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

Emily J. Symmes and Frank G. Zalom

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.

This project addresses two primary pests that affect the production of California's dried plum crop, mealy plum aphids, *Hyalopterus pruni*, and leaf-curl plum aphids, *Brachycaudus helichrysi*. Spring populations of mealy plum aphids and leaf-curl plum aphids inflict significant damage to the crop as a result of both feeding activity and the production of honeydew. Improving monitoring practices and providing reduced-risk alternatives to dormant insecticide treatment may reduce the number of insecticide applications, thereby reducing grower costs and impacts on the environment.

In the major dried plum ('prune') producing regions of California, mealy plum aphids (MPA) and leaf-curl plum aphids (LCPA) exhibit holocyclic heteroecious life cycles involving alternating generations. In late winter/early spring, viviparous females emerge from overwintering eggs laid on prune trees (the primary host) during the previous fall. A series of parthenogenetic (asexual) generations then occurs on prune trees prior to migration of the aphids to their secondary hosts. These spring populations of MPA and LCPA on prune trees are responsible for inflicting injury resulting in damage to the prune crop. Migration of MPA and LCPA to their secondary hosts from the prune crop occurs during late spring to early summer. The aphids remain on the secondary hosts throughout summer, reproducing asexually. The sexual stage of the life cycle begins in fall when winged gynoparae produced on the secondary host migrate back to the primary host (prune) and produce a generation of egg-laying wingless oviparae. Within a few weeks of migration of gynoparae to the primary host, winged males, also produced on the secondary host, migrate to the primary host where they locate and mate with adult oviparae. Overwintering eggs are then laid near the bases of buds and will give rise to damaging spring populations. Given the nature of the aphid life cycle, the current research is focused on management tactics that target the sexual phase of the life cycle, which occurs in prune orchards during fall. Disrupting the life cycle at this point may reduce the abundance of fertile overwintering eggs, leading to a decrease in damaging spring populations.

During the sexual generation, males locate oviparae for mating utilizing a sex pheromone released by oviparous adult females. To date, all aphid species investigated produce and release sex pheromones in which the active components are the cyclopentanoid compounds nepetalactone and nepetalactol (Hardie et al. 1999). Air entrainment bioassays of adult oviparae of a number of aphid species indicate that the ratio of the two pheromone components is relatively species-specific (Sewart-Jones et al. 2007, and references therein). In the cases of MPA and LCPA, air entrainment of adult oviparae indicated that the sex pheromones of the two species also are blends of the (4aS, 7S, 7aR)-nepetalactone isomer and the (1R, 4aS, 7S, 7aR)-nepetalactol isomer (Pickett et al. unpubl.). These have been shown to be the isomers released by the majority of aphid species examined thus far (Sewart-Jones et al. 2007, and references therein). Analyses of laboratory aphid strains indicated that the sex pheromone ratio emitted by MPA oviparae was 3.4:1 (nepetalactone:nepetalactol) and the ratio emitted by LCPA oviparae was 2.6:1 (nepetalactone:nepetalactol) (Pickett et al. unpubl.).

Nepetalactone is a naturally occurring compound found in catnip, *Nepeta cataria* (Lamiaceae). Nepetalactone can be obtained in high yield from fresh plant material via a steam distillation process, after which nepetalactol can be acquired via a chemical reduction of nepetalactone (Birkett and Pickett 2003). Experimental pheromone lure products of the common isomers (those utilized by MPA and LCPA) of each compound have been formulated using polymer extrusion technology. This formulation prevents UV degradation and oxidation of the compounds and provides a slow and consistent release rate. The product is produced in the form of flexible polyvinyl chloride (PVC) 'rope' formulated as 5% extrusions of each compound separately. The rope can then be cut to various lengths to achieve the desired ratio and release rates of the two sex pheromone components. Standard lure lengths provide a minimum release of 200 micrograms/day, stable for up to one month (Birkett and Pickett 2003).

Current monitoring practices for MPA and LCPA in prune orchards, as outlined in the University of California's Pest Management Guidelines (Pickel et al. 2009), include dormant season spur samples aimed at detecting overwintering aphid eggs. The UC guidelines recommend treatment if one aphid egg is found in 100 spur samples. However, the guidelines also state 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.

Presently, the most common practice for managing aphids in prune orchards involves 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. 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 management practices.

The overall objectives of this research focus on utilizing the sex pheromones of MPA and LCPA to improve upon current monitoring recommendations and to investigate whether sex pheromones can be used to effectively disrupt mating and thereby reduce damaging spring populations.

PROCEDURES

Ratio Trials
Orchard trials were conducted during the fall of 2008 in order to determine the ratios of the nepetalactone and nepetalactol pheromone components most attractive to male MPA and LCPA under field conditions. Eighteen replicates of a randomized complete block design were established across four prune orchards in Yolo and Sutter Counties, CA. Pheromone-baited water traps were deployed in the tree canopy utilizing the hand-applied pheromone PVC rope product described previously. The pheromone ratios tested in the experiment (Table 1) were created by cutting the individual nepetalactone and nepetalactol dispensers to particular lengths to achieve the desired ratios while maintaining a constant total release rate. The pheromone ratio treatments included a no-pheromone control, 100% nepetalactone, 100% nepetalactol, equal amounts of each of the two components, the individual ratios identified from laboratory strains of MPA and LCPA oviparae, and approximately double the major component (nepetalactone) of each of the ratios identified from MPA and LCPA oviparae. Traps were changed, processed, and re-randomized weekly, and the numbers of male MPA and LCPA were quantified for each pheromone ratio treatment tested. Trap counts for each species were summed over the trial for analyses.

Monitoring Trials
Experiments to evaluate the use of aphid sex pheromones as a monitoring tool were begun in fall 2010 to examine correlations between number of male aphids trapped during the fall and overwintering egg densities, spring aphid populations, and spring aphid damage. The experiment also was designed to assess the efficacy of various trap types for fall aphid monitoring.

Seven experimental replicates were established in prune orchards in Sutter County, CA. Each replicate consisted of eight 25-tree plots (5x5 trees per plot) with a monitoring trap located in the center tree of each plot. Fall aphid monitoring began 23-Sept-2010 and lasted until 7-Dec-2010. Eight trap-pheromone combinations were included in each replicate, with trap positions in the replicate arranged as a randomized complete block design. Four different trap types were included in the experiment (descriptions below), with each of the four trap types represented twice per replicate, once with a pheromone lure and once with no pheromone lure. Based on fall 2008 ratio trial results, all pheromone-baited traps were treated with the 1:1 (nepetalactone: nepetalactol) pheromone lure ratio at the standardized 200 microgram/day release rate.

Water traps consisted of clear 16-oz. plastic containers with lures suspended approximately one to two inches above the surface of the water using galvanized utility wire. Yellow sticky card traps (Pherocon® AM/NB, Trécé, Inc.) and white sticky traps (Pherocon® V Tent Trap, Trécé, Inc.), deployed as two-sided sticky cards rather than tent traps, had lures mounted through holes punched in the top corners of each trap. White delta traps (Pherocon® IIB, Trécé, Inc.) had lures suspended centrally and approximately one inch above the sticky bottom of the inside of the trap using galvanized utility wire. Traps were hung within the tree canopy and were changed and processed weekly. Lures in pheromone-baited traps were changed every fourth week.

Overwintering egg populations will be quantified in winter 2011 from spur samples taken from all of the 25 trees in each plot. Spring aphid populations and spring aphid damage ratings will be quantified from the same 25-tree plot in spring 2011. Regression analyses will assess the correlation between number of male MPA and LCPA caught in traps during the fall season, overwintering egg densities, spring aphid populations, and spring aphid damage. Analyses also will compare differences among trap types to evaluate the selectivity, specificity, convenience of use, and correlation to later population measures for each trap type and pheromone-baiting status.

Mating Disruption Trials

Experiments to investigate the potential for mating disruption of MPA and LCPA were established in fall 2010 in prune orchards in Sutter County, CA. Five experimental replicates were initiated on 19-Oct-2010, each consisting of two 9-tree plots (3x3 trees per plot). For each replicate, one plot was treated with pheromone (mating disruption) and one plot was left untreated (control). A pheromone-baited check trap was located in the center tree of each plot. Because of the nature of the aphid life cycle and biology (i.e., the fact that mated females are wingless), we are able to use much smaller mating disruption plots than are feasible with other insect pests (e.g., Lepidoptera) because there is no possibility of mated females from outside the treatment area migrating into mating disruption treatment plots and laying eggs. Mating disruption plots were treated with 27 individual pheromone lures (3 lures/tree), a rate equivalent to the approximately 400 hand dispensers per acre recommended for a number of hand-dispensed pheromone lures used in mating disruption. Based on fall 2008 ratio trial results, the 1:1 (nepetalactone:nepetalactol) pheromone lure ratio at the standardized 200 microgram/day release rate was used for all pheromone-baited traps and mating disruption lures. There was a minimum of 150 meters between mating disruption and control plots to minimize the likelihood of pheromone drift between the plots.

The following data will be compared between mating disruption and control plots for this experiment: (1) number of male MPA and LCPA caught in pheromone-baited traps during the fall, (2) overwintering egg densities, (3) spring aphid populations, and (4) spring aphid damage.

Due to the formulation of the pheromone lures being used in these experiments (i.e., the pheromone is ‘contained’ in hand-dispensers rather than sprayed on and dispensers can be easily removed from the environment following the experiments) and the timing of application (pheromone application occurs post-harvest and pheromones are never in the environment at the same time as the harvestable product), clearance for the mating disruption experiment was obtained from the California Department of Pesticide Regulation under the University’s research exemption without the need for an experimental use permit, crop destruction, or other precautions often required for mating disruption or conventional chemical experiments.

RESULTS AND CONCLUSIONS

Ratio Trials

Total trap captures by week (all treatments combined) are shown in Figure 1, indicating that more LCPA were trapped in during the fall of 2008 experiment than MPA. LCPA trap captures remained at relatively high levels throughout October and the first part of November, while MPA captures occurred at a single, distinct population peak in November. Trap captures for both species reached their maximums in November, occurring on the first and second weeks of November for MPA and LCPA, respectively. Trap captures of both species declined significantly after reaching their peaks and remained low after the third week of November. While this information is only based on a single season of trapping, the goal is to combine data from multiple years, perhaps with weather data, in order to most accurately time monitoring and mating disruption efforts.

Total numbers of male MPA and LCPA caught in water traps baited with different ratios of aphid sex pheromone components during the fall of 2008 are summarized in Table 2. Statistical differences among mean numbers of male MPA and LCPA caught by treatment are shown in Figures 2 and 3, respectively. The 1:1 (nepetalactone:nepetalactol) ratio was significantly more attractive to MPA males than any of the other pheromone ratio treatments (Fig. 2). LCPA males were caught in significantly greater numbers in all of the two-component treatments compared to the no-pheromone control and to the nepetalactone only and nepetalactol only treatments, and were, with the exception of the 7:1 (nepetalactone:nepetalactol) ratio, equally attracted to the two-component pheromone blends (Fig. 3).

Monitoring Trials

This trial is ongoing and requires additional data before results are available. Overwintering egg and spring aphid densities and spring aphid damage will be evaluated in 2011 for correlation with fall 2010 trap data. The results of these experiments will indicate whether the information gained from monitoring MPA and LCPA during the fall period can reliably predict the densities of overwintering egg densities, spring aphid populations, and/or spring aphid damage, and thus has the potential to be adopted as a monitoring tool to indicate the need for treatment of the aphid population. Additionally, results from trap comparison analyses will identify the most effective, reliable, and practical trap type(s) for fall aphid monitoring. Complete results of monitoring studies will be provided in the 2011 research report.

Mating Disruption Trials

This trial is ongoing and requires additional data so providing results at this time is premature. Overwintering egg and spring aphid densities will be evaluated in 2011 for additional evidence of efficacy of fall 2010 mating disruption efforts. Significant reduction in the numbers of males caught in pheromone-baited traps in mating disruption plots during the fall will indicate whether mating disruption treatments were effective in disrupting the ability of males to potentially locate females for mating. Further evidence of the efficacy of mating disruption treatments will be confirmed by significant reductions in overwintering egg densities, spring aphid populations, and/or spring aphid damage in the mating disruption plots as compared to control plots. Such results will provide evidence that mating disruption utilizing aphid sex pheromone lures can be achieved for MPA and LCPA and should be further investigated to assess whether a mating disruption program could be used to complement or replace current management tactics targeting these aphid species, particularly the dormant insecticide treatment. Complete results of mating disruption studies will be presented in the 2011 research report.

REFERENCES

Birkett, M. A., and J. A. Pickett. 2003. *Aphid sex pheromones: from discovery to commercial production.* Phytochemistry 62: 651-656

Hardie, J., J. A. Pickett, E. M. Pow, and D. W. M. Smiley. 1999. *Aphids*, pp. 227-250. In J. Hardie and A. K. Minks (eds.), *Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants*. CAB International, Wallingford, UK.

Pickel, C., F. J. A. Niederholzer, W. H. Olson, F. G. Zalom, R. P. Buchner, and W. H. Krueger. 2009. *Insects and mites*. UC IPM Pest Management Guidelines: Prune, UC ANR Publication 3464. University of California Statewide IPM Program, Davis, CA.

Stewart-Jones, A., S. Y. Dewhirst, L. Durrant, J. D. Fitzgerald, J. Hardie, A. M. Hooper, J. A. Pickett, and G. M. Poppy. 2007. *Structure, ratios and patterns of release in the sex pheromone of an aphid, Dysaphis plantaginea*. J. Exp. Biol. 210: 4335-4344.

Table 1. Aphid sex pheromone ratio treatments and associated release rates tested in fall 2008 pheromone ratio trials.

Pheromone Ratio (nepetalactone : nepetalactol)	Release Rate (micrograms/day)		
	Nepetalactone	Nepetalactol	Total
0 : 0	0	0	0
1 : 0	200	0	200
0 : 1	0	200	200
1 : 1	100	100	200
2.6 : 1	145	55	200
3.4 : 1	155	45	200
5 : 1	165	35	200
7 : 1	175	25	200

Figure 1. Weekly totals of male MPA and LCPA caught in water traps during fall 2008 pheromone ratio trials.

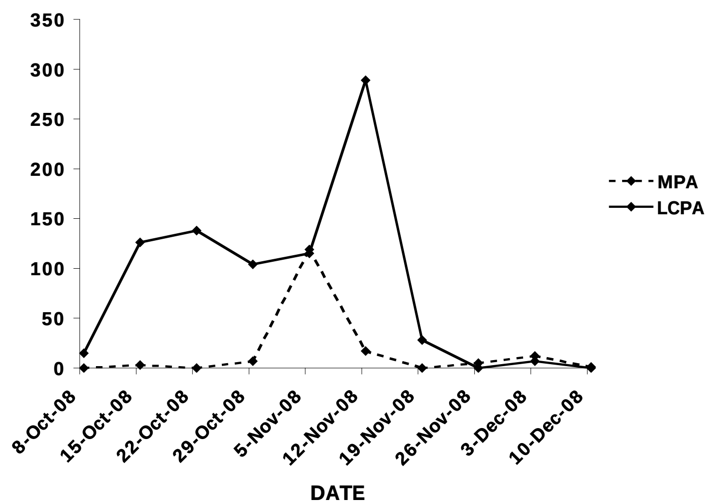

Table 2. Total numbers of male MPA and LCPA caught in water traps baited with different ratios of aphid sex pheromone components during fall 2008 pheromone ratio trials.

Pheromone Ratio (nepetalactone : nepetalactol)	Total Number	
	MPA	LCPA
0 : 0	0	0
1 : 0	4	3
0 : 1	4	0
1 : 1	89	186
2.6 : 1	24	195
3.4 : 1	9	163
5 : 1	11	146
7 : 1	5	122

Figure 2. Mean numbers of male MPA caught in water traps baited with different ratios of aphid sex pheromone components during fall 2008 pheromone ratio trials. Treatments with the same letters are not significantly different (Friedman nonparametric ANOVA on ranked means, followed by least squares means multiple comparisons, $F = 15.03$, df = 7, 119, $P < 0.0001$).

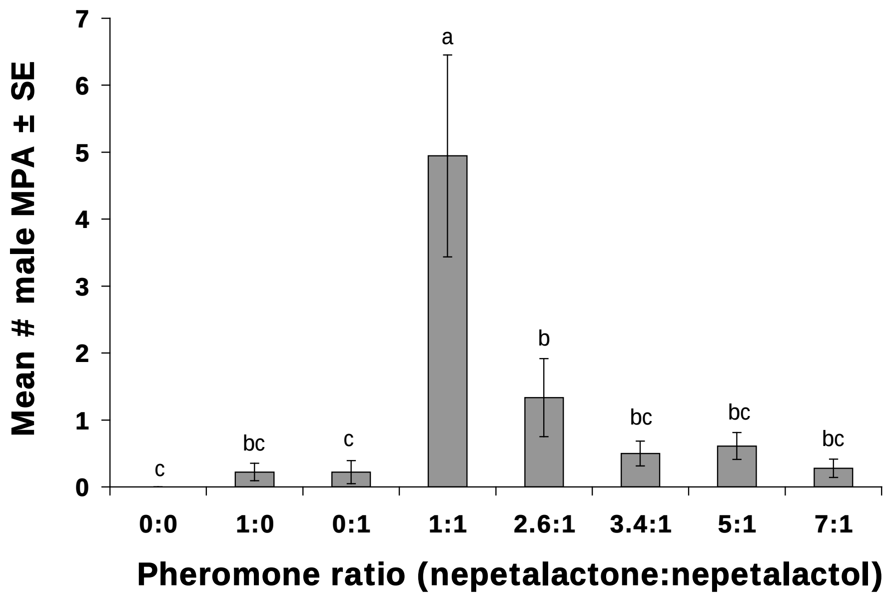

Figure 3. Mean numbers of male LCPA caught in water traps baited with different ratios of aphid sex pheromone components during fall 2008 pheromone ratio trials. Treatments with the same letters are not significantly different (Friedman nonparametric ANOVA on ranked means, followed by least squares means multiple comparisons, $F = 38.07$, df = 7, 119, $P < 0.0001$).