MONITORING AND MANAGEMENT OF MEALY PLUM AND LEAF-CURL PLUM APHIDS USING SEX PHEROMONES

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OBJECTIVES

1. Investigate whether aphid sex pheromones may be used to develop monitoring protocol for mealy plum and leaf-curl plum aphids in dried plum orchards.
2. Explore the use of aphid sex pheromones for mating disruption of mealy plum and leaf-curl plum aphids in dried plum orchards.

Mealy plum aphids (MPA), *Hyalopterus pruni*, and leaf-curl plum aphids (LCPA), *Brachycaudus helichrysi*, are serious pests affecting California's dried plum (i.e., 'prune') crop. Current monitoring practices for MPA and LCPA in prune orchards include dormant season spur samples aimed at detecting overwintering aphid eggs, recommending a treatment threshold of one aphid egg per 100 spur samples. However, current guidelines concede that the absence of aphid eggs in spur samples is not conclusive evidence that aphids will not be a problem, and that orchard history should be used as an additional guideline. Another obstacle concerning dormant sampling for aphid eggs is that it can be difficult and time-consuming, even for well-trained individuals, to detect the eggs. These factors impact the reliability and practicality of the current monitoring protocol and often result in the majority of orchards being treated during the dormant season, often without quantification of the actual overwintering population. Development of a reliable method to assess the population density of return migrants that give rise to the overwintering and subsequent spring populations could be a valuable tool in the management of MPA and LCPA in prune orchards. Typical management practices targeting aphid pests in prune orchards involve the application of a dormant insecticide treatment, usually a pyrethroid or organophosphate with or without oil. As occurs with the application of any management tactic, there are concerns with insecticide treatment during the dormant season, namely runoff and water quality issues. Changing the dormant spray timing to mid-fall could help mitigate runoff issues, but monitoring of MPA and LCPA in the fall becomes even more critical to ensure maximum efficacy of the insecticide application. The use of aphid sex pheromones for monitoring and/or mating disruption has yet to be widely researched, likely because many of the most severe aphid pests affect the secondary host plant, where reproduction is strictly asexual. The fact that MPA and LCPA are pests of the primary host plant provides an ideal system in which to investigate the potential for exploiting the sexual stage of their life cycle to improve monitoring and management practices. Research reports from 2010 and 2011 provide additional detailed background information and results from previous years' studies in the system working toward the above objectives.

This report details two separate experiments conducted during the 2011 – 2012 season. The monitoring experiment examined relationships among numbers of aphids in pheromone-baited traps during fall, overwintering egg densities, and spring aphid populations to determine whether pheromone-based fall monitoring has the potential to be used to predict subsequent aphid populations and/or aphid-related crop damage in order to facilitate treatment decisions. If consistent relationships can be identified between trap catches and later population measures, pheromone-based fall monitoring may augment or replace current aphid monitoring recommendations, which involve laborious and often unreliable overwintering egg samples. The mating disruption experiment compared fall trap catches, overwintering egg densities, and spring aphid populations between pheromone-treated mating disruption plots and no-pheromone control plots. Significantly fewer males in pheromone-baited traps in mating disruption plots (i.e., ‘trap shutdown’) is a measure often used to indicate the effectiveness of disruption treatments. However, more meaningful measures in terms of crop protection are those that indicate substantial reductions in future generations and/or reduced crop damage. In this experiment, overwintering egg densities and spring aphid populations were evaluated in addition to trap shutdown in order to determine the effects of mating disruption treatments on subsequent life stages and generations. Successful disruption of mating resulting in tolerable spring population levels and sub-economic crop damage may provide a viable alternative to traditional dormant or in-season insecticide treatments.

PROCEDURES

Monitoring experiment

The experiment involved 25 replicates in five separate commercial prune orchards in Tehama County, CA. Each replicate consisted of a 5x5 tree plot with a pheromone-baited water trap in the center tree. Based on the results of prior studies, a pheromone ratio of 1:1 (nepetalactone:nepetalactol) was used. Pheromone lures used in this study were the same as those used in previous seasons: flexible polyvinyl chloride (PVC) polymer strips (‘ropes’) formulated as 5% extrusions of each compound separately, cut to length to achieve the 1:1 ratio at an approximate release rate of 200 micrograms per day. Traps were changed and aphids quantified weekly during the fall from 14-October-2011 through 16-December-2011, and pheromone lures replaced every three weeks during this period. Overwintering egg samples and spring population assessments were taken from the 25 trees per experimental replicate. Spur samples were collected in January 2012 for the overwintering egg assessment. A total of twelve spur samples per tree (300 per replicate) were collected; two from each directional tree quadrant (NSEW) and four from the upper tree canopy using extendable pole pruners. Spur samples were examined under a microscope and the numbers of aphid eggs recorded. Spring populations were assessed in April 2012 using a 0 to 10 rating scale based on percent leaf curl incidence as the indicator for LCPA population levels. Spring populations of MPA were not observed and were therefore not included in the spring population assessment. Linear regression analyses were used to examine relationships among fall trap catches, overwintering egg densities, and spring aphid population ratings. Because considerable numbers of non-target aphids have typically been encountered during pheromone trapping in prune orchards over the course of our studies, and because practical applications of pheromone-based monitoring would likely involve quantification of total aphid numbers rather than particular aphid species, the results reported for this experiment included non-target aphid species in addition to the target aphid species, LCPA and MPA, as well as total aphids (non-target and target species combined).

Mating disruption experiment
The experiment involved five replicates, one replicate in each of five separate commercial prune orchards in Tehama County, CA. Each replicate consisted of two 3x3 tree plots: one pheromone-treated mating disruption plot and one no-pheromone control plot. Three pheromone dispensers per tree (27 per plot) were deployed in the mating disruption plots and a pheromone-baited water trap, used to assess trap shutdown, was located in the central tree of each mating disruption and control plot. The hand-applied PVC 'rope' product described above and in previous research reports was used as the disruption dispensers and trap lures in this experiment, each at the 1:1 (nepetalatone:nepetalactol) ratio and approximate 200 micrograms per day release rate. Pheromone dispensers and trap lures were replenished every three weeks throughout the fall experimental period. Water traps were changed and aphids quantified weekly from 14-October-2011 through 16-December-2011 in order to compare fall trap catches between mating disruption and control plots (i.e., assessment of 'trap shutdown'). Spur samples were collected in January 2012 for the overwintering egg assessment. A total of twelve spur samples per tree (108 from each treatment and control plot) were collected; two from each directional tree quadrant (NSEW) and four from the upper tree canopy using extendable pole pruners. Spur samples were examined under a microscope and the numbers of aphid eggs recorded. Spring populations were assessed in April 2012 using a 0 to 10 rating scale based on percent leaf curl incidence as the indicator for LCPA population levels. Spring populations of MPA were not observed and were therefore not included in the spring population assessment. Statistical analyses involved t-tests comparing numbers of aphids trapped during fall, overwintering egg densities, and spring aphid population ratings between pheromone-treated mating disruption plots and no-pheromone control plots. As with the monitoring experiment, for practical application purposes, the results reported for this experiment included non-target aphid species in addition to the target aphid species, LCPA and MPA, as well as total aphids (non-target and target species combined).

RESULTS & CONCLUSIONS

Monitoring experiment
Total numbers of aphids trapped during the fall monitoring period are shown in Table 1. Male LCPA were caught in high numbers in the pheromone-baited traps, and represented the majority of aphids trapped. Very few LCPA gynoparae were detected, which was expected based on previous studies that indicated no attraction of gynoparaous forms of the species to pheromone-baited traps. Negligible numbers of MPA were trapped in this experiment. Low MPA populations are apparently typical for prune growers in Tehama County; LCPA populations tend to predominate in this area. As in previous years' studies involving fall pheromone trapping of aphids in prune orchards, a considerable number of male aphids of non-target aphid species also were trapped, as were measurable numbers of non-target gynoparae. Weekly totals of trap catches of male LCPA and non-target

males and gynoparae are shown in Figure 1. The overwhelming majority of male LCPA were trapped in a single week in late October, while numbers of non-target males and gynoparae were trapped relatively consistently throughout the fall. It is not unexpected that the pooled non-target collection data is more distributed throughout the fall trapping period because it is likely composed of a number of different aphid species which do not actually colonize or reproduce in prune orchards and whose peak abundance in the orchards likely differs based on the migration and activity period of the particular species (think of them as accidental tourists intercepted in the pheromone traps while attempting to locate their respective overwintering hosts). The fact that LCPA activity appears to occur within a very limited time period in the fall is beneficial for management, allowing monitoring efforts for the purposes of population quantification and population reduction tactics such as mating disruption to be focused within a very specific time interval. In addition, this is valuable information for growers who prefer to apply their ‘dormant’ treatments during the fall before wet winter weather becomes an obstacle (a practice that can reduce insecticide run-off and agricultural impacts to water quality). Timing of fall insecticide applications should occur after peak LCPA and/or MPA activity has been detected in the orchard in order to achieve maximum efficacy.

Linear regression analyses revealed no discernable relationships among fall trap catches of aphids (male LCPA and total aphids), overwintering egg densities, and spring aphid population ratings when data from all 25 replicates across the five orchards were pooled (Figure 2). Linear regression analyses examining the data separately for each experimental orchard site are displayed in Table 2. When analyzed individually, the data indicate positive relationships between fall trap catches and spring aphid populations (male LCPA, $R^2 = 0.96$; total aphids, $R^2 = 0.97$) in one particular orchard (Site 4, Table 2), highlighting the variability among orchards and the difficulty in establishing consistent correlations using fall trap catches for implementing pheromone-based monitoring protocol. Results of the monitoring experiment also highlight the lack of reliability in using overwintering egg samples for determining whether treatment is recommended, as no relationship between overwintering egg density and spring aphid populations was detected when data from all 25 experimental replicates were pooled ($R^2 = 0.03$, Figure 2), although a positive relationship appeared to be exist in one of the experimental orchards when analyzed separately ($R^2 = 0.87$, Site 3, Table 2).

The monitoring experiment, although unable to provide consistent results required to identify treatment thresholds and implement dependable monitoring protocol, did demonstrate that pheromone-based fall trapping can provide valuable information regarding pest aphid activity (LCPA in this case), which can be useful for directing future monitoring and management efforts and establishing optimal timing of insecticide treatments. Results of this study also emphasize the limitations of the current aphid monitoring recommendations for prune orchards, which rely on overwintering egg thresholds to determine the need for treatment intervention, and suggest that future studies should focus efforts on fall trapping, spring population, and crop damage measures rather than egg samples.

Mating disruption

The numbers of aphids trapped during fall in pheromone-treated mating disruption and no-pheromone control blocks are shown in Table 3. Overall, LCPA males represented the majority of aphids trapped during the experiment; a measureable number of non-target male species were also trapped. Few gynoparae of any species were trapped and no MPA were detected in this experiment. Seasonal trap catches in treated and control blocks are shown in Figure 3. Similar to the monitoring experiment, male LCPA were trapped almost entirely within a single week in late October, while the assemblage of non-target male aphid species were trapped somewhat consistently throughout the season. Differences between the mean numbers of aphids trapped in mating disruption and control blocks are shown in Figure 4. Statistically, there was no difference in the number of male LCPA trapped in pheromone-baited traps during fall in treatment and control plots ($P = 0.14$), likely because of the large variation that existed between the replicates (Table 4, Figure 4). However, a total of 421 male LCPA were trapped in the pheromone-treated plots while only 42 were trapped in the control plots. This is the opposite of what was expected if the pheromone treatment was effective in disrupting mating (i.e., successful trap shutdown), but these results do suggest that the pheromone treatment was behaviorally active and attracted more males into the area, although it was not sufficient to disrupt the males' ability to locate pheromone sources or females. Significantly fewer non-target male aphids were trapped in the pheromone-treated plots ($P = 0.016$), however identification of the particular non-target aphid species trapped is necessary to establish the biological relevance of this (i.e., effectiveness of mating disruption on individual non-target aphid species). There was no statistical difference in egg density, as only three eggs were detected in this experiment; all three were found in the control plot of one replicate (Table 4). Mean spring population ratings in treated and control blocks are shown in Figure 5. There is a marginally significant effect ($P = 0.09$) of the pheromone-treatment on spring LCPA populations, with evidence of larger populations in the pheromone-treated mating disruption plots. These results are again contrary to those expected if pheromone-treatment was effective in disrupting mating, suggesting rather that the method of release and/or amount of pheromone used in this experiment was effective in attracting more male LCPA into the experimental plots but was not sufficient to reduce mate location or mating. Although mating disruption in the current study was not achieved, there is a strong indication that the pheromone lures do indeed elicit behavioral activity in male LCPA, as evidenced by large numbers trapped and relatively higher spring populations in the pheromone-treated plots. This provides an opportunity to continue to investigate whether mating disruption is feasible, perhaps by altering the amount of pheromone used for disruption (by increasing release rates or point sources), or investigating alternative pheromone dispensers. Future mating disruption experiments also should be designed to account for the high degree of orchard variation (Figure 4), and should focus efforts on the trap shutdown, spring population, and crop damage measures, rather than overwintering eggs, which are often detected in very low numbers and do not necessarily relate to subsequent aphid population densities (Table 2, Table 4, Figure 2). Additional population reduction tactics that exploit the apparent attraction of males to pheromone-treated areas may also be possible. Some examples include attract and kill and trap-out methods, as well as entomopathogen dispersal, in which pests are attracted to a source where they contact pathogenic organisms (e.g., insect-pathogenic fungi such as *Beauvaria bassiana* and *Metarhizium anisopliae*) and subsequently spread the pathogens throughout the pest population.

BUDGET SUMMARY

Funding for this project in 2011 and 2012 was provided by the CDFA Specialty Crop Block Grant Program.

Table 1. Numbers of aphids trapped during fall 2011 (monitoring experiment).

Aphid species	Total aphids trapped
LCPA male	1454
LCPA gynoparae	3
MPA male	1
MPA gynoparae	2
Other male	440
Other gynoparae	86

Table 2. Linear regressions of fall trap catches, overwintering egg densities, and spring aphid population ratings, analyzed separately for each orchard site (monitoring experiment).

	Male LCPA vs.				Total Aphids vs.				Egg Density vs. Spring Population	
	Egg Density		Spring Population		Egg Density		Spring Population			
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
Site 1 (n=6)	N/A	N/A	0.0043	0.00451	N/A	N/A	0.004	0.00388	N/A	N/A
Site 2 (n=5)	-0.0018	0.16942	0.0013	0.06258	-0.0017	0.17864	0.0013	0.06678	0.3041	0.06525
Site 3 (n=5)	0.0016	0.30188	0.0015	0.23863	0.0013	0.24677	0.0012	0.18387	0.9947	0.87376
Site 4 (n=3)	-0.0103	0.0235	0.0214	0.96125	-0.0116	0.0366	0.0193	0.97476	-0.1117	0.11891
Site 5 (n=6)	-0.0237	0.19902	0.0027	0.02084	-0.0293	0.22933	0.0042	0.07124	0.0984	0.14732

Table 3. Numbers of aphids trapped in pheromone-treated mating disruption and no-pheromone control plots during fall 2011 (mating disruption experiment).

Aphid species	Total aphids trapped	
	Control	Treated
LCPA male	40	421
LCPA gynoparae	1	1
MPA male	0	0
MPA gynoparae	0	0
Other male	180	30
Other gynoparae	21	6
Total aphids	242	458

Table 4. Fall trap catches, overwintering egg densities, and spring aphid population ratings in pheromone-treated mating disruption and no-pheromone control plots, shown separately for each orchard site (mating disruption experiment).

	Male LCPA		Other male		Total aphids		Egg Density		Spring Rating	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Site 1	12	15	12	15	79	23	0	0	3	4.7
Site 2	1	319	1	319	11	321	0	0	0	1.8
Site 3	4	75	4	75	32	77	0	0	0	0
Site 4	1	11	1	11	46	29	0	0	2	4
Site 5	22	1	22	1	74	8	2.8	0	0.56	2.6

Figure 1. Weekly totals of aphids trapped during fall 2011 (monitoring experiment).

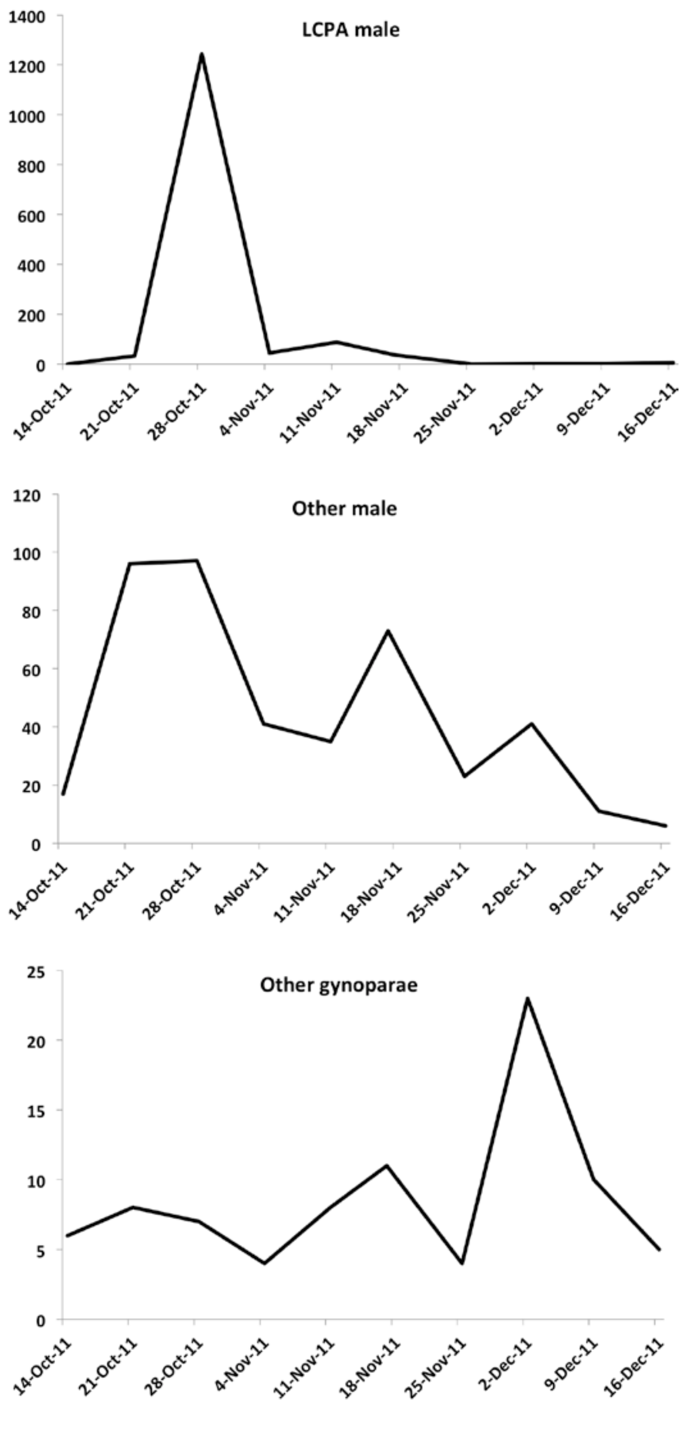

Figure 2. Linear regression analyses examining relationships among fall trap catches (male LCPA and total aphids), overwintering egg densities, and spring aphid population ratings (monitoring experiment).

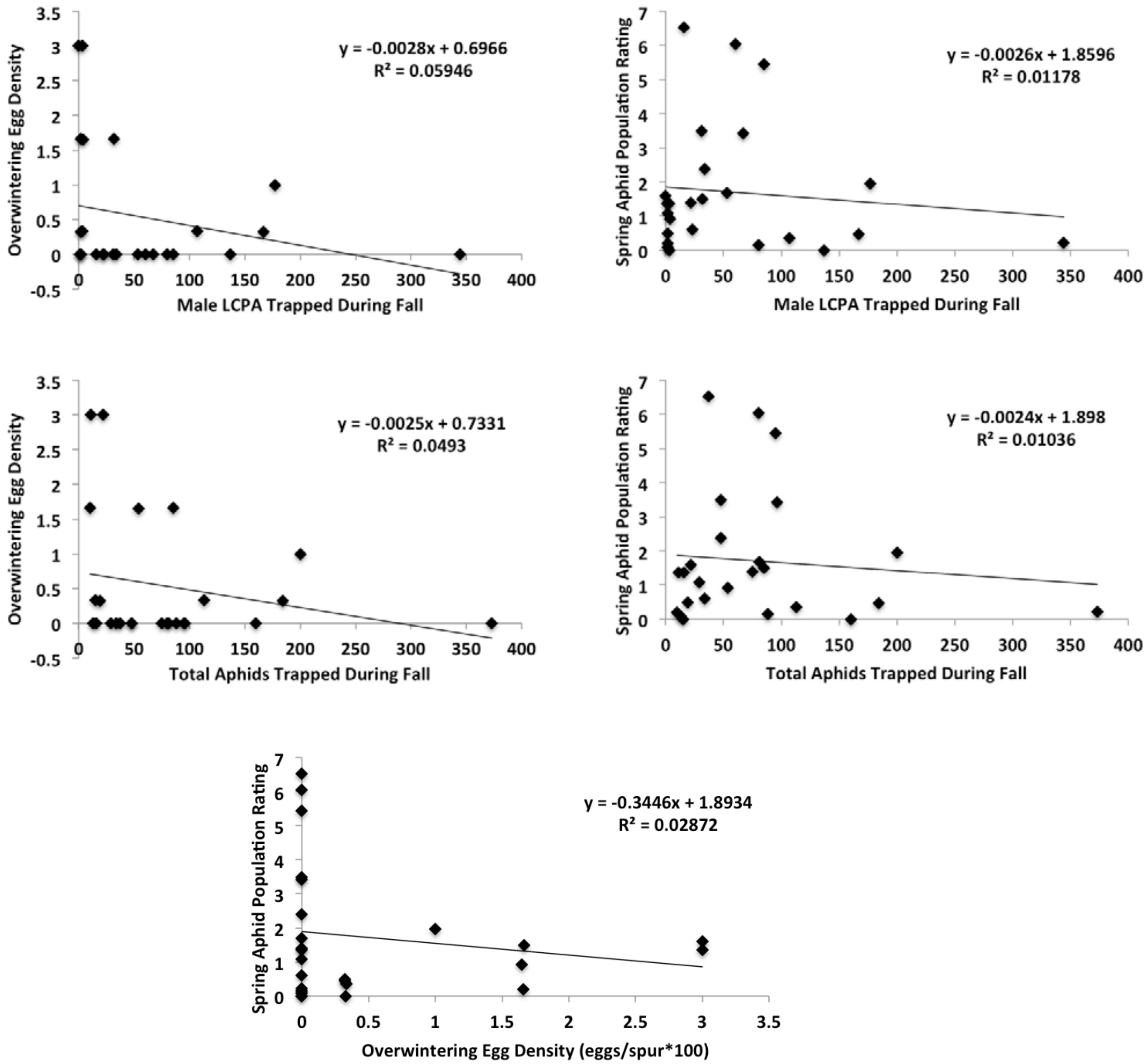

Figure 3. Weekly totals of aphids trapped during fall 2011 in pheromone-treated mating disruption and no-pheromone control plots (mating disruption experiment).

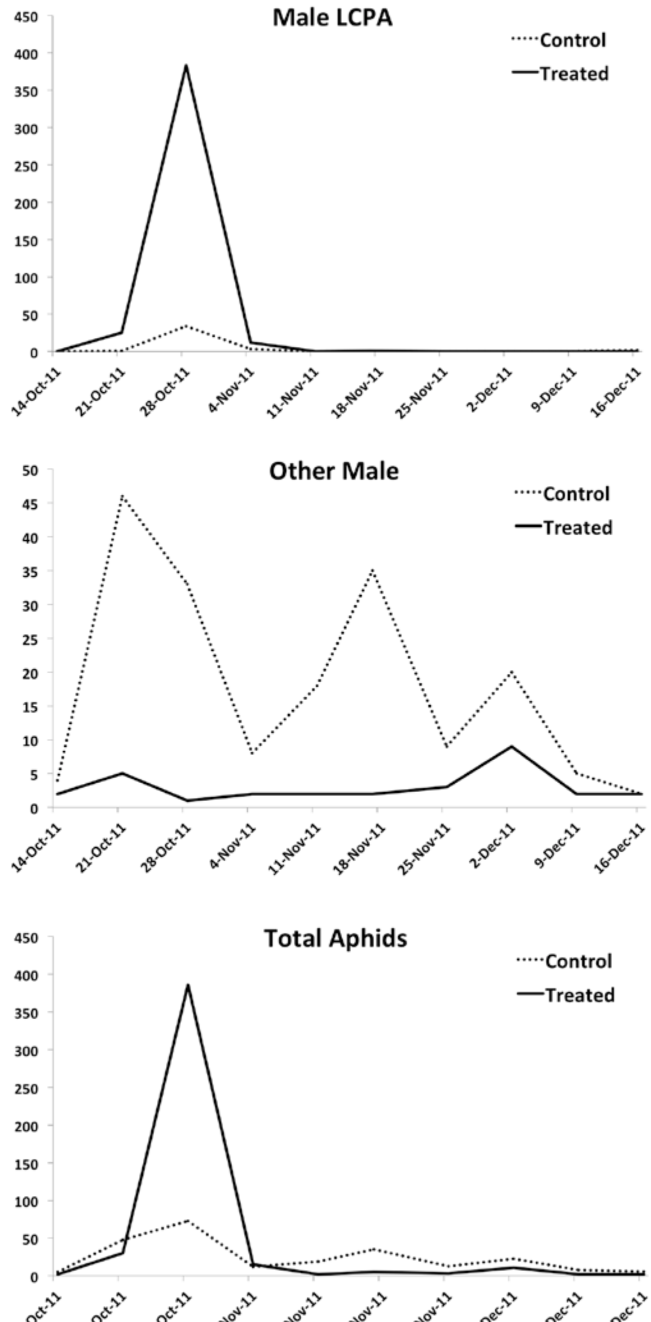

Figure 4. Mean numbers of aphids trapped in pheromone-treated mating disruption and no-pheromone control plots during fall 2011 (mating disruption experiment).

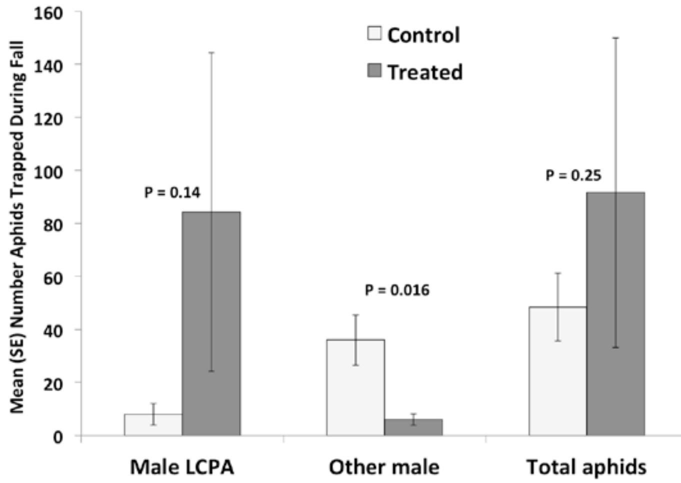

Figure 5. Mean spring aphid population ratings in pheromone-treated mating disruption and no-pheromone control plots during fall 2011 (mating disruption experiment).