanislaus and San Joaquin counties in 2012 (Grant et al. 2012).

Paired plots were examined at four blocks (orchards) in each of the two counties. A conceptual plot arrangement is illustrated for a 40-acre square block (Figure 1). Most of the block received the additional early insecticide treatment, whereas this treatment was withheld from a smaller portion. The less stringent treatment (i.e., no early application) was applied to the smaller portion because it was presumed that the mobility of the navel orangeworm (Sappington and Burks 2014) would require this larger buffer to observe differences in adults in the untreated portion. These treated and untreated paired plots were monitored with grids of five pheromone traps (Figure 1) arranged in a square of 110 to 130 feet on each side (0.3 to 0.4 acres, depending on tree spacing), with a fifth trap placed approximately in the middle. Pheromone traps were as described in the previous section. Groups of traps were used because previous research found that apparent differences in abundance in adjacent blocks were detected better by groups of traps than by individual traps (Burks and Higbee 2015).

A “grower standard” insecticide program included insecticide applications timed to the codling moth 1B and 2A flights. The early treatment program included an additional treatment, intended to coincide with the onset of navel orangeworm oviposition (slightly ahead of a codling moth 1A application). To the extent possible, reduced-risk materials with alternating modes (http://www.irac-online.org/) of action were used at the full label rate. For example, at the early treatment plot at Site 3 received Intrepid (methoxyfenozide), Alticor (chlorantraniliprole), and

Delegate (spinosad) were used on 7 April, 4 June, and 24 June to target navel orangeworm oviposition and codling moth flights 1B and 2A, respectively. Similar patterns and timings were used at other sites. Intrepid Edge (methoxyfenozide and chlorantraniliprole) was used at 13 oz./acre (equivalent to methoxyfenozide a.i. in 16 oz./acre Intrepid 2F) for the early navel orangeworm treatment at sites 5, and at sites 4 and 5 this treatment included half Intrepid Edge and half Intrepid 2F. Intrepid 2F was used for the early treatment at all other sites.

This conceptual design was applied as closely as possible to mature blocks, of various area, with a history of navel orangeworm pressure (as identified by cooperating growers and PCAs) (Table 1). A fifth block was in Tulare County did not receive the early insecticide treatment, but was monitored and sampled as the other block to examine association of pheromone trap counts and damage (see subsequent section). Husk-split and harvest sample were taken (as described in the previous section) from trees in the 0.3 to 0.4 acre monitoring area.

Association of pheromone trap counts and damage. Since no significant effect of the early insecticide treatment was detected, the paired plots in the blocks established for this purpose were treated as separate observations. Based on in-season observations, harvest damage was compared with pheromone traps in the period near husk-split. For the Tulare County plots, damage at first shake was compared to average males per trap per week for the weeks of 18 and 25 August. Pheromone data was taken from the week of 8 September for the Stanislaus County plots because irrigation practices resulted in different monitoring periods during August for the blocks in Stanislaus County.

Statistical analysis. The proportion of insect-damaged walnuts was compared between the husk-split and first shake samples in Stanislaus County using a generalized linear mixed model (GLMM) with a binomial distribution, and with harvest as a repeated measure within the block (orchard) (Gbur and Stroup 2012). This analysis was not suitable for the Tulare County sites because of too many cases of complete separation (i.e., a 0 response). The data were therefore recoded as 2 × 2 contingency tables, and Fisher's Exact Test (Zar 1999) was used to test, on a site-by-site basis, whether there was greater damage in the harvest sample compared to the husk-split sample. The effect of the early insecticide treatment was tested using a mixed model ANOVA, with the insecticide treatment as a fixed effect and the block as a random effect. The cumulative number of males captured and the proportion of damaged kernels (with arcsine transform) were the response variables for the insecticide trial. Statmate (Graphpad Software, San Diego, CA, http://www.graphpad.com/scientific-software/statmate/) was also used to examine experimental power for this insecticide trial. The Spearman rank correlation method was used to examine the association of pheromone trap counts with subsequent damage. This non-parametric method was more appropriate than Pearson correlation, which is more susceptible to influence by extreme observations. Statistical analysis was performed using the SAS System (SAS Institute Inc. 2013). This association was examined separately between the previously-established sites and the new insecticide study sites in Tulare County because the trap arrangement was different between the two plots. The Stanislaus County insecticide test sites were also examined separately because it was necessary to use a different trapping period to compare to damage. Spearman correlation was also used to examine the association of first-harvest damage with harvest date.

RESULTS

Seasonal abundance and patterns of damage. Patterns of seasonal abundance of males in pheromone traps were broadly similar across three years in the previously-established plots in Tulare County (Figure 2). For this comparison, only sites not in mating disruption in 2012 and 2013 were used. Higher counts were seen between March and May, a decline by early June, and an increase between mid-July and August. The late-season pattern was similar in the insecticide test plots in both Tulare (Figure 3) and Stanislaus (Figure 4) counties. However, fewer males were seen in the Tulare County insecticide plots from March to May, whereas the early-season patter in the Stanislaus County insecticide test plots were more similar to the previously-established plots in Tulare County.

Patterns of insect damage were also broadly similar between the sites. Among the previously-established Tulare County sites, there were four instances in which the first harvest samples had significantly more insect damage ($P < 0.1$) than the husk-split samples, compared to one instance in which the converse was true (Table 2). At Site 1, in which the husks-split damage was greater, 20% of the insect damage was diagnosed as navel orangeworm. In contrast, over 60% of the insect damage was identified with navel orangeworm at the remaining nine previously-established sites. In the Tulare County insecticide test sites, no insect damage was found in any husk-split samples, while some insect damage was found in all samples from the first commercial harvest (Table 3). This difference was significant ($P < 0.1$) for six of the nine individual plots. Over 70% of the insect damage was associated with navel orangeworm in each of these nine plots. There was also a significant ($P < 0.1$) overall increase in damage between the husk-split and first commercial harvest in the eight Stanislaus County insecticide test plots (Table 4). At Site 13, 45% of insect damage was identified as navel orangeworm in the first commercial harvest at the untreated plot, and in the treated plot 50% was identified as navel orangeworm damage both in both the husk-split and first commercial harvests. In all other samples from Stanislaus County, ≥67% of damage was identified with navel orangeworm.

Impact of an early insecticide application. There was no practical difference between the number of males captured prior to August in the plots receiving the early insecticide treatment and the grower standard treatment (Figure 5A). There was nominally less kernel damage at first shake in the plots which received the early insecticide treatment than in the grower standard (Figure 5B). This difference, however, was also not statistically significant.

Association of damage with pheromone trap counts. The association of late summer pheromone trap counts with damage at first commercial harvest was weak and not statistically significant in the previously-established Tulare County sites (Figure 6) and in the insecticide trial sites in Stanislaus County (Figure 7). The association of damage at first harvest with pheromone traps was also not statistically significant among the Tulare County insecticide plots, but the plot and the greater value of the Spearman ρ suggest a greater association of damage with pheromone trap counts at these sites (Figure 7). Damage at first harvest was not significantly associated ($P >$ 0.1) with harvest date among the Tulare County samples (both previously-established and the new insecticide trial plots), but there was a nearly significant association of damage at first harvest with harvest date among the Stanislaus County insecticide trial plots ($\rho = 0.66$, $P = 0.08$, $N = 8$).

DISCUSSION

Research on navel orangeworm management in walnuts in recent years has found high damage with both mating disruption and conventional insecticide treatments (Burks et al. 2012, 2013). Recent studies of navel orangeworm management in almond have found value in spring applications (Higbee and Siegel 2012, Zalom and Nicola 2014). The recent introduction of a pheromone lure for navel orangeworm (Higbee et al. 2014, Burks and Higbee 2015) has also broadened possibilities and interest in monitoring for this pest. Here we established additional plots to examine the value of an earlier insecticide application for control of navel orangeworm in almonds, with the intention of also using these additional plots to provide greater information on the relationship between pheromone trap counts and subsequent damage. As a trade-off against the expanded sampling effort, we sampled only the first commercial harvest and not subsequent harvests.

Seasonal abundance and patterns of damage. While the relative number of males captured in spring vs. late summer was different in the newer Tulare County insecticide trial plots, a consistent increase in pheromone trap capture in August was seen at all sites. This observation has implications for monitoring (discussed in a subsequent section). Overall the damage in 2014 for the first commercial harvest the 10 previously-established Tulare County sites is similar to that reported in 2013, and less than that reported in 2012 (respectively 2.9 ± 2.2, 2.8 ± 1.7, and 6.8 ± 3.3). As in previous years, most of the identifiable damage was associated with navel orangeworm. Also, as in 2013, damage increased between the husk-split sample and the first commercial harvest. This timing for damage is important, because most codling moth damage would be expected prior to husk-split whereas most navel orangeworm damage to sound walnuts would be expected after husk-split. If navel orangeworm damage comes primarily from infestation of fruit previously damaged by codling moth, then this increase between husk-split and first commercial harvest would not be expected.

Impact of an early insecticide application. The data suggest a greater impact of the early treatment on damage at first harvest compared to males captured in pheromone traps in early season. At the current value of walnuts, a 0.3% difference in damage is potentially important. However, under the conditions observed in 2014, 30 blocks would be required for an experiment with a power of 80%.

Association of damage with pheromone trap counts. The data suggested a greater association of trap counts with damage in the Tulare County insecticide trial plots than in the other locations examined. There was also a near-significant association of damage with harvest date in the Stanislaus County insecticide plots, but not in the Tulare County locations. This greater association with harvest date in Stanislaus County may be due the harvests being stretched over a longer period at that location. That, and the involvement of different varieties, may present confounding variables and may explain why a trend was more evident in the Tulare County Insecticide trials, which involved a single variety in a more uniform set of orchards harvested within a shorter interval. These data nonetheless suggest that factors in addition to abundance are important to kernel damage. For example, in the Tulare County insecticide plots, the observation with greatest damage and the observation with the highest pheromone trap counts (but more modest damage) occurred in the same block, indicating that the influence of cultural differences (insecticide application, harvest date, etc.) was particularly minimal. Greater navel orangeworm damage was observed in Tulare County in 2012, despite lower degree-day accumulation than more recent years. In 2012 an unusually hot period in August resulted in many injured walnuts. Conceivably it could conceivably also have caused more vulnerability in to navel orangeworm entry in the shells of sound walnuts.

In general, the results from the current study are consistent with recommendations for a treatment for codling moth for both the 1A and 1B flights in orchards with a history of lepidopteran damage. Future research needs include a more refined understanding of the relationship between late season navel orangeworm pheromone trap counts, walnut vulnerability and damage; and examination of efficacy of insecticides that could potentially be used between husk split and over multiple harvests.

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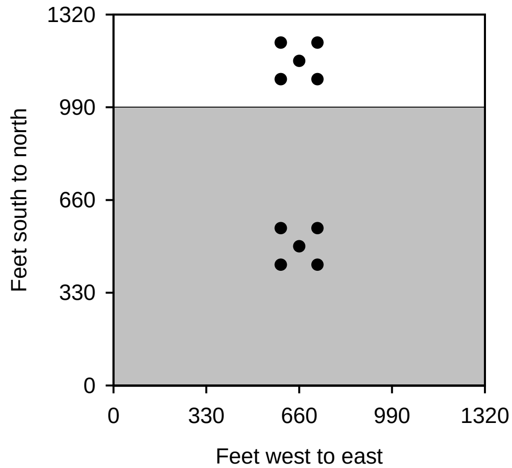

Figure 1. Plot arrangement for the 2014 study of effect of an early insecticide treatment on navel orangeworm abundance and damage. A conceptual diagram of the general plot design for a test of a grower's standard regime of insecticide by itself (white area, 10 acres), or with an additional early treatment (gray area, 30 acres). The standard treatments were applied at codling moth 1B, 2A timings, and at husk split. The additional early treatment, targeting eggs and neonates from overwintering (flight 1) navel orangeworm, was timed slightly earlier than codling moth 1A.

Table 1. Research sites used in the 2014 study of the effect of an early insecticide treatment on navel orangeworm abundance and damage.

County	Site	Longitude, Latitude	Variety	Size (acres)
Tulare	3	36.3351, -119.2290	Serr	31
	4	36.2480, -119.2781	Serr	43
	5	36.2404, -119.2715	Serr	27
	6	36.2369, -119.2513	Serr	35
	7[a]	36.2562, -119.3872	Serr, Sunland	46
Stanislaus	10[b]	37.6579, -120.7443	Hartley	25
	11	37.6297, -120.8762	Howard	18
	12	37.6393, -120.8829	Serr	10
	13	37.6177, -120.7211	Vina	30

[a]No early insecticide application; the Serr portion was used only for association of monitoring and damage. [b]Orchard included Hartley, Franquette, and Vina, but only Hartley was sampled.

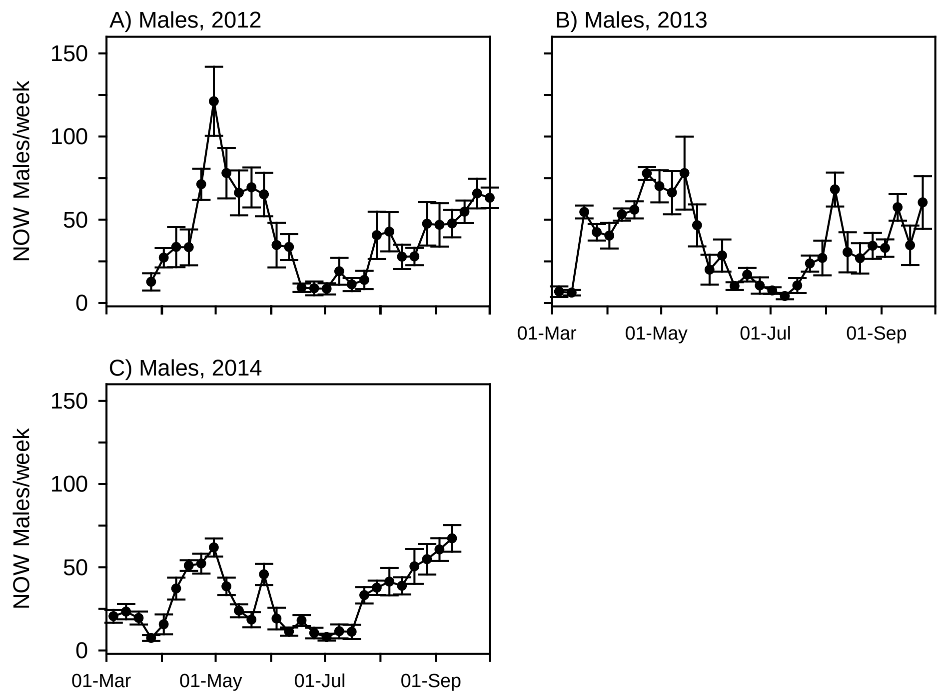

Figure 2. Weekly counts (mean and SE) of males in individual pheromone traps in six Tulare County sites monitored from 2012 to 2014.

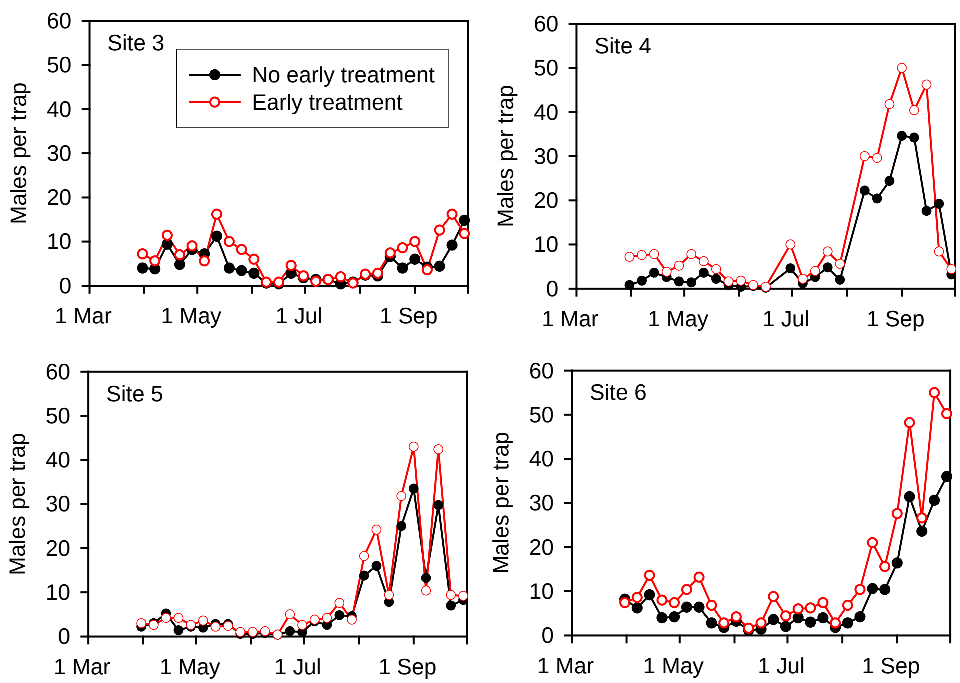

Figure 3. Weekly counts of males in pheromone traps in insecticide test sites in Tulare County. Plots received either a grower standard insecticide regime (closed circles), or an additional early treatment (open circles). Figures are the mean of 5 traps, which were treated as a single unit for analytical purposes. Compared to the sites monitored over multiple seasons (Figure 2), fewer adults were trapped in spring.

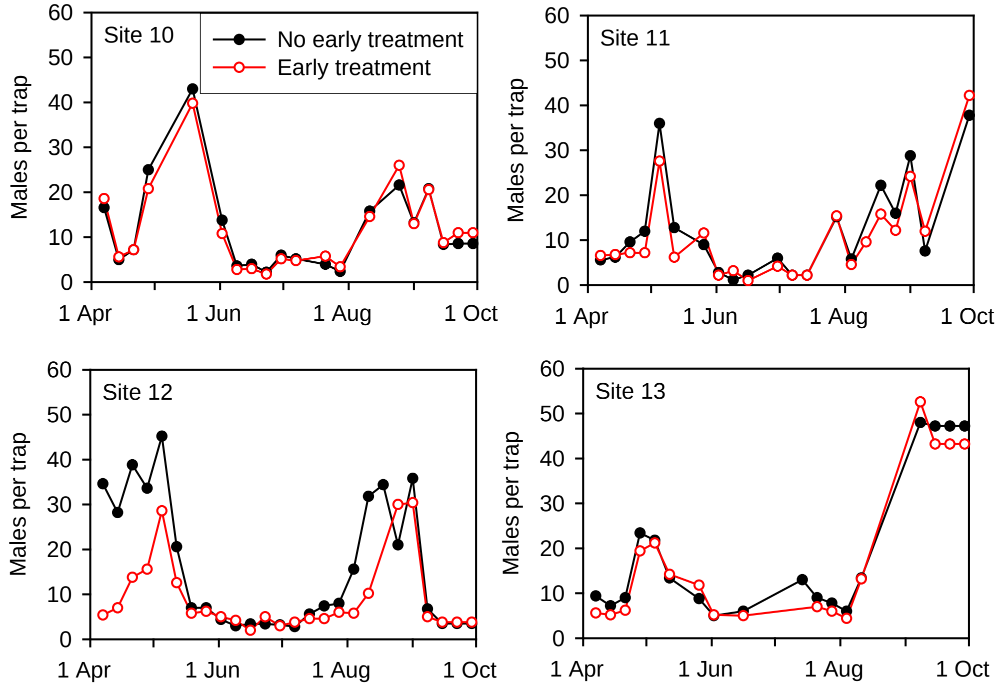

Figure 4. Weekly counts of males in pheromone traps in insecticide test sites in Stanislaus County. Plots received either a grower standard insecticide regime (closed circles), or an additional early treatment (open circles). Figures are the mean of 5 traps, which were treated as a single unit for analytical purposes. Unlike the Tulare County sits (Figure 3), the overall pattern of male seasonal abundance was similar to the pattern observed in Tulare County sites monitored over multiple seasons (Figure 2).

Table 2. Sample size and percent insect damage at husk-split and first shake damage in 10 Tulare County sites in 2014 (same as sites examined in 2012 and 2013)

	Sample size (Walnuts) and date		*Percent Insect Damage*	
Site	**Husk-split**	**First shake**	**Husk-split**	**First shake**
1	709 (8/26)	1025 (9/10)	1.41***	0
2	424 (8/22)	607 (9/5)	0	0
3	519 (8/26)	1014 (9/9)	0.19	0
4	603 (8/29)	1014 (9/6)	0.33	1.08
5	584 (8/21)	613 (8/28)	0.17	0.16
6	515 (8/25)	601 (9/8)	6.41	22.13***
7	630 (8/25)	510 (9/15)	0	0.59ns
8	611 (8/26)	616 (9/18)	0	0.00
9	813 (8/25)	496 (9/15)	1.23	2.62ns
10	550 (8/22)	663 (9/5)	1.27	2.87ns

Significance of difference in proportion of kernel damage between the husk-split and first shake samples for individual plots (Fisher's Exact Test): ns $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Note that these are different sites from Table 2.

Table 3. Sample size and percent insect damage at husk-split and first shake damage in 9 Tulare County insecticide test plots in 2014

		Sample size (Walnuts) and date		*Percent Insect Damage*	
Site	**Plot**	**Husk-split**	**First shake**	**Husk-split**	**First shake**
3	Early treatment	408 (8/29)	1003 (9/5)	0	0.3
	Grower Standard	400 (8/29)	1002 (9/5)	0	0.4
4	Early treatment	400 (8/26)	999 (9/8)	0	2.3***
	Grower Standard	400 (8/26)	1002 (9/8)	0	1ns
5	Early treatment	398 (8/20)	801 (9/3)	0	1.12*
	Grower Standard	402 (8/22)	1001 (9/3)	0	1.2*
6	Early treatment	399 (8/22)	1002 (9/9)	0	1.5**
	Grower Standard	397 (8/22)	1003 (9/9)	0	0.5
7	N/A	402 (8/27)	1000 (9/10)	0	0.9ns

Significance of difference in proportion of kernel damage between the husk-split and first shake samples for individual plots (Fisher's Exact Test): ns $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Note that these are different sites from Table 2.

Table 4. Percent insect damage at husk-split and first shake damage in 8 Stanislaus County insecticide test plots in 2014

		Percent Insect Damage	
Site	**Plot and date of first shake**	**Husk-split**	**First shake**
10	Early treatment (10/8)	0.5	1.2
	Grower Standard (10/8)	0.3	1.7
11	Early treatment (10/1)	0.4	0.9
	Grower Standard (10/1)	0.4	0.4
12	Early treatment (9/15)	0.1	0.3
	Grower Standard (9/15)	0.3	0.2
13	Early treatment (9/14)	0.4	1.1
	Grower Standard (9/14)	0.2	0.2

All percentages based on 1000-nut samples. There was a marginally significant difference in the overall proportion of damaged kernels between the husk-split and first shake samples (GLMM with binomial distribution, $F = 5.44$, df = 1,7; $P = 0.0524$).

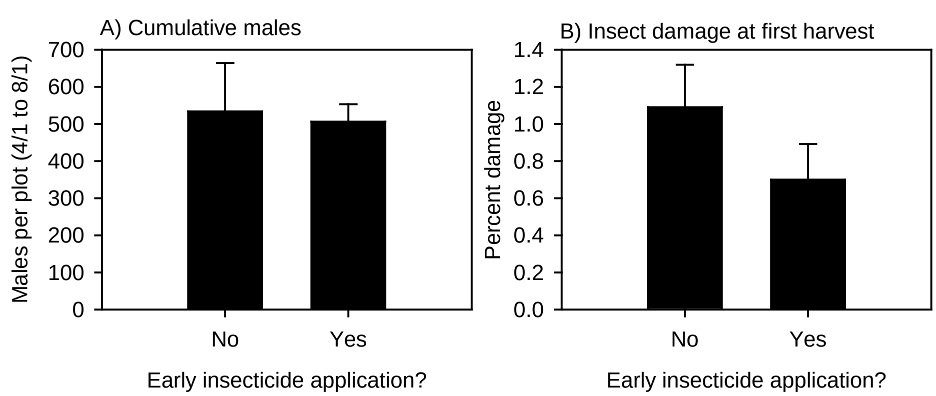

Figure 5. Effect of an additional early insecticide treatment on A) the cumulative total of males captured prior to 1 August ($F = 0.08$, df = 1,7; $P = 0.78$); and B) damage to walnut meats at first harvest ($F = 3.03$, df = 1,7; $P = 0.13$).

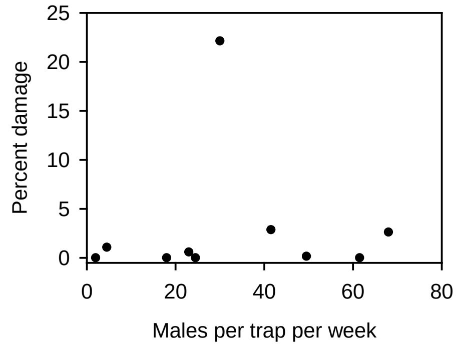

Figure 6. Association of damage with pheromone trap counts in 2014, in ten Tulare County sites monitored from 2012 to 2014. Male counts per week reflect average males per trap per week for weeks of 18 and 25 August, 2014 ($\rho = 0.29$, $P = 0.41$).

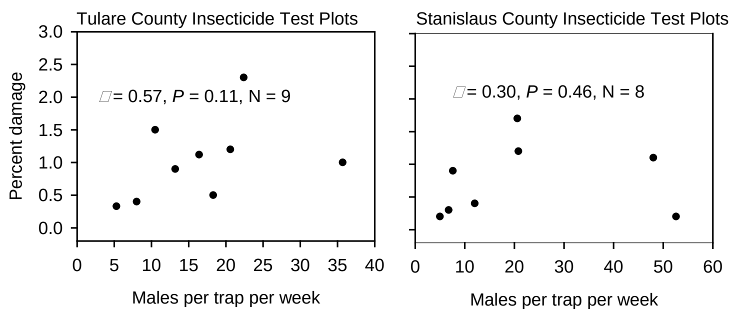

Figure 7. Association of damage with pheromone trap counts in individual plots used in insecticide tests in Tulare and Stanislaus counties in 2014. Male counts per week reflect average males per trap per week for weeks of 18 and 25 August for Tulare County; and the average males per week per trap for the week of 8 September in Stanislaus County.