Title

Biodiversity and Ecosystem Services in Agriculture: Evaluating the Influence of Floral Resource Provisioning on Biological Control of Erythroneura Leafhoppers (Hemiptera: Cicadellidae) and Planococcus Mealy Bugs (Hemiptera: Pseudococcidae) in Califor...

Author

Miles, Albie Felix

Publication Date

2013

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By

Albie Felix Miles

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy and Management

in the Graduate Division of the

University of California, Berkeley

Committee in charge:

Professor Miguel A. Altieri, Chair
Professor John Hurst
Dr. Kent M. Daane

Fall 2013

Abstract

Biodiversity and Ecosystem Services in Agriculture: Evaluating the Influence of Floral Resource Provisioning on Biological Control of *Erythroneura* Leafhoppers (Hemiptera: Cicadellidae) and *Planococcus* Mealybug (Hemiptera: Pseudococcidae) in California Vineyards

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Albie Felix Miles,
Doctor of Philosophy in Environmental Science Policy and Management
University of California, Berkeley

Professor Miguel A. Altieri, Chair

The global intensification of viticulture has led to the creation of monocultures characterized by an absence of non-crop vegetation in and around vineyards. The increased concentration of crop host resources, and the loss of non-crop habitats that support arthropod natural enemies can lead to increase pest pressure and crop loss. To manage recurring pest problems, many producers rely on synthetic insecticides posing a range of environmental quality and human health risks.

With increasing concern over the environmental impacts of viticulture, rising production costs, and increased regulation of pesticides, the demand for effective ecologically based pest management strategies has grown. Grape producers have sought to use ecologically based pest management strategies, including floral resource provisioning, to promote biological control of key vineyard pest. Despite growing interest, few ecologically based pest management strategies have been scientifically evaluated for the ability to consistently regulate pest populations or to clarify the underlying biology of control mechanisms when successful.

The research tested the natural enemies hypothesis in an attempt to explain why lower pest densities are observed in some diversified farming systems. The research evaluated the influence of floral resource provisioning (FRP) and chemical ecology strategies on biological control of *Erythroneura* leafhoppers (Hemiptera: Cicadellidae) and *Planococcus* mealybug (Hemiptera: Pseudococcidae) in California vineyards. Field and laboratory studies quantified the impacts on crop damage, pest and natural enemy abundance, and natural enemies fitness theorized to be enhanced through floral resource provisioning in agroecosystems. Multiple two-year studies measured the impact of intercropping three flowering ground covers, lacy phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*), and common carrot (*Daucus carota*) on biological control of leafhoppers and vine mealybug by the parasitoids *Anagrus spp.* (Hymenoptera: Mymaridae) and *Anagyrus pseudococci* (Hymenoptera: Encyrtidae). Using identical intercropping treatments, the research included three large scale and fully replicated research designs located in the central San Joaquin, the northern San Joaquin, and the Napa Valley of California. Laboratory studies quantified the impacts of FRP on the fitness of *Anagyrus pseudococci*, a key parasitoid natural enemy of vine mealybug. The central San Joaquin Valley

field study measured the impact of FRP and pheromone based mating disruption on biological control of vine mealybug. The northern San Joaquin Valley field study measured the impact of FRP and methyl salicylate on biological control of *Erythroneura* leafhoppers. The Napa Valley field study measured the effect of methyl salicylate alone on biological control of *Erythroneura* leafhoppers.

The overall findings of the study were that FRP alone (and in combination with chemical ecology strategies) had little significant impact on natural enemy fitness, abundance, parasitism rates or pest abundance. Laboratory studies found that despite evidence of nectar feeding on FRP species, the enhancement of *A. pseudococci* longevity was found to correlate only with exposure to a honey solution and *Ammi majus*. Mean longevity of *A. pseudococci* was found to be negatively impacted by exposure to phacelia. Survivorship analysis showed that survival probability was significantly greater in the honey solution, buckwheat, wild carrot and bishop's weed treatments. Exposure to Phacelia was found to significantly reduce the survivorship probability of *A. pseudococci*. FRP treatments were found to have no significant impact on parasitism rate of vine mealybug (VMB), with the exception of *Ammi majus,* exhibiting a significant negative impact on parasitism. FRP treatments did not have any significant effect on second-generation sex ratios of *A. pseudococci*. The central San Joaquin Valley field study showed that FRP resulted in no significant differences on parasitism rates of sentinel vine mealybug (VMB), overall VMB densities or crop damage in 2009. In 2010, leaf densities of VMB were found to be significantly lower in FRP plots at harvest time (September). FRP was shown to reduce late summer (August) VMB density over that of pheromone-based mating disruption. However, a lower mean rate of parasitism was measured in the FRP treatment plots in late summer of 2010. No significant difference in crop damage levels were found between FRP and control plots in 2010. The Napa Valley field study found no significant impact of the PredaLure ® (MeSA) alone on biological control of *Erythroneura* leafhoppers. With the exception of a greater cumulative mean of non-*Anagrus spp.* parasitoids found in the MeSA plots in 2009, no evidence was found supporting the conclusion that MeSA had any significant impact on *Erythroneura* leafhopper nymphs, key natural enemies or adult leafhopper densities at harvest time in either 2009 or 2010. The northern San Joaquin Valley "attract and reward" field study field study found that FRP alone and the combination of (FRP) + PredaLure ® (MeSA) had no significant impact on key natural enemy abundance, *Erythroneura* leafhopper nymph density or leafhopper egg parasitism rate by *Anagrus spp.* in 2010 or 2011.

The findings suggest that the integration of the selected flowering plant resources in vineyard monocultures, though they may serve to convey a wide range of other ecosystem services, is not presently a reliable strategy for effectively managing *Erythroneura* leafhoppers or *Planococcus* mealybug. Although enhanced pest regulation may occur at higher pest densities, consistent economic control of *Erythroneura* leafhoppers or *Planococcus* mealybug has yet to be demonstrated using FRP alone or in combination with other chemical ecology strategies in California vineyards. As many ecological factors influence natural pest regulation in vineyards, including landscape context, intra-guild competition, vineyard age and cultural practices, much more applied ecological research will be necessary to develop a more comprehensive scientific understanding of how to consistently manage the biotic and abiotic environment to consistently reduce crop damage in vineyards and other cropping systems.

Biodiversity and Ecosystem Services in Agriculture:
Evaluating the Influence of Floral Resource Provisioning on Biological Control of *Erythroneura* Leafhoppers (Hemiptera: Cicadellidae) and *Planococcus* Mealybug (Hemiptera: Pseudococcidae) in California Vineyards

Table of Contents

Chapter 1:
Habitat Diversity at the Field and Landscape Level:
Conservation Biological Control Research in California Viticulture

Habitat Diversity at the Field and Landscape Level: Conservation Biological Control Research in California Viticulture

Albie Miles, Houston Wilson, Miguel Altieri and Clara Nicholls

1.1 INTRODUCTION*:* THE NEED FOR ECOLOGICALLY BASED VITICULTURE IN CALIFORNIA

The intensification of viticulture in California has led to the creation of grape monocultures characterized by an absence of non-crop plant diversity in and around vineyards. The continued expansion of vineyards into California native plant communities has also led to an aggregate reduction of non-crop habitats at the landscape scale (Heaton and Merenlender 2000). Such increased concentration of plant host resources and the reduction of non-crop habitats supporting natural enemies have been shown to increase pest densities, with associated crop losses and reduce overall crop productivity (Root 1973, Russel 1989, Corbett and Rosenheim 1996a, Altieri and Nicholls 2004). To manage recurring pest problems, California grape growers rely principally on the use of synthetic pesticides, including organophosphate and carbamate insecticides, known to pose a range of environmental quality and human health risks (Bentley 2009, CDPR 2009, UC IPM 2010b, Eskenazi et al. 2010).

With increasing concern over the environmental impacts of viticulture, rising production costs, and increased regulation of pesticides, the demand for research driven by ecologically based pest management (EBPM) strategies has steadily grown (Broome and Warner 2008, Meadows 2008, Ross and Golino 2008, Brodt and Thrupp 2009). In addition to the use of insecticides accepted under the United States Department of Agriculture, National Organic Program, California grape growers have sought to use EBPM strategies, including on-farm diversification to promote biological control (Altieri et al. 2005, Ross and Golino 2008). Despite growing interest and adoption, few field-and farm-scale EBPM strategies in use today have been scientifically evaluated for their ability to consistently regulate pest populations below economic thresholds. With the exception of the general principles (Altieri et al. 2011), California grape growers lack specific guidelines on how to successfully diversify their vineyards or conserve non-crop habitats in the surrounding landscapes to ensure biological control of important arthropod pests.

1.2 KEY HYPOTHESES INFORMING RESEARCH IN VINEYARD DIVERSIFICATION IN CALIFORNIA: NATURAL ENEMIES AND RESOURCE CONCENTRATION

Two main hypotheses have been used for evaluating the effect of on-farm vineyard diversification strategies on biological control in California: 1) the Natural Enemies Hypothesis (NEH) (Andow 1991a), and 2) the Resource Concentration Hypothesis (RCH) (Root 1973). The NEH predicts a positive correlation between plant species richness, natural enemy abundance and the regulation of herbivore pests through increased predation and parasitism. The RCH predicts that herbivore pests are more likely to find and remain on agricultural host plants grown in pure stands (monocultures) than in more biologically diversified (polycultures) cropping systems. The RCH predicts that most specialized herbivore species are likely to attain the highest relative densities in monocultures when compared to diversified farming systems (Root 1973). In more complex agroecosystems, the dilution of plant host resources, inter-specific competition, and more favorable environmental conditions for natural enemies are understood to be complementary factors that serve to regulate herbivore pest densities (Russel 1989, Altieri and Nicholls 2003, Costello and Daane 2003).

1.3 VINEYARD DIVERSIFICATION STUDIES IN CALIFORNIA: FIELD-LEVEL RESEARCH

Multiple on-farm diversification studies have measured the impact of overwintering and summer cover crops on biological control of *Erythroneura* leafhoppers (Table 8.1).

In a 2-year study, Flaherty (1969) measured the impact of the weedy Johnson grass (*Sorghum halepense* (L.) Persoon) on population densities of the Willamette mite (*Eotetranychus willamettei* (McGregor)) in a Tulare County ‘Thompson Seedless’ vineyard. Researchers measured population densities of predators and pests in both weed infested and grass-free vines, concluding that the Johnson grass supported populations of alternate prey (the twospotted spider mite) which moved between the weedy vegetation and the vine canopy. Provided with an alternate food source, the predatory mites (*Metaseiulus occidentalis* (Nesbitt)) were maintained at higher densities and were better dispersed throughout the vineyard area influenced by the weedy vegetation when compared to plots with no Johnson grass. Predatory mites were thus able to respond more rapidly to an increased abundance of the Willamette mite pest and control them at lower densities resulting in significantly lower Willamette mite densities in the diversified (with Johnson grass) versus the simple (no Johnson grass) plots. To further substantiate that enhanced predation by predatory mites was the cause of lower pest mite densities, researchers evaluated the impact of insecticide applications (thus reduced predatory mite populations) on population densities of Willamette mites. Plots with and without Johnson grass that were treated with insecticide showed both lower densities of predatory mites and consistent and significantly higher populations densities of Willamette mite, indicating ecological release of herbivore mites from predation (Flaherty 1969).

Roltsch et al. (1998) conducted several experiments to determine the effect of resident weedy vegetation and cover crops on spider densities and biological control of variegated leafhopper (*Erythroneura variabilis* Beamer) in a San Joaquin Valley ‘Thompson Seedless’ vineyard. Building upon prior studies suggesting that vineyard spiders could be influenced by the ground cover habitats, researchers sought to evaluate the impact of the planted cover crops, common vetch (*Vicia sativa* L.), purple vetch (*Vicia benghalensis* L.) and oat (*Avena sativa* L.) on leafhopper densities and spider abundance and diversity. Agelenid (*Holonena nedra* Chamberlin & Ivie), and theridiid (*Theridion* spp.) spiders were found to be more abundant in the vine canopy in ground cover plots. A corresponding inverse relationship was found for leafhopper densities, with the highest densities found in control plots (no cover). Further corroboration of spiders playing a key role in regulating leafhopper densities was found with a strong positive correlation between high late-season leafhopper densities and low spider abundance in insecticide (dimethoate) treated vineyards, indicating an ecological release of leafhoppers from predation by spiders (Roltsch et al. 1998). No further mechanistic studies were conducted to empirically validate enhanced predation by spiders in the presence of ground covers.

An in-field diversification study examining the impact of overwintering cover crop mixtures and resident weedy vegetation on variegated leafhopper (*E. variabilis)* was reported by Hanna et al. (2003). Prior research had established that spiders are the most abundant generalist natural enemy in vineyards and other agroecosystems and are the only natural enemy, other than *Anagrus* spp. (Mymaridae), present in sufficient densities to regulate *Erythroneura* leafhoppers (Costello and Daane 1999, 2003). Researchers thus set out to evaluate the impact of cover cropping on spider and leafhopper abundance using a fall-planted mix of purple vetch (*V. benghalensis),* common vetch (*V. sativa*) and 'Cayuse' oat (*A. sativa).* Using the cover crop mixture and bare-ground as main plots and vine exclusion as sub plots (to restrict spiders), researchers evaluated the relative impact of each treatment on spider and *E. variabilis* densities. Parasitism rates by *Anagrus* spp. were found to be similar in all plots throughout the year and other generalist natural enemies were found to be rare. Spider exclusion resulted in an average 35% increase in the density of first generation *E. variabilis* nymphs only. Yet, despite a 1.6-fold increase in spider densities on vines with cover crops (no exclusion), the cover crop did not significantly affect the density of *E. variabilis* on grape vines. Researchers suggest that this was due to insufficient spider enhancement from the cover crop and low overall leafhopper abundance during the study period. Interestingly, the cover crop mix had no significant impact on vine vigor/nutrient status, in contrast to the findings of Costello and Daane (1999, 2003). While this study provided support for the hypothesis that in-field diversification can enhance spider abundance, it does not always lead to lower pest densities, perhaps because of the complexity and variability of trophic interactions (e.g. inter- and intra-guild predation) in agroecosystem (Hanna et al. 2003).

Nicholls et al. (2000) conducted a 2-year comparative study of the effect of floral resource provisioning on biological control in an organic wine grape vineyard in Hopland, California. Comparing two 1-ha vineyard blocks (with and without flowers), researchers measured the impact of the summer cover crops, annual sunflower (*Helianthus annuus* L.) and annual buckwheat (*Fagopyrum esculentum* Moench) on population densities of western grape leafhopper (*Erythroneura elegantula* Osborn), western flower thrips (*Frankliniella occidentalis* (Pergande)), and key natural enemies (parasitoids and generalist predators). Researchers reported an estimated 15% lower density from mid to late season (July-August) leafhopper nymphs in cover cropped vineyards when compared to monocultures and a significantly lower density of thrips (32%) for both years of the study. The study also found a greater abundance and richness of generalist natural enemies (*Orius* spp., Coccinellid beetles, and thomisid spiders) in the treated vs. control plot. Although researchers found a higher density of *Anagrus* spp[1] wasps in the control plots, no significant difference in rates of parasitism were found between treatment and control plots. Lower density of leafhopper nymphs in the treatment plot (with cover crops) were attributed to impacts of generalist predators, namely spiders and *Orius* spp. anthocorids. Lower

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[1] Early research referred to all species of *Anagrus* wasps, a key egg parasitoid of *Erythroneura* leafhoppers, found in vineyard as '*Anagrus epos* Girault.' Recent taxonomic revisions of *Anagrus epos* by Triapitsyn (1998) have revealed a complex of species, including the two most common grape leafhopper parasitoids in California: *A. erythroneurae* and *A. daanei.* As such, *Anagrus* spp. will hereafter be referred to as simply '*Anagrus*.'

Table 1.1. Summary of research on effects of vineyard diversification on biological control in California vineyards: 1998-2005

Description of study	Reference	Main effect	Economically important level of biocontrol?	Reported mechanism influencing biocontrol
Effect of weedy vegetation (Johnson grass) on population densities of Willamette mites (*Eotetranychus willamettei*)	Flaherty 1969	98% reduction in Willamette mites	Yes	Natural enemies: Weedy grasses support populations of alternate prey used by predatory mites (*Metaseiulus occidentalis*). Predatory mites were maintained at high density and well dispersed in system thus able to respond rapidly to increased abundance of pest mites, controlling pest at lower densities
Effect of cover crops (vetch and oats) on population densities of *Erythroneura variabilis*	Roltsch et al. 1998	40-50% reduction in 2nd and 3rd generation leafhopper density (year 1), 2nd generation only (year 2)	No	Natural enemies: Spider densities negatively correlated with leafhopper densities in presence of cover crop
Effect of cover cropping (vetch and barley) on *Erythroneura* leafhoppers	Costello and Daane 1998	15-20% reduction in nymph leafhopper density	No	Resource concentration: Reduced vine vigor influencing plant host quality. No significant difference in natural enemy abundance
Effect of cover cropping (vetch and barley) on *Erythroneura* leafhoppers.	Costello and Daane 2003	15-20% reduction in nymph leafhopper density	No	Resource concentration and natural enemies: Reduced vine vigor influencing plant host quality. Predation by spiders suggested as contributing factor to enhanced biocontrol
Effect of cover cropping (vetches and oats) on *Erythroneura* (variegated) leafhoppers	Hanna et al. 2003	35% higher density of 1st generation of leafhopper nymphs in exclusion sub-plots	No	Natural enemies: 35% higher density of 1st generation leafhopper nymphs were found in exclusion sub-plots, suggesting role of spiders in regulating leafhoppers. Higher density of spiders in cover crop plots, but no significant effect on biocontrol. Suggested as resulting from intraguild predation and low seasonal density of leafhoppers
Effect of floral resource provisioning (*Helianthus annuus* and *Fagopyrum esculentum*) on *Erythroneura* leafhoppers and thrips	Nicholls et al. 2000, Altieri et al. 2005	15% reduction in nymph leafhopper densities (for periods). 32% reduction in thrips	No (leafhoppers) Yes (thrips)	Natural enemies: Spiders correlated with low leafhopper densities, and *Orius* spp. correlated with reduced thrips densities in treatment plots

density of thrips in treatment plots were attributed to the impact of the generalist *Orius* spp. predators. The researchers also studied the impact of mid-season mowing of the flowering cover crops on pest and beneficial insects, reporting a significant but temporary increase in density (18%) of both generalist predators and *Anagrus* parasitoids, and a subsequent lower (27%) leafhopper nymph density in the vine canopy after mowing (Nicholls et al. 2000, Altieri et al. 2005).

Daane and Costello (1998) assessed the influence of purple vetch (*V. benghalensis*) and barley (*Hordeum vulgare* L.) cover crops and resident weedy vegetation on vine vigor, natural enemy and leafhopper abundance in four San Joaquin Valley vineyards. They found that season-long cover cropping reduced late season leafhopper nymph densities by 15-20%. Though a treatment effect was clearly determined, the level of leafhopper reduction was not considered economically important and the mechanisms leading to pest reduction were not clearly established. No significant differences in the density of leafhopper predators or *Anagrus* spp. parasitoids were found on vines in cover cropped versus control plots. Additionally, no consistent differences in parasitism rates by *Anagrus* spp. wasps were observed between treatments and control plots, leading researchers to conclude that natural enemy fitness, behavior and density were not significantly enhanced by cover cropping and therefore did not play an important role in regulating leafhopper densities. Assessments of the impact of cover cropping on vine vigor (indicated by petiole nitrogen and vine shoot biomass) however, showed significantly lower vigor and the lowest late-season leafhopper density on vines with season long cover crops and resident weedy grasses. Additionally, researchers found the lowest total number of leafhopper eggs on grape vines in cover cropped plots (Daane and Costello 1998). In a follow-up study, Costello and Daane (2003) re-evaluated the influence of the same cover crops (purple vetch and barley) on leafhopper abundance to determine how their presence had reduced leafhopper density, and to isolate the relative influence of cover crops on the nutrient status of vines (i.e. plant host quality) from the impact of cover crops on natural enemy fitness on biological control. Three treatments were established and compared in the 2-year study: ground cover (vetch and barley), no-cover (tilled control) and ground cover with exclusion (i.e. with barriers limiting arthropods and spiders moving into the vine canopy). They showed mid- and late-season leafhopper densities were significantly reduced in plots with the ground cover compared with the no-cover. Neither leafhopper egg parasitism by *Anagrus* spp. nor spider density (on vines or ground) could explain differences in leafhopper density. Vine vigor, however, was determined to be significantly lower in cover crop than in the no-cover plots, and late-season leafhopper density was highest in ground cover/exclusion plots. Grapevine vigor had the strongest correlation with leafhopper density, with low vigor resulting from the apparent competition between the cover crops, resident weedy vegetation and grapevines and not from the impact of natural enemies. Higher late season leafhopper density in the cover/exclusion plots was, however, attributed to the reduced predation by spiders. The study suggests that cover crops may have a significant impact on soil quality and vine growth, complementing any function they serve in enhancing the natural enemies of vineyard pests (Costello and Daane 2003, Daane et al. 2005).

1.4 LANDSCAPE ECOLOGY AND CONSERVATION BIOLOGICAL CONTROL IN

CALIFORNIA VINEYARDS

The intensification of production has not only produced simplified individual cropping systems (i.e., monocultures), but in addition the regional adoption of such practices has led to the aggregate simplification of entire agricultural landscapes (Tscharntke et al. 2005). The process of agroecosystem simplification is particularly acute in wine grape regions as the geographic branding of wine (e.g. premiums paid for wine produced in Napa County) further encourages regional land use conversion from natural habitat to high-value wine grape production. This loss of both agrobiodiversity and natural habitats that surround agroecosystems can lead to the loss of multiple ecosystem services, including biological control (Kremen et al. 2002, Altieri and Nicholls 2004).

The term landscape ‘heterogeneity’ (alternately landscape ‘complexity’ or ‘diversity’) has been used in the ecology and conservation literature to describe the area, arrangement and/or composition of natural habitats surrounding agroecosystems (Bianchi et al. 2006). Studies of landscape effects on ecosystem services typically quantify ecological features within a 1-3 km radius around a crop field, although some studies have measured landscape features at scales ranging from as little as 0.4 km to at most 25 km (Thies and Tschantke 1999, Ostman et al. 2001, Steffan-Dewenter et al. 2002). Landscapes are generally quantified in terms of the relative proportion of various habitat types within a given area (e.g., 32% oak woodland within a 1.5 km radius of a crop field), although some studies simply utilize categorical terms to describe a landscape (e.g., ‘complex’ and ‘simple’ landscapes) (Thies and Tscharntke 1999).

While researchers previously hypothesized that landscape heterogeneity could have a significant impact on biological control (van Emden 1965), it is only more recently that they have begun to address this relationship empirically. Bianchi et al. (2006) conducted a review of the ecological literature measuring the influence of landscape heterogeneity on arthropod populations and biological control in agriculture. Their analysis showed that in 74% of the cases studied, increased natural enemy diversity and abundance were correlated positively with increased landscape heterogeneity. However, in only 45% of the studies reviewed, increased landscape heterogeneity correlated positively with decreased pest densities, reduced crop damage or increased yield. While landscape heterogeneity has been shown to have a significant and positive influence on natural enemy diversity and abundance at the field level, meta-analyses conducted to date have shown that landscape heterogeneity does not consistently result in enhanced biological control (Bianchi et al. 2006, Chaplin-Kramer et al. 2011). The relationship between landscape heterogeneity and enhanced pest regulation in agriculture is therefore considered to be specific to the cropping system and life-history characteristics of key pests and their natural enemies (With et al. 2002, Hunter 2002, Tscharntke et al. 2007). A more detailed understanding of how specific biophysical features of landscapes influence arthropod populations will be essential for the development of cost-effective habitat enhancement strategies aimed at improving biological control and other ecosystem services to agriculture.

1.4.1 Research on overwintering habitat for *Anagrus* spp.

Several studies have evaluated the contribution of natural enemy refuges to pest regulation in California grape systems. A majority of the existing work has focused on the effect of *Anagrus* overwintering habitat and whether its proximity to vineyards influences biological control of *Erythroneura* leafhoppers. This is because this parasitoid must locate alternate leafhopper host eggs to complete winter diapause. Although *Anagrus* can complete multiple generations by parasitizing *Erythroneura* eggs during the spring and summer, these pest leafhoppers overwinter as adults while *Anagrus* overwinters as larvae (UC IPM 2010a). Overwintering habitat that supports alternate leafhopper host(s) may be limited (due to plant community composition) or lie at a great distance from vineyards. Low quality or distant overwintering habitat for *Anagrus* may lead to delayed spring colonization of vineyards, allowing early grape leafhopper populations to develop unchecked. This can result in leafhopper damage to young grape shoots and/or large populations of adult leafhoppers at the end of the growing season, which can interfere with harvest activities (UC IPM 2010a).

Researchers have attempted to address this management problem by investigating how habitat patches that serve as natural enemy refuge can contribute to early-season control of grape leafhoppers. Studies primarily evaluate the use of blackberry and prune refuges (*Rubus* spp. and *Prunus* spp., respectively) around California vineyards. Although some of this section draws from the broader North American literature, many of the known alternate host plants for overwintering *Anagrus* can be found in California. An overview of known alternate host plants (and associated leafhoppers) for overwintering *Anagrus* wasps is included in Table 8.2.

1.4.1.1 Studies of wild blackberry refuges.

A 1966 study of blackberry refuges revealed a gradient of parasitoid activity that declined with increasing distances from the refuges. Leafhopper egg parasitism was observed up to 6.4 km away from the blackberry stands. Beyond this distance egg parasitism rates declined substantially. Researchers concluded that the observed trend was likely due to *Anagrus* dispersing outward from the blackberry refuge. The study did not include any direct measurements of dispersal (e.g. mark-recapture) or quantitative assessments of *Anagrus* densities (Doutt et al. 1966).

In a related survey of *Anagrus* dispersal, Doutt and Nakata (1973) monitored vineyards for parasitoid activity at increasing distances from a large riparian area. It was assumed that the riparian habitat harbored a high density of wild blackberry, although no formal information on plant species composition was reported for the riparian area. Sampling vineyards at increasing distances from the riparian habitat (up to 32 km), researchers observed leafhopper egg parasitism 3-4 weeks earlier in vineyards located at closer proximity (< 8 km) to the riparian forest. This finding again led researchers to conclude that *Rubus* spp. were harboring overwintering populations of *Anagrus* wasps and that these parasitoids were dispersing into nearby vineyards earlier in the spring. In addition, researchers observed earlier leafhopper egg parasitism in vineyards located downwind from the riparian ecosystem when compared with vineyards upwind at similar or closer distances. This finding led to the suggestion that dominant wind direction also plays an important role in *Anagrus* dispersal (Doutt and Nakata 1973).

Although none of the studies above measured whether early season parasitism significantly influenced pest densities, the findings led to the development of recommendations that growers establish blackberry refuges around their vineyards to promote early season biological control of leafhoppers. More than a decade after the recommendations were made, further scientific evaluation of the plantings showed that the blackberry refuges did not consistently enhance biological control (Flaherty et al. 1985). Researchers posited that the on-farm blackberry refuges were unsuccessful because many were planted outside of their native riparian habitats and that reduced canopy cover and lower soil moisture levels reduced the

Table 1.2. Summary of plant and leafhopper host associations for *Anagrus* spp.

Plant species	Common name	*Anagrus* species	Host species	Region	Ref.
Aceraceae					
Acer glabrum	Douglas maple	*A. atomus*	?	BC	3
Acer saccharum	Sugar maple	*A. daanei*	?	NY	1, 2
		Anagrus spp.	?	NY	2
Betulaceae					
Alnus sp.	Alder	*A. erythroneurae*	?	BC	3
Betula occidentalis	Water birch	*A. atomus*	?	BC	3
		A. avalae	?	BC	3
		A. erythroneurae	?	BC	3
Betula pendula	European white birch	*A. atomus*	?	BC	3
Ostrya virginiana	Hophornbeam	*A. atomus*	?	NY	2
Cornaceae					
Cornus racemosa	Gray dogwood	*A. yawi*	?	NY	2
Cornus stolonifera	Red osier dogwood	*A. daanei* (?)	?	BC	3
		A. erythroneurae	?	BC	3
Fabaceae					
Robinia pseudoacacia	Black locust	*A. daanei*	?	NY	2
		A. epos	?	NY	2
		A. nigriventris	?	NY	2
		Anagrus spp.	?	NY	2
Fagaceae					
Quercus rubra	Northern red oak	*Anagrus* spp.	?	NY	2
Juglandaceae					
Juglans nigra	Black walnut	*Anagrus* spp.	?	NY	2

Lamiaceae					
Lavendula angustifolia	Lavender	*A. atomus*	?	BC	3
Mentha spp.	Garden mint	*A. atomus*	?	BC	3
		A. erythroneurae	?	BC	3
Mentha spp.	Mint	*A. atomus*	?	BC	3
		A. erythroneurae	?	BC	3
Nepeta cataria	Catnip	*A. atomus*	?	BC	3
		A. erythroneurae	?	BC	3
Nepeta x *mussinii*	Persian catnip	*A. atomus*	?	BC	3
		A. erythroneurae	?	BC	3
Salvia officinalis	Garden sage	*A. atomus*	?	BC	3
		A. erythroneurae	?	BC	3
Oleaceae					
Fraxinus americana	White ash	*Anagrus* spp.	?	NY	2
Rosaceae					
Crateagus sp.	Hawthorn	*Anagrus* spp.	?	NY	2
Fragaria x *ananassa*	Strawberry	*A. atomus* or *A. erythroneura*	?	BC	3
Malus domestica	Apple	*A. atomus*	?	BC	3
		A. avalae	?	BC	3
		A. erythroneurae	?	BC	3
Malus pumila	Apple	*Anagrus* spp.	?	NY	2
Malus spp.	Apple	*A. atomus*	*Typhlocyba pomaria*	BC, ON	1
			Empoasca maligna	MI	1
			?	BC	1
		A. avalae	*T. pomaria*	ON	1
		A. daanei	*T. pomaria*	MI	1
			?	MI	1
		A. erythroneurae	*T. pomaria*	CA, WA	1
		Anagrus sp.	*T. pomaria*	MI	1

		A. tretiakovae	?	MI	1
Prunus avium	Sweet cherry	*A. erythroneurae*	?	BC	3
Prunus domestica	Cultivated plum	*A. erythroneurae*	?	BC	3
Prunus dulcis	Almond	*A. atomus*	?	CA	1
		A. daanei	?	CA	1
		A. erythroneurae	?	CA	1
Prunus persica	Peach	*A. atomus*	*Edwardsiana prunicola*	CA	1
			Typhlocyba quercus (?)	CA	1
			?	CA	1
		A. avalae	*E. prunicola*	CA	1
			?	CA	1
		A. erythroneurae	*E. prunicola*	CA	1
			?	CA	1
		A. tretiakovae	*Erythroneura plena*	MD	1
Prunus serotina	Black cherry	*Anagrus* spp.	?	NY	2
Prunus virginiana	Choke cherry	*A. atomus*	?	BC	3
		A. avalae	?	BC, OR, WA	3, 4
		A. daanei	?	BC	3
		A. tretiakovae	?	OR, WA	4
Purshia tridentata	Antelope bitterbrush	*A. atomus*	?	OR, WA	4
Rosa eglanteria	Sweetbrier	*A. atomus*	?	OR, WA	4
		A. erythroneurae	?	OR, WA	4
Rosa multiflora	Multiflora rose	*A. atomus*	?	NY	2
		A. daanei	?	NY	2
Rosa rugosa	Rugose Rose	*A. atomus*	?	OR, WA	4
		A. erythroneurae	?	OR, WA	4
		A. tretiakovae	?	OR, WA	4
Rosa spp.	Rose	*A. atomus*	*Edwardsiana rosae*	CA, NY,	1
			?	BC, NY, OR, WA	3, 4
		A. avalae	*E. rosae*	BC, OR(?)	1
			?	BC, OR, WA	3, 4

		A. daanei	*E. rosae*	CA, NY	1
		A. erythroneurae	?	BC, OR, WA	3, 4
		A. nr. sp. *daanei*	?	OR, WA	4
		A. tretiakovae	?	OR, WA	4
Rosa woodsii	Wood's rose	*A. atomus*	?	OR, WA	4
		A. erythroneurae	?	OR, WA	4
Rubus armeniacus	Himalayan blackberry	*A. atomus*	?	OR, WA	4
(*R. discolor, R. procerus*)		*A. avalae*	?	OR, WA	4
		A. erythroneurae	?	OR, WA	4
		A. nigriventris	?	OR, WA	4
		A. nr. sp. *columbi*	?	OR, WA	4
		A. tretiakovae	?	OR, WA	4
Rubus laciniatus	Evergreen blackberry	*A. atomus*	?	OR, WA	4
Rubus spp.	Blackberry	*A. atomus*	*Dikrella* spp.	CA	1
			?	BC, CA	1, 3
		A. daanei	*Dikrella* spp. (?)	CA	1
			?	CA	1
		A. erythroneurae	*D. californica* (?)	CA	1
			D. cruentata (?)	CA	1
			Dikrella spp.	CA	1
			?	BC, CA	1, 3
		A. nigriventris	?	CA	1
Rubus spp.	Tayberry	*A. atomus*	?	BC	3
		A. erythroneurae	?	BC	3
Rutaceae					
Zanthoxylum americanum	Common pricklyash	*A. daanei*	?	NY	2
		Anagrus spp.	?	NY	2
Salicaceae					
Salix babylonica	Weeping willow	*A.* nr. sp. *avalae*	?	OR, WA	4
Salix nigra	Willow	*A. erythroneurae*	?	NY	2
Salix spp.	Willow	*A. atomus*	?	OR, WA	4
		A. erythroneurae	?	OR, WA	4
		A. nr. sp. *nigriventris*	?	BC, OR, WA	3, 4

		Anagrus sp.	?	BC	3
Ulmaceae					
Ulmus pumila	Siberian elm	*A. atomus*	?	BC	3
Vitaceae					
Parthenocissus quinquefolia	Virginia creeper	*A. daanei*	?	BC	3
Vitis cv. Castel	Grape	*A. daanei*	*Erythroneura* spp. (?)	NY	2
		Anagrus spp.	*Erythroneura* spp. (?)	NY	2
		A. tretiakovae	*Erythroneura* spp. (?)	NY	2
Vitis cv. GR-7	Grape	*A. daanei*	*Erythroneura* spp. (?)	NY	2
		Anagrus spp.	*Erythroneura* spp. (?)	NY	2
		A. tretiakovae	*Erythroneura* spp. (?)	NY	2
Vitis cv. Seyval blanc	Grape	*A. daanei*	*Erythroneura* spp. (?)	NY	2
Vitis labrusca Bailey cv. Concord	Grape	*A. daanei*	*Erythroneura* spp. (?)	NY	2
		A. nigriventris	*Erythroneura* spp. (?)	NY	2
		A. tretiakovae	*Erythroneura* spp. (?)	NY	2
Vitis labrusca Bailey cv. Delaware	Grape	*A. tretiakovae*	*Erythroneura* spp. (?)	NY	2
Vitis labrusca Bailey cv. Niagara	Grape	*A. daanei*	*Erythroneura* spp. (?)	NY	2
		A. erythroneurae	*Erythroneura* spp. (?)	NY	2
		A. tretiakovae	*Erythroneura* spp. (?)	NY	2
Vitis riparia	Grape	*A. tretiakovae*	*Erythroneura* spp. (?)	NY	2
Vitis spp.	Grape	*A. daanei*	*Erythroneura bistrata*	NY	1
			E. comes	NY	1
			E. elegantula	CA	1
			Erythroneura spp.	CA, NY	1
			E. variabilis	CA	1
			E. ziczac	BC, NY	1
			?	BC, CA, NY	1
		A. epos	*Dikrella*. sp.	NM	1
			E. comes	NY	1

			Erythroneura spp.	BCA, SON	1
			E. variabilis	SON	1
			E. vulnerata	CO	1
			?	NM, SON	1
		A. erythroneurae	*D. cockerellii*	NM	1
			Dikrella sp.	NM	1
			E. bistrata	NY	1
			E. comes	NY	1
			E. elegantula	CA	1
			Erythroneura spp.	BCA, CA	1
			E. variabilis	BCA, CA	1
			?	BCA, CA, NY, SON	1
		A. flaveolus	?	SON	1
		A. nigriventris	*E. comes*	NY	1
		A. tretiakovae	*D. cockerellii* (?)	COA	1
			E. bistrata	NY	1
			E. comes	DE, NY	1
Vitis spp.	Grape	*A. tretiakovae*	*Erythroneura* spp.	AZ, COA, NY	1
			E. variabilis	AZ, SON	1
			E. ziczac	NY	1
			?	AZ, BCA, MI, NM, NY, SON	1
		A. yawi	*E. comes*	NY	1
Vitis vinifera	Grape	*A. daanei*	?	BC	3
Vitis vinifera L. cv. Chardonnay	Grape	*A. daanei*	*Erythroneura* spp. (?)	NY	2
		A. epos	*Erythroneura* spp. (?)	NY	2
		A. erythroneurae	*Erythroneura* spp. (?)	NY	2
		A. tretiakovae	*Erythroneura* spp. (?)	NY	2

State and province abbreviations used

Canada: BC = British Columbia; ON = Ontario.

United States: AZ = Arizona; CA = California; CO = Colorado; DE = Delaware; MD= Maryland; MI = Michigan; NM = New Mexico; NY = New York; OR = Oregon; WA = Washington.

Mexico: BCA = Baja California: COA = Coahuila: SON = Sonora.

References

[1] Triapitsyn 1998, [2] Williams and Martinson 2000, [3] Lowery et al. 2007, [4] Wright and James 2007

(?) Unverified species or region of collection.

quality of the refuges which contributed to lower populations of both blackberry leafhopper (*Dikrella cruentata* (Gillette)) and *Anagrus*. Flaherty et al. (1985) attempted to substantiate this hypothesis by providing shade structures to *Rubus* plantings. Findings suggest that while the shade treatment did enhance *D. cruentata* populations on the blackberry, *Anagrus* densities were not significantly increased.

Due to its inability to consistently control leafhopper populations, California growers largely abandoned the planting of blackberry around Central Valley vineyards by the late 1980s. Additionally, identification of *Rubus* spp. as a systemic host of Pierce's Disease (*Xylella fastidiosa* Wells et al.) led to its removal from many riparian habitats of the Northern and Central Coast grape growing regions (Purcell and Saunders 1999, Baumgartner and Warren 2005).

1.4.1.2 Experiments involving prune refuges.

Counter to previous findings, Kido et al. (1983) reported high early season leafhopper parasitism in vineyards adjacent to prune orchards and revealed an additional alternate host for overwintering *Anagrus*, the prune leafhopper (*Edwardsiana prunicola* (Edwards)), which was reproducing in French prune (*Prunus* spp.) orchards neighboring vineyards. Following this discovery, Kido et al. (1984) conducted a non-replicated 2-year study quantifying population densities of *E. prunicola* and *Anagrus* in two vineyards adjacent to prune orchards. Only one vineyard-orchard pair was studied each year. Based on observations of leafhopper egg parasitism 3-4 weeks earlier in vineyards adjacent (< 30 m) to the prune orchards, researchers concluded that *Anagrus* populations remained active in the prune trees throughout the growing season and dispersed into the nearby vineyards to parasitize grape leafhoppers eggs in the spring. Kido et al. (1984) concluded that French prunes could be used like *Rubus* spp. to enhance overwintering habitat for *Anagrus* wasps and thereby increase biological control of leafhoppers.

Building upon the above assessments, Wilson et al. (1989) monitored *Anagrus* activity in two vineyards, one adjacent and the other located at some distance away from a prune orchard (exact distance not reported). The study showed that the prune orchard harbored high densities of *Anagrus* wasps and that leafhopper egg parasitism occurred approximately 3-4 weeks earlier in the nearby vineyard. Like others, the study concluded that *Anagrus* could successfully overwinter in French prune refuges, and potentially contribute to early-season control of grape leafhoppers. The effect of wind speed was also measured on *Anagrus* colonization. Prune trees subjected to low velocity winds were found to have a higher abundance of *Anagrus*. Based on these and the findings of Doutt and Nakata (1973), researchers advised growers to plant French prune trees upwind from their vineyards to augment populations of *Anagrus* and enhance biological control of leafhoppers.

Prior to 1990, all research conducted on the *Anagrus*-leafhopper system had been based on non-replicated comparisons and indirect assessments of *Anagrus* dispersal from overwintering refuges. While early season leafhopper egg parasitism was reported to be enhanced with proximity to *Rubus* spp. and *Prunus* spp. refuges, no assessment of vineyards pest densities were conducted.

The first direct assessment of *Anagrus* movement was carried out by Corbett and Rosenheim (1996a) using rubidium (Rb) to mark prune refuges adjacent to two vineyard sites over a 2-year period. Early season *Anagrus* populations were monitored at increasing distances away from the refuges to quantify the proportion of the *Anagrus* population found in the vineyard that originated in the Rb-marked prune trees. Consistent with the previous prune refuge studies, a higher density of *Anagrus* was found in vine rows directly adjacent (10-20 m) and downwind from prune trees. Only a small percentage of these parasitoids, however, were positively marked with Rb. Given the conflicting evidence, the authors concluded that although prune trees did directly contribute to vineyard *Anagrus* populations, the presence of regional riparian habitats appeared to be a greater source of *Anagrus*. The apparent 'prune tree effect' was partially the result of the prune stands acting as windbreaks for aerially dispersing *Anagrus* assumed to be coming from nearby riparian habitats (Corbett and Rosenheim 1996a).

Murphy et al. (1996, 1998a, b) completed a more comprehensive evaluation of the effect of prune refuges on biological control. In these studies, 18-24 pairs of vineyard blocks with and without nearby prune orchards were evaluated over two years. Researchers tested the hypotheses suggested in previous studies by evaluating the influence of prune trees on *Anagrus* abundance in vineyards, measuring leafhopper parasitism rates, and quantifying the abundance of leafhopper nymphs at increasing distances away from the prune refuge plantings. These studies again showed that *Anagrus* was more abundant approximately 3-4 weeks earlier in vineyards adjacent to prune orchards (Murphy et al. 1996). A similar effect was seen in parasitism rates, where leafhopper egg parasitism was significantly higher and occurred approximately 3-4 weeks earlier in vineyards adjacent to the prune orchards (Murphy et al. 1998a). Although significant differences in *Anagrus* density and parasitism rates between treatment and control blocks diminished later in the growing season (second and third leafhopper generations), early season effects of prune refuges could potentially influence late-season leafhopper populations. The final component of this study showed, however, that leafhopper nymph densities were not found to be significantly different between treatment and control sites (Murphy et al. 1998b). These results raised additional questions about the source habitats for *Anagrus* and highlighted the need to carry out more thorough evaluations of alternate overwintering habitat and conduct further mark-recapture studies to better understand *Anagrus* dispersal across the landscape.

Corbett and Rosenheim (1996b) conducted another mark-recapture study of *Anagrus*, this time using fluorescent dust to mark wasps emerging from vineyard grape leaves. While not a study of *Anagrus* dispersal from any type of refuge *per se*, this study did provide new information on the biology of this minute parasitoid. They also found that, in a vineyard, *Anagrus* appeared to disperse up to 24.5 m/day and, contrary to all prior evidence, had a significant tendency to disperse up-wind. However, these novel findings are not definitive. In their discussion, Corbett and Rosenheim (1996b) suggested that, because their data on dominant wind speed and direction was from a nearby weather station, it may not have been representative of wind characteristics within the study vineyard itself. Like the prune refuges, the vineyard canopy structure may have altered wind speed and direction within the vineyard and this might have subsequently influenced *Anagrus* dispersal.

Prune orchards can still be found near some commercial vineyards in California. While these orchards could potentially provide a patchwork of overwintering habitat for *Anagrus* wasps, their area relative to the vineyards is small and their contribution to biological control is likely negligible. Researchers have suggested that small refuges (prune, blackberry or otherwise) may not be viable over the long-term, as their entire population of alternate overwintering hosts risk being eliminated by overwhelming populations of *Anagrus* produced in large vineyards during the summer (Mills and Daane 2005).

1.4.1.3 North American research on alternate overwintering habitat for *Anagrus* spp.

Studies evaluating the impact of habitat patches on biological control of leafhoppers examined only two plant genera, *Rubus* spp. and *Prunus* spp., both in the Rosaceae. This limited range of known overwintering host plants for *Anagrus* has encouraged researchers to seek out new alternate host plants that could be utilized for habitat enhancement in proximity to vineyards. This work is especially important in light of recent taxonomic revisions to the *Anagrus* complex, which revealed that not all *Anagrus* species overwintering near vineyards are necessarily the same that parasitize *Erythroneura* leafhoppers in vineyards (Triapitsyn 1998).

Overwintering habitat assessments have been conducted in various viticulture regions in North America, revealing a range of new plant and host associations for *Anagrus* (Table 8.2). Whereas *Anagrus* is consistently encountered on plants in the Rosaceae, this parasitoid also appears to be associated with plants in many other families. At present, *Anagrus* appears to reproduce exclusively on eggs from species in the Ciccadellidae. A summary of known plant and leafhopper host associations for *Anagrus* spp. is presented on Table 8.2.

1.4.2 Measuring the effect of plant corridors, flower islands, and native vegetation

Nicholls et al. (2001) evaluated the influence of non-crop habitat on biological control in a northern California organic vineyard. They focused on two separate non-crop habitats: 1) a vegetational corridor bisecting the vineyard, and 2) a riparian forest abutting the vineyard. The corridor consisted of 65 different species of flowering plants. No description of plant species composition was provided for the riparian forest. Natural enemy and pest populations were monitored at increasing distances away from the corridor and the riparian forest. Furthermore, they reported that the abundance of generalist predators decreased at increasing distances away from both the forest and corridor, while *Anagrus* densities increased towards the center of the vineyard plots. Leafhopper egg parasitism rates did not exhibit any significant spatial trends relative to the two non-crop habitats, although parasitism rates were generally higher towards the center of the vineyard blocks. Thrips, *F. occidentalis*, and grape leafhopper adult densities both increased at greater distances away from the two non-crop habitats (Fig. 8.1). *Anagrus* dispersal was evaluated through an indirect assessment of movement, and no clear information was provided about dominant wind direction relative to non-crop habitats. In that study, Nicholls et al. (2001) concluded that the distribution of *Anagrus* was likely following that of the leafhoppers resulting from a density-dependent relationship between the parasitoid and host rather than any influence from non-crop habitat.

To further understand the spatial patterns of biological control in vineyards, Altieri et al. (2005) evaluated population densities of pest and beneficial insects at increasing distances away from a 0.25 ha on-farm ‘flower island’ in a northern California vineyard. The island was composed of 33 species of flowering shrubs and herbs predominantly from the Asteraceae, Agavaceae, and Lamiaceae. The assemblage of flowering plants was selected to provide floral resources from April to late September. Natural enemy populations (*Orius* spp., Coccinellidae, Syrphidae, *Anagrus* wasps), leafhopper egg parasitism, and thrips density were recorded at increasing distances away from the island (10, 30 and 60 m) over a single growing season. Results showed that natural enemy densities and leafhopper egg parasitism rates both decreased and abundance of thrips increased at greater distances away from the island (Fig. 8.2). The researchers thus suggested that the flower island may have served as a source of pollen, nectar or alternate prey for natural enemies which led to the observed changes in leafhopper egg parasitism and thrips densities.

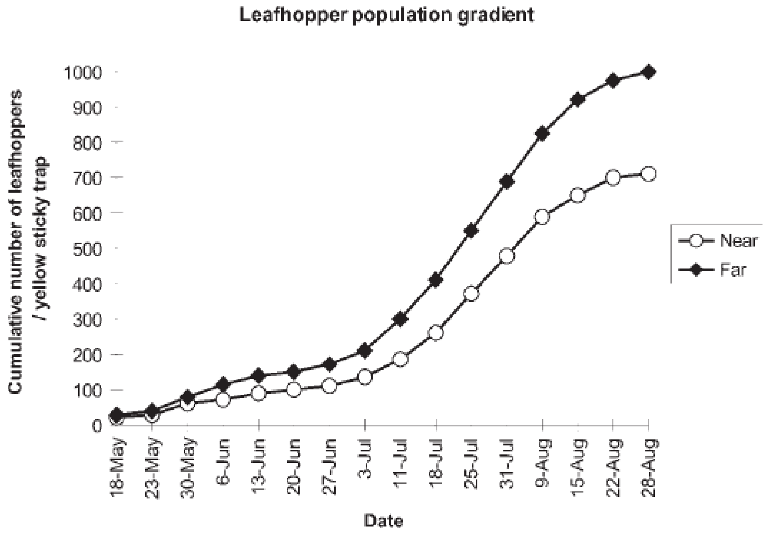

Fig. 1.1. Season patterns of adult leafhoppers in a vineyard 'Near' and 'Far' from a corridor of flowering vegetation (Hopland, California 1996) (From Altieri et al. 2005).]

While much conservation biological control research in California viticulture has focused on the *Anagrus*-leafhopper system, other research has been conducted to evaluate the relationship between natural habitats and vineyard spider populations. Spiders are known to be the most abundant generalist predator in vineyards and natural habitats could be contributing to these vineyard populations (Costello and Daane 1995, Roltsch et al. 1998, Costello and Daane 1999). Hogg and Daane (2010, 2011) evaluated how oak woodland-chaparral, and riparian habitats contributed to vineyard spider populations. Spiders were sampled throughout the growing season in natural habitats and at multiple distances into vineyards. They reported that spider dispersal into vineyards appeared to occur later in the growing season (July and August) and that spider species diversity and abundance significantly differed between natural and vineyard habitats. The observed differences in species composition became more pronounced with increasing distance away from the natural habitats (up to 250 m), and vineyards were found to be dominated by just a few spider species. Researchers suggested that natural habitats serve as an important source of vineyard spider populations. While no assessment of pest densities was conducted in these two studies, the researchers noted that the observed changes in vineyard spider species composition relative to distance away from adjacent source habitats likely has implications for biological control.

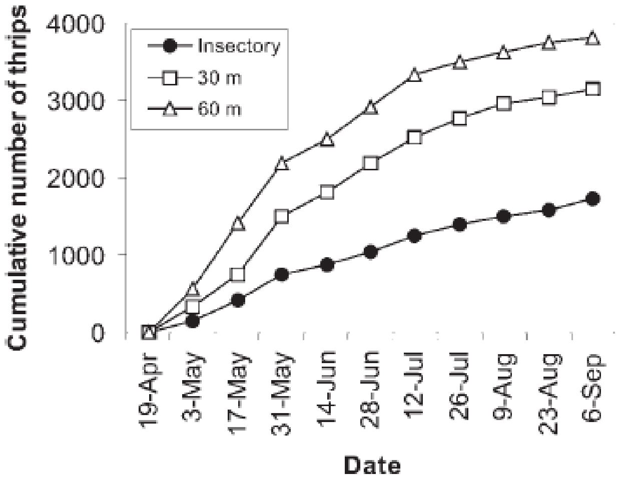

Fig. 1.2. Cumulative number of thrips per yellow sticky trap in 2004 at Benziger vineyard (Glen Ellen, California).

1.4.3 Landscape restoration to enhance ecosystem services to California vineyards

Habitat restoration in California agriculture is characterized by the establishment of mixed-use hedgerows intended to promote biological control, pollination and other ecosystem services. Hedgerows typically consist of combinations of annuals, herbaceous, and woody perennial shrubs and trees. These plantings have been found to attract populations of important natural enemies of vineyard pests, including *Orius* spp., *Geocoris* spp., Coccinellidae, Chrysopidae, Nabidae, Syrphidae and various spiders (Dufour et al. 2000, Robins et al. 2001, Earnshaw 2004). Despite the limited data on the aggregation of natural enemies, the impact on biological control of vineyard pests remains largely unexplored. Given the lack of scientific data on the impact of such plantings, growers and government programs supporting such efforts may not be fully realizing the outcomes they are intended to achieve.

Additionally, the inconsistent findings of many of the previous vineyard diversification studies described above may be in part due to a failure of researchers to adequately account for the influence of the surrounding landscape on biological control. Identifying the key qualities and quantities of non-crop habitats that support natural enemies will be an essential step in developing scientifically based landscape restoration programs that effectively enhance biological control and other ecosystem services to vineyards. Despite the many important contributions of ecologists and biological control specialists to date, many research gaps remain. Filling these gaps will be essential in providing the empirical evidence needed to define the specific types of habitat enhancement that leads to cost effective regulation of important vineyard pests.

1.5 CURRENT DIVERSIFICATION RESEARCH AT UC BERKELEY: FIELD-SCALE ANALYSIS

Building upon the prior field- and landscape-scale studies in conservation biological control in vineyards discussed above, researchers at the university of California, Berkeley, have recently initiated the first comprehensive, multi-scalar study of the impact of floral resource provisioning (FRP) and landscape complexity in Napa, Sonoma, San Joaquin and Fresno County wine grape systems.

The floral resource provisioning theory predicts that the addition of flowering plants to simplified agroecosystems improves biological control by providing insect parasitoids or predators with key food sources (e.g., nectar, pollen) that would otherwise limit fitness of natural enemies (Barbosa 1998, Landis et al. 2000, Altieri and Nicholls 2004, Heimpel and Jervis 2005, Lee and Heimpel 2008). The floral resource provisioning systems attract the interests of researchers and growers because of its theoretical appeal and success in some cropping systems (Tonhasca and Byrne 1994, Gurr and Wratten 2000, Letourneau et al. 2010). The floral resource provisioning schemes also attract some skepticism in the scientific community as the outcomes of hundreds of on-farm diversification studies have been mixed (Andow 1991a, Lavandero et al. 2005, Wäckers et al. 2005, Straub et al. 2008). However, in a recent meta-analysis, Letourneau et al. (2010) showed that on-farm diversification strategies consistently supported a greater abundance and diversity of natural enemies and increased pest control. Further, when FRP strategies do appear successful, the ecological processes underlying enhanced pest regulation often remains unsubstantiated or not fully understood (Gurr et al. 2000, 2004; Landis et al. 2000, Nichols et al. 2000). Finally, the relationship between FRP and pest densities in vineyards and other cropping systems may also be explained by multiple alternative hypotheses (Wratten et al. 1998, Corbett 1998, Costello and Daane 2003, Gurr et al. 2004, Heimpel and Jervis 2005, Bianchi et al. 2006). The current scientific consensus is that FRP can enhance biological control, but its success is both context and system specific (Altieri and Nicholls 2004, Tscharntke et al. 2007). Moreover, while FRP programs have the potential to decrease reliance on pesticides, the uncertainty of the effectiveness of this and other diversification schemes restricts large-scale implementation.

Prior studies in field-scale diversification in California vineyards were limited by a number of key factors. First, in Costello and Daane (1998, 2003), non-flowering cover crops (i.e., barley) were used, and less consideration was given to seasonal availability of floral resources, flower morphology and accessibility, and/or the quality of floral resources needed to enhance the fitness of natural enemies (Wäckers 2004, Begum et al. 2006, Vattala et al. 2006). The findings of Nicholls et al. (2000) were limited to a comparative analysis of two large vineyard blocks without full substantiation of the cause of enhanced biological control. Additionally, in all the prior on-farm diversification research in California, the landscape context (i.e., the area and diversity of non-crop habitat) was not fully taken into account (Tscharntke et al. 2005, 2007, Chaplin-Kramer et al. 2011).

To address some of the limitations and build upon prior studies in vineyard diversification, the current UC Berkeley conservation biological control project will assess the impact of floral resource provisioning and landscape complexity in several key grape producing regions. At the field level, the study will measure the impact of four flowering ground covers (annual buckwheat (*F. esculentum*), lacy phacelia (*Phacelia tanacetifolia* Bentham), sweet alyssum (*Lobularia maritima* (L.) Desvaux), and common carrot (*Daucus carota* L.)) on biological control of *Erythroneura* leafhoppers (*E. elegantula* and *E. variabilis*) and vine mealybug (*Planococcus ficus* (Signoret)) by the parasitoid wasps *Anagrus* spp. and *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) in California vineyards. The research includes eight split-block trials on commercial vineyards in Napa and Sonoma County and two fully replicated research designs, one located in Lodi and the other at the UC Kearney Agriculture Center in Fresno County. The research will test multiple hypotheses (i.e., natural enemies and resource concentration) of biological control in vineyards to advance scientific knowledge of cost-effective and ecologically based pest management. The study will quantify the impacts of FRP on population densities of pest and beneficial insects and analyze the biological mechanisms (e.g., longevity, fecundity, parasitism rates) theorized to be enhanced through FRP. Comparative cost-benefit analyses (FRP vs. conventional practices) will evaluate the cost-effectiveness of the tested strategies. In addition, the study will measure natural enemy movement from flowering cover crops to the vine canopy and substantiate nectar feeding through laboratory studies and anthrone testing. In a separate replicated and complementary study, researchers are testing the effect of methyl salicylate lures (a beneficial insect attractant) on natural enemies, pest densities and biological control (James 2003, 2006, Cook et al. 2006, James and Price 2004). Data from laboratory studies indicate that FRP has a significant positive impact on the longevity of *A. pseudococci* females (A. Miles et al., unpublished data).

1.6 CURRENT DIVERSIFICATION RESEARCH AT UC BERKELEY: LANDSCAPE ANALYSIS

The landscape component of the UC Berkeley conservation biological control project will evaluate the influence of landscape heterogeneity on the effectiveness of a field-scale FRP treatment to enhance biological control of grape leafhopper (*E. elegantula*), and vine mealybug (*P. ficus*) in northern California wine grape vineyards. An in-field FRP treatment plot will be compared to a control plot in 20 separate vineyards situated along a gradient of landscape heterogeneity. The FRP treatment will consist of three annual flowering plant species: lacy phacelia (*P. tanacetifolia*), bishop's weed (*Ammi majus* L.) and wild carrot (*D. carota*). This combination of species was selected to provide floral bloom throughout the entire growing season. These species are also drought tolerant, require no additional irrigation and can readily be integrated with standard vineyard management practices in northern California. Populations of the two pests and their key natural enemies will be monitored along with parasitism rates, crop damage and yield. Vine vigor will also be assessed in order to evaluate the influence of plant nutrient status on pest densities. Additionally, an assessment of *Anagrus* dispersal from natural habitats into adjacent vineyards will be conducted. Finally, *Anagrus* overwintering habitat will be assessed. Plant species commonly found in northern California vineyard landscapes will be sampled and evaluated for overwintering parasitoids. Plant material found to support significant *Anagrus* populations will be further evaluated to determine the associated insects that serve as alternate-hosts for the parasitoid. In combination, these studies are intended to generally evaluate how vineyard landscape composition influences the ability of field-scale FRP to enhance biological control of key wine grape pests. The goal of this research is to determine thresholds of landscape heterogeneity within which the use of field-scale FRP is most cost-effective for enhancing biological control.

1.7 CONCLUSION: FIELD AND LANDSCAPE-LEVEL DIVERSIFICATION FOR CONSERVATION BIOLOGICAL CONTROL

Results of the California studies reviewed above show a pattern consistent with the larger national and international conservation biological control literature: treatment effects from diversification strategies are discernable, yet cost-effective biological control is not consistently achieved (Andow 1991a) English-Loeb et al. 2003, Begum et al. 2006, Berndt et al. 2006, Straub et al. 2008). Nevertheless meta analyses showed that diversification had a moderate effect on the abundance of plant herbivores (Tonhasca and Byrne 1994, Letourneau et al. 2010). Other meta-analyses of landscape factors have also shown that while natural enemy abundance, richness, predation and parasitism rates do increase significantly with landscape heterogeneity, pest densities are not found to be consistently lower (Bianchi et al 2006, Chaplin-Kramer et al. 2011). Despite the large body of existing research, significant gaps remain in the conservation biological control literature. Findings from the research proposals outlined herein will help provide the necessary information for advancing the science of conservation biological control and developing more cost-effective ecologically based pest management strategies for California vineyards.

1.8 PROPOSALS AND CONSIDERATIONS FOR FUTURE RESEARCH: CONSERVATION BIOLOGICAL CONTROL IN CALIFORNIA VINEYARDS

The following are guidelines and specific proposals for research that would serve to advance the science and practice of conservation biological control in California viticulture. Proposals include both natural and social science studies.

As the effect of field-scale habitat enhancement strategies can be influenced by features in the surrounding landscape, future research must consider the influence of non-crop habitats that lie beyond the individual field or vineyard boundary. Broad correlative studies of landscape heterogeneity, natural enemy and pest density must be conducted along with detailed evaluations of the ecological processes theorized to influence biological control. To provide reliable data for use in developing effective pest management strategies, studies must be conducted for a minimum of two years and include full replication at the field- and landscape-scale. Field-scale evaluations of diversified cropping systems should assess both natural enemies and pest densities along with empirical tests of parasitism and predation (Bianchi et al. 2006). Measures should be taken to determine the impact of treatments on herbivore population densities, crop yield and quality. Studies measuring the impact of intercropping must account for the influence of non-crop vegetation on plant nutrient status along with impacts on the fitness of natural enemies (Daane and Costello 1998, Altieri and Nicholls 2003). Multi-trophic interactions must also be considered as increased diversity and abundance of natural enemies in complex agricultural habitats can lead to intraguild predation and subsequent release of pests from biological control (Finke and Denno 2004, Straub et al. 2008).

Studies involving habitat manipulation should evaluate both the natural enemies and resource concentration hypotheses. Invertebrate response to landscape heterogeneity should be evaluated in a way that can address both of these hypotheses. At a minimum, this would require separately examining insect response to the relative area, diversity and connectivity of both natural habitat and agricultural land at the landscape scale. The high probability of idiosyncratic and species specific response to the landscape will require that observed trends be evaluated relative to a number of alternate measures of landscape heterogeneity, including perimeter-to-area ratio, mean patch size, and distance away from natural habitats (for details see Concepción et al. 2008). As non-crop habitats cannot be assumed to benefit only predators and parasitoids, studies should simultaneously measure the impact of non-crop vegetation on the fitness of insect pests (van Emden 1965, Baggen et al. 1999, Roschewitz et al. 2005).

As habitat diversity will influence insect movement at both the field- and landscape-scale, researchers are encouraged to consider the movement and distribution of arthropods in relation to the elements of heterogeneity under study (Corbett 1998, Dover and Settele 2009). The results of Corbett and Rosenheim (1996a) demonstrate the importance of empirical assessments of parasitoid dispersal from non-crop habitats. Quantifying insect movement between in-field habitat and crop and from non-crop habitats into cropping systems will be critical to developing a more nuanced understanding of the impact of heterogeneity at multiple spatial scales. Recent advances with relatively inexpensive marking systems (Hagler and Jones 2010) will help make this a reality.

Controlled field and laboratory trials are essential for determining the physiological influence of non-crop vegetation on key pests and natural enemies (Wäckers et al. 2005). Quantifying the influence of multiple species of flowering plants on parasitoid longevity, fecundity, parasitism rates and sex ratios of key biological control agents can help form the empirical basis for understanding enhanced biological control in field trials. To further substantiate nectar feeding, researchers should consider anthrone or HPLC testing to determine changes in parasitoid gut-sugar levels in the presence of flowers (Stepphun and Wäckers 2004, Heimpel and Jervis 2005). Ideally, such work would be conducted under conditions most resembling the vineyard environment (Lee and Heimpel 2008).

It is important for applied research in conservation biological control to include on-farm and participatory trials in commercial vineyard settings. Such dialog with growers encourages the development of practices suitable for large-scale implementation and facilitates a social learning process between researchers and growers that may improve the relevancy of research and advance grower adoption of successful ecologically-based pest management practices (Röling and Wagemakers 2000, Warner 2007a, b). Cost-benefit analysis, including data on impact to other ecosystem services (e.g., soil quality, etc.) will provide a more holistic basis for grower decision making regarding the true costs and benefits of vineyard diversification (Fiedler et al. 2008, Gurr et al. 2003, Jackson et al. 2007).

Habitat enhancement tactics may also be successfully combined with the many new chemical ecology approaches (e.g., pheromones) to further enhance biological control (Daane et al. 2008). ‘Attract and reward’ strategies, for example, combine the use of herbivore-induced plant volatile compounds (HIPVC) with in-field FRP and has shown much promise in enhancing the effectiveness of diversification schemes (James 2006, Khan et al. 2008). One such HIPVC, methyl-salicylate (MeSA) has been shown to increase abundance of some natural enemies in grape vineyards as well as in other cropping systems (James and Price 2004, James 2006, Lee 2010).

Future research must also include relevant economic and social assessments which may assist in developing ecologically-based pest management practices suitable for commercial adoption and provide a sound basis for the formulation of public policy (Cullen et al. 2008). To date, little work has been done to evaluate the impacts of public policy on vineyard habitat management or the ability of public institutions to adequately respond to grower research needs and coordination of agricultural restoration efforts at the regional scale. Finally, it will be critical to gather information on consumer perceptions of product quality and value associated with agricultural goods produced using ecologically-based farming practices (Forbes et al. 2009, Zucca et al. 2009, Howard and Allen 2010, Delmas and Grant 2010).

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Chapter 2:

Quantifying the impact of floral resource provisioning on the fitness of *Anagyrus pseudococci*, a key natural enemy of the vine mealy bug, *Planococcus ficus*

Abstract:

Conservation biological control aims to enhance the effectiveness of arthropod biological control agents, such as parasitoids, through managing environmental conditions, including provisioning of floral resources (FRP). The suitability of a given flowering plant species for provisioning nectar and enhancing the fitness of natural enemies, however, is dependent on the morphology of the parasitoid and the flowering plant, as well as on the quantity and quality of the nectar provided. To test the parasitoid nectar-feeding hypothesis and determine the suitability of flowering plants for field application in California vineyards, the study first quantified floral nectar feeding by the vine mealybug parasitoid, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encritidae), from ten species of flowering plants through gut sugar analysis under laboratory conditions. The study also measured the impact of floral resource provisioning on the longevity and parasitism rate by *A. pseudococci* of the invasive vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). The flowering plants measured in the study included mustard (*Brassica juncea*), yarrow (*Achillea millefolium*), rose clover (*Trifolium hirtum*), cilantro (*Coriandrum sativum*), sweet alyssum (*Lobularia maritima*), lacey phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*), annual buckwheat (*Fagopyrum esculentum*), wild carrot (*Daucus carota*), and English lavender (*Lavandula angustifolia*). Results from anthrone testing of gut contents show that *A. pseudococcus* was able to feed on English lavender, lacey phacelia, wild carrot and annual buckwheat, each containing significantly higher fructose concentrations than the control. *A. pseudococci* exposed to alyssum, lacey phacelia, bishop's weed, buckwheat, carrot, and lavender treatments, showed significantly higher combined fructose and sucrose levels when compared to the control group (water only). Despite strong evidence of nectar feeding, only the honey solution served to increased mean longevity of *A. pseudococci.* Nectar feeding from lacey phacelia significantly reduced the mean longevity of *A. pseudococci* when contrasted with the control. Kaplan-Meier estimates showed that honey solution, buckwheat, bishop's weed and carrot all significantly increased the probability of survival of *A. pseudococci*. FRP treatments were found to have no significant impact on parasitism rate of vine mealy bug, with the exception of *Ammi majus,* exhibiting a significant negative impact on parasitism. FRP treatments did not have any significant effect on second-generation sex ratios of *A. pseudococci*. Relevance to the parasitoid nectar-feeding hypothesis is discussed along with the implications of these results conservation biological control in California vineyards.

Keywords:

Conservation biological control, floral resource provisioning; floral nectar; parasitoid nectar feeding hypothesis; *Anagyrus pseudococci*; *Planococcus* mealy bugs; vine mealy bug; fructose; sucrose; longevity; parasitism; anthrone testing; Hymenoptera; Encritidae; viticulture.

1. Introduction:

Externalized environmental and economic cost of pesticide use in the U.S. is estimated to be over $9.6 billion USD annually (Pimentel 2009). Meanwhile, biological control of agricultural pests through the actions of natural enemies is estimated to be valued at over $4.5 billion USD annually (Isaacs et al. 2008). With increased investment in research and development, there is great potential to increase the effectiveness of biological control and other ecosystem services to agriculture (Birch et al. 2011, Gurr et al. 2012, Wyckhuys et al. 2012, Miles and Carlisle 2013, submitted).

A leading hypotheses explaining decreased pest densities in biologically diversified farming systems is that increased plant diversity can enhance the fitness and effectiveness of natural enemies of arthropod pests - the 'natural enemies hypothesis' (Russell 1989). Increased plant diversity in agroecosystems can provide natural enemies with key resources such as refuge, favorable microclimates, alternative hosts or prey, or plant-based foods such as pollen and nectar (Altieri and Nicholls 2004, New 2005, Lundgren 2009). Targeted farming system diversification is increasingly identified as a scientifically underdeveloped but important strategy for enhancing biological control of arthropod pests while improving the multi-functionality and long-term ecological sustainability of agroecosystems (Altieri 1999, Fiedler et al. 2008, Crowder et al. 2010, Letourneau et al. 2011, Kremen and Miles 2012, Wäckers and van Rijn 2012, Wyckhuys et al. 2012).

Many species of natural enemies of agricultural pests feed on non-host food, including floral resources (Vattala et al. 2006, Kehrli and Bacher 2008). It has been repeatedly demonstrated that nectar feeding is key to the survival of many adult parasitoids and can have a positive impact on fitness through an enhanced rate of egg maturation, prolonged reproductive longevity, or both (Wäckers et al. 2005, Lee and Heimpel 2008a, Tomkins et al. 2010, Harvey et al. 2012).

The invasive vine mealybug (VMB), *Planococcus ficus* (Hemiptera: Pseudococcidae), is a primary grape vine pest throughout the Mediterranean regions of the world (Daane et al. 2012). VMB honeydew excretion, supporting sooty mold growth, can significantly compromise both crop quality and quantity leading to substantial economic losses for growers (Daane et al. 2008). VMB is also a vector of grapevine leaf roll viruses, and thus can be an economically damaging pest even at low population densities (Tsai et al. 2008, Daane et al. 2012). Insecticides used for the control of VMB are frequently inadequate, drive the development of genetic resistance, disrupt the actions of natural enemies and pose a risk to non-target organisms (Daane et al. 2004, Bostanian et al. 2012). *Anagyrus pseudococci* (Giralt) is one of the most effective, well-studied, widely distributed and commercially available parasitoids of VMB, holding much potential for enhancing natural pest regulation in vineyards (Walton et al. 2012).

With *A. pseudococci* and selected species of flowering plants, we tested the parasitoid nectar-feeding hypothesis (PNFH) for its potential deployment in California vineyards to manage VMB. The PNFH posits that the presence of nectar producing plants in or around agroecosystems can enhance biological control of pests by supplying parasitoids with otherwise limiting carbohydrates. The PNFH includes 2 necessary and testable components: a.) improved biological control of pests (the outcome), and b.) nectar feeding by the parasitoid (the mechanism) (Heimpel and Jervis 2005). The objectives of the current laboratory experiments were, therefore, to quantify nectar feeding and measure its impacts on survivorship, parasitism and offspring sex ratios of the VMB parasitoid, *A. pseudococci* (Onagbola et al. 2007). Key questions we sought to answer include: does *A. pseudococci* feed on floral nectar? If so, which agronomically suitable plant species provide the greatest quantity of fructose and/or sucrose? Does nectar feeding effect the longevity of *A. pseudococci*? How does nectar feeding by *A. pseudococci* impact the rate of parasitism, and thus mortality, of VMB? Quantifying the above impacts of FRP on biological control will contribute to a deeper theoretical understanding of the underlying ecological mechanisms that influence natural enemy-pest population dynamics at field-level (Lu et al. 2013) while evaluating new conservation biological control strategies for VMB.

2. Methods and materials:

Sources and methods for flowering plants: Plants of mustard (Brassicaceae: *Brassica juncea*), yarrow (Asteraceae: *Achelia millifolium*), rose clover (Fabaceae: *Trifolium hirtum*, celantro (Apiaceae *Coriandrum sativum*), fennel (Apiaceae: *Foeniculum vulgare*), sweet alyssum (Brassicaceae: *Lobularia maritime*), lacey phacelia (Boraginaceae: *Phacelia tanacetifolia*), bishop's weed (Apiaceae*: Ammi majus*), annual buckwheat, (Polygonaceae*: Fagopyrum esculentum*), wild carrot (Apiaceae*: Daucus carrota*), and English lavender (Laminaceae: *Lavandula angustifolia*) were all grown from seed under uniform green house and field conditions. All have agronomic potential for growing in or around Mediterranean climate vineyards, many of which have been studied previously for their biological control potential in other cropping systems (Fiedler et al. 2008). Because the different species of plants bloomed asynchronously, separate experiments were conducted between January-October of 2010. Each nectar feeding, longevity and parasitism experiment conducted during this period included a separate control group (water only) to account for possible variability among generations of insects. All experiments were conducted under controlled laboratory conditions at the Natural Resources Laboratory at the University of California at Berkeley.

Sources and methods for *Planococcus ficus* (VMB) and *A. pseudococci* for nectar feeding experiment: Colonies of VMB were raised under controlled laboratory conditions on butternut squash (*Cucurbita moschata* L.) cleaned in a 0.5% bleach solution to reduce mold growth, and then triple rinsed in water. Squash were inoculated with ~200 1st instar VMB and held in sleeved rearing cages (45 × 45 × 45 cm) at a constant temperature of 27 ± 2 °C, 40% relative humidity, and 16:8 photoperiod. To control timing of oviposition and emergence, after 7 days the VMB inoculated squash were transferred into separate but identical rearing cages and ~20 adult female *A. pseudococci* were introduced. After 16 days of gestation, peak emergence of *A. pseudococci* occurred at which time 5 female *A. pseudococci* (<24h) were removed and placed in separate 14 (O.D.) x 30.5 cm clear extruded acrylic cylinders (Professional Plastics, Pasadena, Ca). Each cylinder was provisioned separately with 1 small bouquet of one FRP species (above) and a source of distilled water. A fine plastic mesh glued to the top of each cylinder provided ventilation (see appendix 1). After 72 h exposure to the FRP treatments, the 5 female *A. pseudococci* were removed, frozen and stored at -18 °C for subsequent gut sugar analysis. To qualify and quantify nectar feeding by *A. pseudococci,* cold and warm anthrone testing methods were used to detect levels of sucrose and fructose following protocols outlined by Van Handel (1967) and Heimpel et al. (2004). The study was organized in a randomized complete block design (Tomkins et al. 2010) with a minimum of 3 replicates of each treatment. Individual blocks consisted of the following treatments: control (water only), mustard (*Brassica juncea*), yarrow (*Achelia millifolium*), rose clover (*Trifolium hirtum*), cilantro (*Coriandrum sativum*), sweet alyssum (*Lobularia maritima*), lacey phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*), annual buckwheat (*Fagopyrum esculentum*), wild carrot (*Daucus carrota*), and English lavender (*Lavandula angustifolia*).

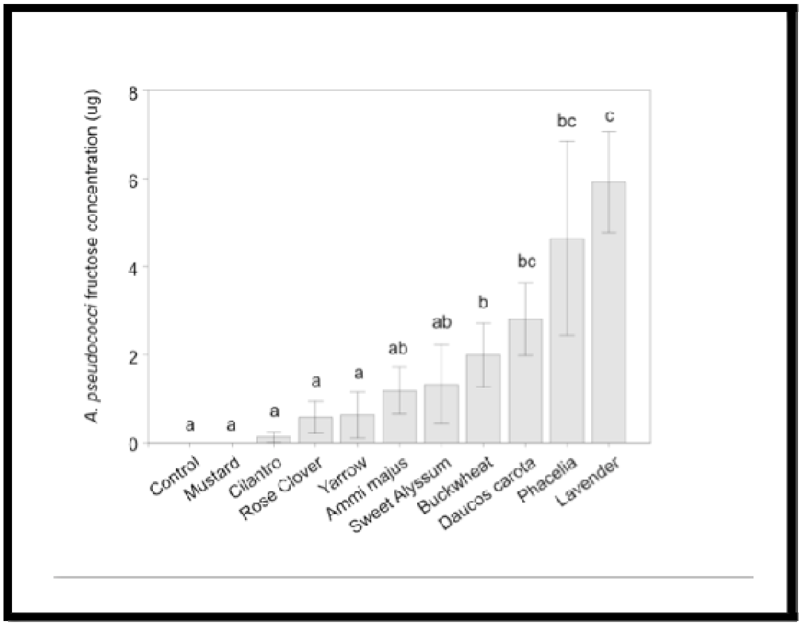
Sources and methods for *Planococcus ficus* (VMB) and *A. pseudococci* longevity and parasitism experiment: Sprouted potatoes, *Solanum tuberosum* L. (~3.5 cm diameter), were cleaned in a

0.5% bleach solution and then triple rinsed in water. Each potato was inoculated with ~50 1st – 2nd instar VMB and held at 22 ± 2 °C for ~7 days. *A. pseudococci* (<24h) were derived from the same process outlined above. Newly emerged (<24h) *A. pseudococci* parasitoids (3 female: 2+ males) were placed in identical 14 (O.D.) x 30.5 cm clear extruded acrylic cylinders (Professional Plastics, Pasadena, Ca) + 1 small bouquet of one FRP treatment (above) + 1 sprouted potato inoculated with ~50 2nd – 3rd instar VMB placed in a 4 oz fluon-coated plastic soufflé cup (Solo P400, Mission Restaurant Supply, San Antonio, Tx) + distilled water. Each cylinder was placed on a white paper plate to allow for ease of viewing parasitoids. A fine plastic mesh was glued to the top of each cylinder providing ventilation. Containers were checked daily for *A. pseudococci* mortality and recorded until all female parasitoids were dead. Depending on the abundance of each flower, experiments had 6-12 replicates of the FRP species treatment and the control (see tables 1-8). VMB-inoculated potatoes exposed to oviposition by *A. pseudococci* were removed from the experimental cylinders every 72 h and replaced by new potatoes inoculated with ~50 2nd and 3rd instar VMB not exposed to oviposition. Once removed from the experimental cylinders, potato samples containing parasitized VMB were stored at constant temperature (27 ± 2 °C, 40% relative humidity, 16:8 photoperiod) for 20 days to allow for gestation of *A. pseudococci.* Samples were subsequently frozen and held at -18 °C to arrest development, transferred to 80% EtOH solution and refrigerated at 3 ± 2 °C. VMB were removed from potato pieces and examined under a dissecting microscope where tallies of parasitized and non-parasitized VMB were made. The study was organized in a randomized complete block design (Tomkins et al. 2010). Individual blocks consisted of the following treatments (water included in all blocks): control (water only); honey solution (50:50 honey to water by volume); sweet alyssum (*Lobularia maritima*), lacey phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*), annual buckwheat (*Fagopyrum esculentum*), wild carrot (*Daucus carota*) and common fennel (*Foeniculum vulgare*).

Statistical analysis: For studies of parasitoid nectar feeding, ANOVA was used to evaluate the influence of floral nectar feeding on parasitoid gut sugar levels and means were subsequently separated using Tukey's Honestly-Significant-Difference (HSD) test. Influence of the FRP treatments on mean parasitoid longevity, parasitism rate and *Anagyrus* offspring sex ratio were all analyzed using ANOVA with Type II error structure due to unequal replication amongst the treatments. Means were separated using the Tukey-Kramer method. Parasitism rates and sex ratios are based on averages per potato per replicate. Parasitoid survivorship curves were derived using the Kaplan-Meier method. Survival curves were then compared using a log-rank test and Shared Frailty Model following methods outlined by Rondeau et al. (2012). The Shapiro-Wilks test was used to assess normality of the data. Longevity data (# of days lived) was log(x+1) transformed and parasitism and sex ratio data were arcsine-square-root transformed prior to analysis. All analyses was conducted in "R".

3. Results:

Nectar feeding by *A. pseudococci*: Fructose is not detected in the haemolymph of unfed insects using cold anthrone tests (Lee et al. 2004). Fructose levels exceeding 1 µg, as measured through cold anthrone testing, indicate that floral nectar feeding by *A. pseudococci* occurred with a minimum of 4 species of flowering plants. *A. pseudococci* exposed to lavender, phacelia, wild carrot and buckwheat were found to contain significantly higher fructose concentrations than the control (water only) and all other flowering plant species with the exception of bishop’s weed and alyssum. In descending order, the highest fructose concentrations measured in *A. pseudococci* resulted from exposure to lavender, phacelia, carrot and buckwheat flowers (Fig. 1a and Table 1). When contrasted to the control (water only), significantly higher sucrose levels were also detected via warm anthrone testing in *A. pseudococci* when exposed to alyssum, bishop’s weed, buckwheat, phacelia, wild carrot and lavender (Fig. 2, Table 2). *A. pseudococci* was found to have significantly higher combined fructose and sucrose levels when exposed to alyssum, phacelia, bishop’s weed, buckwheat, wild carrot and lavender (Fig. 3, Table 3).



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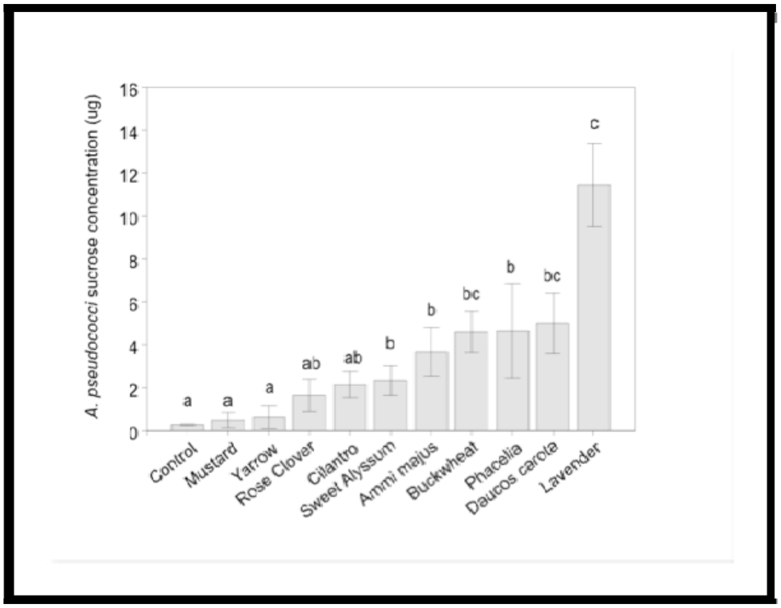

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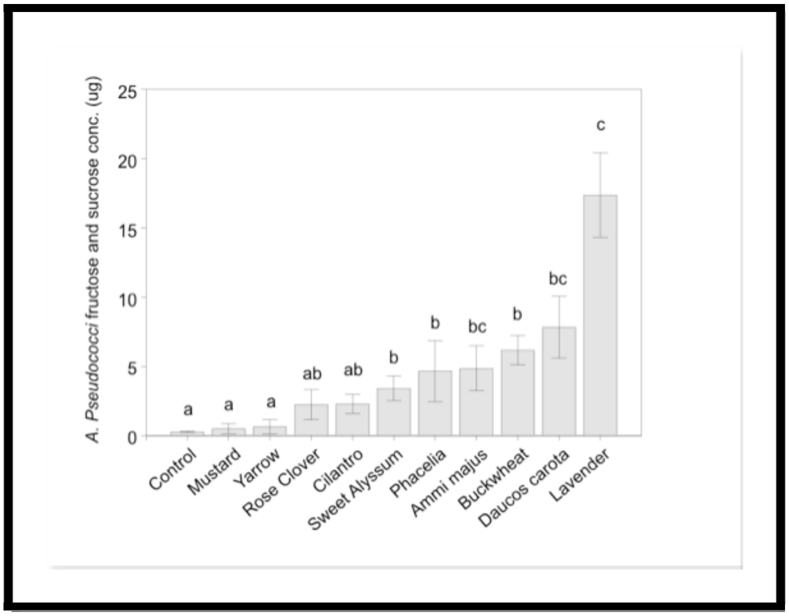

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Figure 1. (a) *A. pseudococci* fructose concentrations (µg), as measured by cold anthrone testing, when separately exposed to ten different species of flowering plants for 72 hr. (b) *A. pseudococci* sucrose concentrations (µg), as measured by warm anthrone testing, when separately exposed to ten different species of flowering plants for 72 hrs. (c) Combined fructose and sucrose concentrations detected in *A. pseudococci* when separately exposed to ten different flowering plant species. Means were compared using Tukey's HSD test. Bars sharing the same letter do not differ at P = 0.05, error bars = +/- SE, n = 10-62.

Longevity and survivorship of *A. pseudococci:* ANOVA indicated that, relative to the Control, parasitoid longevity was significantly enhanced in the "Honey solution" and *Ammi majus* treatments (F = 3.8699, $DF_{7,147}$, P = 0.0006773) (Fig. 2, Table 4).

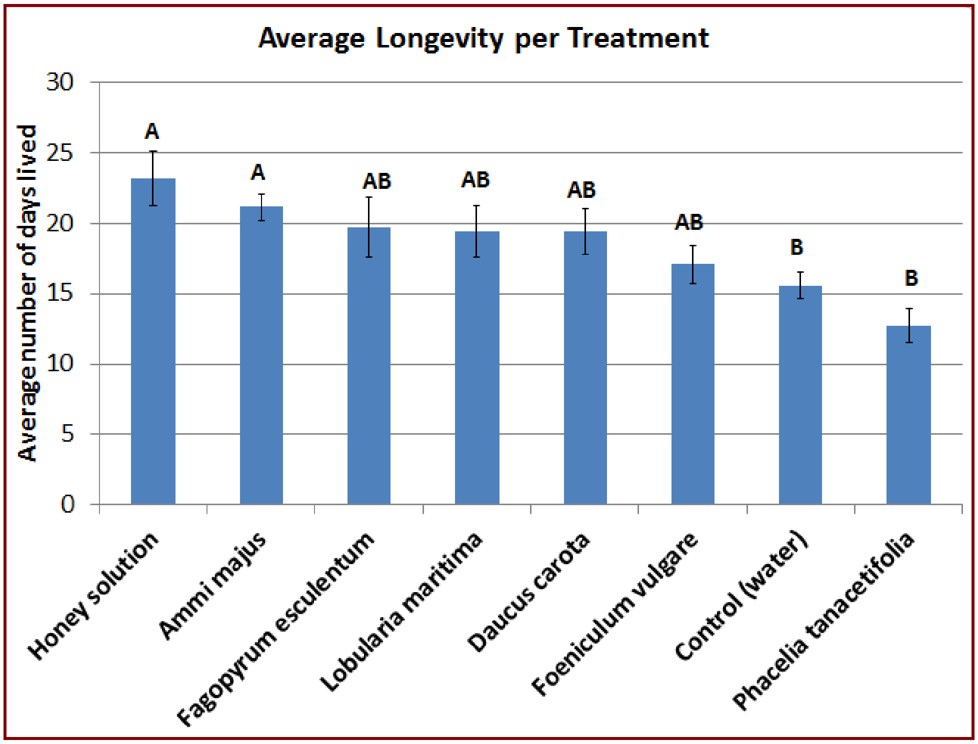

Figure 2. Mean longevity (in days) of *A. Pseudococci* when exposed to seven separate treatments of flowering plants and control (water only). Bars sharing the same letter do not differ at P = 0.05, error bars = +/- SE, n = 11-47.

The log-rank test on parasitoid survivorship indicated significant differences in the probability of survival between treatments ($\chi^2 = 39.8$, DF_7, $P < 0.001$). To compare differences between individual treatments, a Shared Frailty Model was used. The frailty model indicated that parasitoid survivorship was significantly increased under the honey solution, wild carrot, buckwheat and bishop's weed treatments and decreased in the phacelia treatment (Fig. 3).

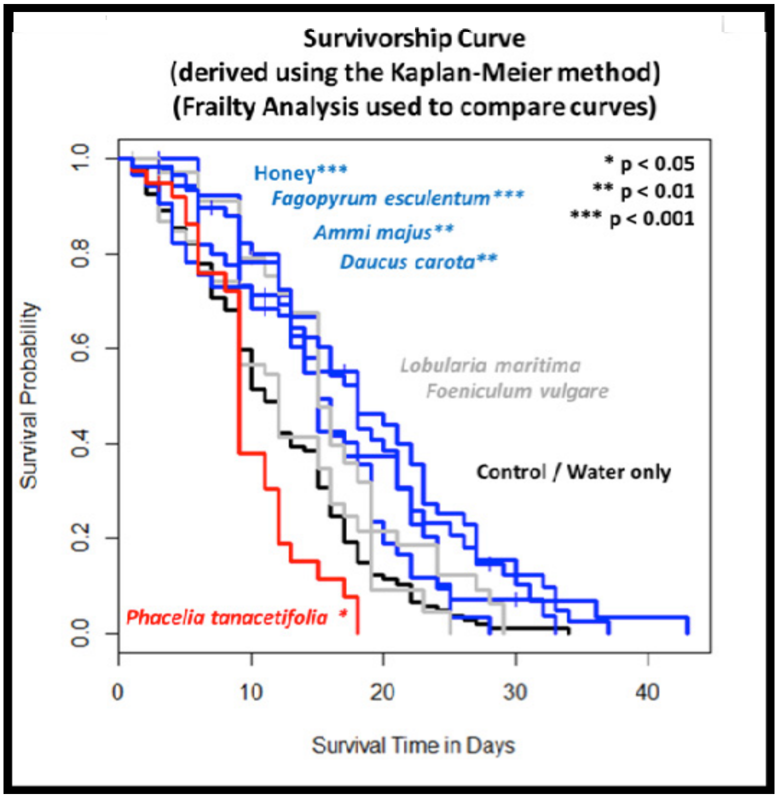

Figure 3. Kaplan–Meier estimates of the survival probability of *A. pseudococci* when exposed to different treatments. Survival curves were compared using a Shared Frailty Model.

Parasitism of vine mealy bug by *A. pseudococci*: Relative to the Control, there was a significant decline in parasitism rates in the *Ammi majus* treatment (F = 2.2735, $DF_{7,70}$, P = 0.03813) (Fig. 4, Table 6).

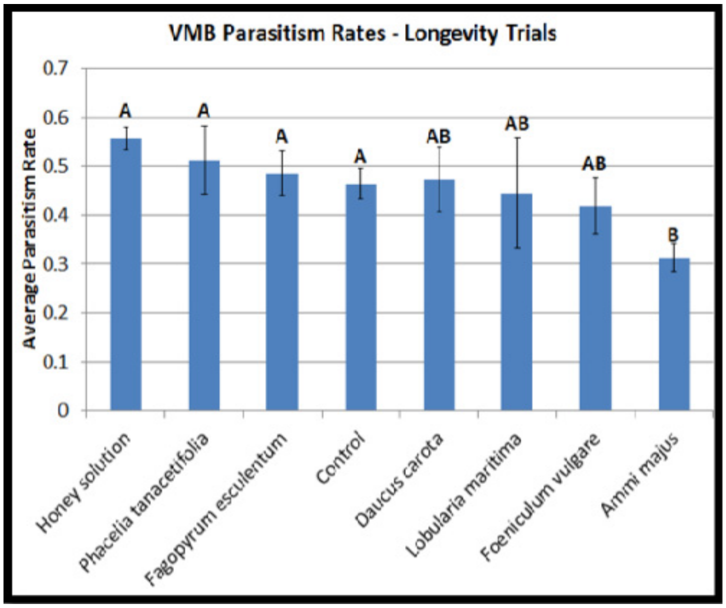

Figure 4: Mean parasitism of *A. Pseudococci* when exposed to seven separate treatments of flowering plants and control (water only). Bars sharing the same letter do not differ at P = 0.05, error bars = +/- SE, n = 6-12. Relative to the Control, parasitism rates significantly declined in the *Ammi majus* treatment (F = 2.2735, $DF_{7,70}$, P = 0.03813).

2^{nd} generation sex ratios of *A. pseudococci*: ANOVA of the mean percentages of male and female offspring indicated that treatments did not have any effect on second generation sex ratios (F = 1.9895, $DF_{7,70}$, p=0.0687) (Figure 5, Table 7).

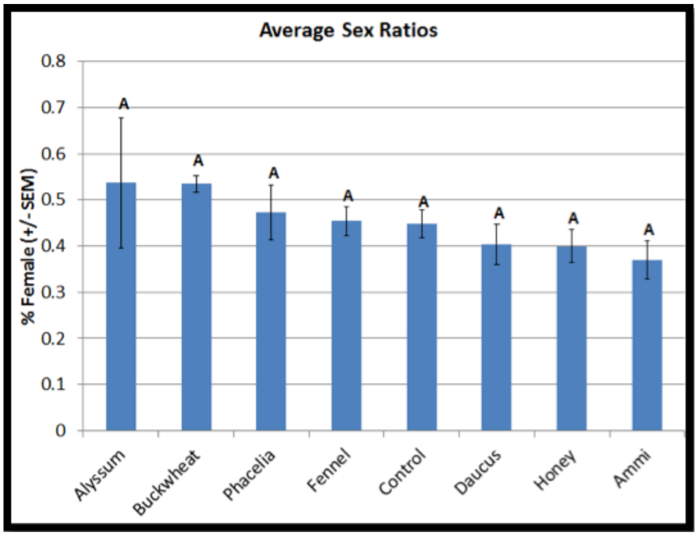

Figure 5: Mean sex ratios of offspring of *A. Pseudococci* adults when exposed to separate treatments of flowering plants and control (water only). Bars sharing the same letter do not differ at P = 0.05, error bars = +/- SE, n = 6-12. FRP treatments did not have any effect on second generation sex ratios (F = 1.9895, $DF_{7,70}$, p=0.0687).

4. Discussion:

The findings of the study show that *A. pseudococci* can feed on a variety of annual and perennial nectar sources, including bishop's weed, phacelia, and wild carrot, all deployed in subsequent field studies (see Miles et al., in prep.) *A. pseudococci* exposed to lavender, phacelia, wild carrot and buckwheat were all found to contain significantly higher fructose concentrations than the control (water only) and all other flowering plant species with the exception of bishop's weed and alyssum. When contrasted to the control, significantly higher sucrose levels were also detected via warm anthrone testing in *A. pseudococci* when exposed to alyssum, bishop's weed, buckwheat, phacelia, wild carrot and lavender. When compared to the control, *A. pseudococci* was found to have significantly higher combined fructose and sucrose levels when exposed to alyssum, phacelia, bishop's weed, buckwheat, wild carrot and lavender. Consistent with other studies of floral nectar feeding, the plant families Apiaceae, Laminanceae and Polygonaceae all appear to be important nectar sources also utilized by *A. pseudococcii*.

Despite clear evidence of nectar feeding, the enhancement of *A. pseudococci* longevity was found to correlate only with exposure to the honey solution and *Ammi majus*. It is notable that longevity of *A. pseudococci* in the control was slightly higher than that of phacelia, which resulted in the lowest mean longevity. Survivorship analysis showed that survival probability was significantly greater in the honey solution, buckwheat, wild carrot and bishop's weed treatments when using the Kaplan-Meier estimates and Shared Frailty Model. Exposure to Phacelia significantly reduced the survivorship probability of *A. pseudococci*. This finding is consistent with prior nectar feeding studies showing phacelia having a strong negative impact on parasitoid longevity (Vattala et al. 2006). As a whole, these findings indicate that qualitative differences in carbohydrate sources and/or flower morphology can significantly impact the longevity of natural enemies and is consistent with prior work showing that natural enemies exhibit inter-specific variation in their preferences and responses to different species of flowering plants (Vattala et al. 2006; Araj et al 2008; Luo et al. 2010; Sivinski et al. 2011).

With the exception of bishop's weed, resulting in significantly reduced rate of parasitism, all other FRP treatments and the honey solution resulted in no significant differences in the rate of parasitism of VMB by *A. pseudococci*. Exposure to FRP treatments resulted in no statistically significantly effect on second-generation sex ratios of *A. pseudococci*.

Despite the honey solution and some FRP species enhancing (and in 1 case decreasing) the longevity and/or survivorship probability of *A. pseudococci*, the overall findings indicate that the FRP species selected for this study did not significantly enhance the fitness or the biological control potential of *A. pseudococci* under controlled laboratory conditions. The study thus fulfills only one of two key requirements of the parasitoid nectar-feeding hypothesis - nectar feeding by the parasitoid - but this apparently does not lead to improved biological control. Additional longer-term FRP research is warranted to assess the bio-control value of other species, especially those that may serve to generate multiple ecological services (Campbell et al. 2012, Gurr et al. 2012). *For a summary of all statistical analyses, see Table 8.

Acknowledgements: The authors would like to sincerely thank the following individuals and institutions for their generous support of this research: The California Department of Food and Agriculture (CDFA); the Organic Farming Research Foundation (OFRF); The United States Environmental Protection Agency (EPA); the many UC Berkeley undergraduate research interns; collaborating growers and their firms; California Sustainable Wine Growers Group; UC Kearney Agriculture Research Center staff and researchers, including Glenn Yokota and Dale Pattigan; staff and research assistants with the UC Berkeley Natural resources lab, Korry Kassir and John Hutchins; and the Robert van den Bosch Scholarship for providing research scholarships for Albie Miles.

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Appendix:

Acrylic cylinders use in experiment. Each cylinder was provisioned with 1 small bouquet of one FRP species (listed above), newly emerged (<24h) *A. pseudococci* parasitoids (3 female: 2+ males) + 1 VMB-inoculated potato with ~ 50 2nd and 3rd instar VMB in 4 oz, fluon coated, plastic soufflé cup and a source of distilled water. A fine plastic mesh glued to the top of each cylinder provided ventilation. There were a minimum of 12 replicates for each treatment and control (water only).

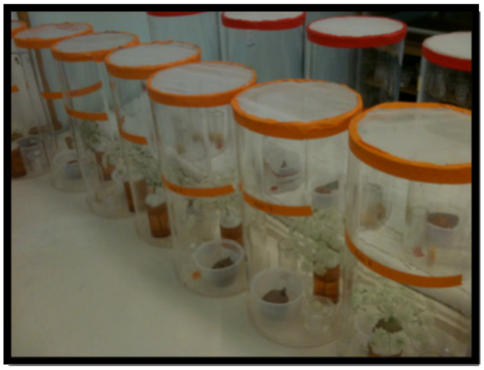


Table 1. Mean **fructose** concentration in micrograms (µg) measured in *A. pseudococci,* via cold anthrone test, after 72 hr. exposure to inflorescence.

Flower	Mean	SEM	N	Min	Max
Control	0.000	0.000	62	0	0
Mustard	0.000	0.000	10	0	0
Cilantro	0.128	0.114	14	0	1.598
Rose Clover	0.578	0.360	11	0	3.534
Yarrow	0.628	0.526	13	0	6.817
Bishop's Weed	1.179	0.532	11	0	5.017
Alyssum	1.324	0.905	12	0	10.57
Buckwheat	1.996	0.721	11	0	6.066
Wild Carrot	2.808	0.835	14	0	8.513
Phacelia	4.646	2.209	11	0	21.636
Lavender	5.922	1.147	28	0	21.339

Table 2. Mean **sucrose** concentration in micrograms (µg) measured in *A. pseudococci,* via warm anthrone test, after 72 hr. exposure to inflorescence.

Flower	**Mean**	**SEM**	**N**	**Min**	**Max**
Control	0.271	0.055	62.000	0.000	2.167
Mustard	0.487	0.359	12.000	0.000	4.240
Yarrow	0.628	0.526	13.000	0.000	6.817
Rose Clover	1.647	0.742	11.000	0.000	6.911
Cilantro	2.150	0.614	14.000	0.000	7.341
Alyssum	2.338	0.694	15.000	0.000	10.262
Bishop's Weed	3.672	1.143	11.000	0.090	9.755
Buckwheat	4.608	0.962	14.000	0.000	10.965
Phacelia	4.646	2.209	11.000	0.000	21.636
Wild Carrot	5.004	1.411	14.000	0.000	15.874
Lavender	11.456	1.934	28.000	0.087	35.509

Table 3. Mean **fructose and sucrose** concentration in micrograms (µg) measured in *A. pseudococci,* via cold and warm anthrone test, after 72 hr. exposure to inflorescence.

Flower	**Mean**	**SEM**	**N**	**Min**	**Max**
Control	0.271	0.055	62	0	2.167
Mustard	0.487	0.358	12	0	4.24
Yarrow	0.628	0.526	13	0	6.817
Rose Clover	2.226	1.088	11	0	10.445
Cilantro	2.278	0.698	14	0	8.939
Alyssum	3.397	0.887	15	0	10.765
Phacelia	4.646	2.209	11	0	21.636
Bishop's Weed	4.851	1.627	11	0.09	14.772
Buckwheat	6.176	1.058	14	0	10.965
Wild carrot	7.812	2.237	14	0	24.387
Lavender	17.378	3.056	28	0.087	56.848

Table 4. Analysis of the effect of different plant species and honey solution on *A. pseudococci* longevity.

Treatment	**n**	**Mean longevity ±SE**
Honey solution	18	23.2 ± 1.9 a
Ammi majus	15	21.1 ± 1.0 a
Fagopyrum esculentum	23	19.7 ± 2.1 ab
Daucus carota	16	19.4 ± 1.6 ab
Lobularia maritima	14	19.4 ± 1.8 ab
Foeniculum vulgare	11	17.1 ± 1.4 ab
Water	47	15.6 ± 0.9 b
Phacelia tanacetifolia	11	12.7 ± 1.2 b

Table 5. Kaplan–Meier estimates of the survival probability of *A. pseudococci* when exposed to different treatments.

Treatment	**coef**	**exp(coef)**	**SE coef (H)**	**SE coef (HIH)**	**z**	**p**	
Ammi majus	-0.5678	0.5668	0.1808	0.1955	-3.141	1.69e-03	**
Daucus carota	-0.5182	0.5956	0.1772	0.1917	-2.925	3.44e-03	**
Lobularia maritima	-0.2056	0.8142	0.1787	0.1946	-1.151	2.50e-01	
Fagopyrum esculentum	-0.6310	0.5320	0.1685	0.3000	-3.745	1.81e-04	***
Foeniculum vulgare	-0.3424	0.7101	0.2168	0.2272	-1.579	1.14e-01	
Phacelia tanacetifolia	0.4909	1.6338	0.2125	0.2235	2.310	2.09e-02	*
Honey solution	-0.8604	0.4230	0.1738	0.1914	-4.952	7.36e-07	***
Control							

Table 6: Analysis of the effect of different plant species and honey solution on VMB parasitism rates by *A. pseudococci*.

Treatment	**n**	**Average parasitism rate ±SE**
Ammi majus	12	0.31±0.03 a
Daucus carota	9	0.47±0.07 a
Honey Solution	11	0.56±0.02 a
Foeniculum vulgare	10	0.42±0.06 a
Control (water only)	12	0.47±0.03 a
Phacelia tanacetifolia	6	0.51±0.07 a
Fagopyrum esculentum	12	0.49±0.05 a
Lobularia maritima	6	0.45±0.11 a

Table 7: Analysis of the effect of different plant species and honey solution on sex ratios of offspring of *A. pseudococci*.

Treatment	**n**	**Average sex ratio ±SE F / (F+M)**
Ammi majus	12	0.37±0.04 a
Daucus carota	9	0.40±0.04 a
Honey Solution	11	0.40±0.04 a
Foeniculum vulgare	10	0.45±0.03 a
Control (water only)	12	0.45±0.03 a
Phacelia tanacetifolia	6	0.47±0.06 a
Fagopyrum esculentum	12	0.53±0.20 a
Lobularia maritima	6	0.54±0.14 a

Table 8 : Summary of statistical analyses. “x” indicates significant difference relative to the Control. “(+) / (-)” indicates whether the difference was positive or negative.

	Experiment 1			**Experiment 2**			
Treatment	**Fructose**	**Sucrose**	**Fruc.+Suc.**	**Longevity**	**Survivorship**	**Parasitism**	**Sex-ratio**
Control							
Honey solution (50%)	NA	NA	NA	x (+)	x (+)		
Phacelia tanacetifolia	x (+)	x (+)	x (+)		x (-)		
Ammi majus		x (+)	x (+)	x(+)	x (+)	x (-)	
Daucus carota	x (+)	x (+)	x (+)		x (+)		
Fagopyrum esculentum	x (+)	x (+)	x (+)		x (+)		
Lobularia maritima		x (+)	x (+)				
Foeniculum vulgare							
Achillea millefolium				NA	NA	NA	NA
Brassica juncea				NA	NA	NA	NA
Trifolium hirtum				NA	NA	NA	NA
Lavandula angustfolia	x (+)	x (+)	x (+)	NA	NA	NA	NA
Coriandrum sativum				NA	NA	NA	NA

Chapter 3:
Evaluating the Influence of Floral Resource Provisioning and Pheromone-based Mating Disruption on Biological Control of *Planococcus* Mealybug (Hemiptera: Pseudococcidae) in California Vineyards

Abstract:
Conservation biological control aims to enhance arthropod pest regulation through managing environmental conditions to enhance the effectiveness the natural enemies such as parasitoids and predators. Laboratory studies have demonstrated that the provisioning of floral resources can enhance the survival probability of the parasitoid, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encritidae), a key natural enemy of invasive vine mealy bug, *Planococcus* ficus (Signoret) (Hemiptera: Pseudococcidae). Previous studies have also shown that pheromone-based mating disruption and inoculative releases of *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Cocccinellidae) can increase predation rates while reducing vine mealybug (VMB) densities and crop damage in California vineyards. The 2-year study measured the effect of floral resources provisioning (FRP), pheromone-based mating disruption (PBMD) and augmentative releases of mealybug destroyer (*C. montrouzieri*) on biological control of VMB. A combination of fall planted Lacey phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*) and wild carrot (*Daucus carota*) were used as alley groundcovers providing season-long floral resources. Results show that FRP resulted in no significant differences on parasitism rates of sentinel VMB, overall VMB densities or crop damage in 2009. In 2010, leaf densities of VMB were found to be significantly lower in FRP plots at harvest time (September). FRP was shown to reduce late summer (August) VMB density over that of pheromone-based mating disruption. However, a lower mean rate of parasitism was measured in the FRP treatment plots in late summer of 2010. No significant difference in crop damage levels were found between FRP and control plots in 2009 or 2010. The relevance of these results for enhancing biological control of VMB in California vineyards is discussed.

Keywords:
Floral resource provisioning; conservation biological control; pheromone-based mating disruption*; Planococcus* mealybug; Hemiptera; parasitoid nectar feeding; *Anagyrus pseudococci*; *Cryptolaemus montrouzieri*; parasitism; Hymenoptera; viticulture; biodiversity; agroecosystems.

1. Introduction:

Conservation biological control aims to enhance the effectiveness of arthropod natural enemies by managing environmental conditions to provide otherwise limiting resources such as refuge, favorable microclimates, overwintering sites, alternative hosts or prey, and plant-based foods such as pollen or nectar (Altieri and Nicholls 2004, New 2005, Bukovinszky et al. 2012, Chaplin-Kramer et al. 2012). Farming system bio-diversification, at the field or landscape scale, is increasingly recognized as a scientifically underdeveloped but potentially important strategy for enhancing biological control of arthropod pests while enhancing the provisioning of multiple ecosystem services to and from agroecosystems (Tscharntke et al. 2007, Fiedler et al. 2008, Crowder et al. 2010, Campbell et al. 2012, Kremen and Miles 2012, Tomkins et al. 2012).

Many species of natural enemies of arthropod pests feed on non-host food, including floral resources (Kehrli and Bacher 2008, Harvey et al. 2012). Nectar feeding has been shown to enhance the reproductive success of natural enemies such as parasitoids and hoverflies through enhanced rate of egg maturation, prolonged reproductive longevity, or both (Wäckers et al. 2005, Lee and Heimpel 2008, Sivinski et al. 2011). Habitat management via floral resource provisioning (FRP) has been demonstrated to enhance the abundance and diversity of arthropod natural enemies (Landis et al. 2000), increase rates of predation and parasitism (Heimple and Jervis 2005), and decrease pest densities and crop damage in multiple cropping systems (Johnson et al. 2010, Letourneau et al. 2011, Hogg et al. 2011).

Pheromone-based mating disruption (PBMD) is increasingly utilized as a commercially viable strategy for pest management programs in many cropping systems (Welter et al. 2005, Witzgall et al. 2010). Prior studies in California vineyards have demonstrated that PBMD can reduce the reproductive success, population densities and crop damage from vine mealybug, an internationally important wine grape pest (Walton et al. 2006, Daane et al. 2008).

The invasive vine mealy bug (VMB), *Planococcus* ficus (Hemiptera: Pseudococcidae), is a key grape vine pest throughout the Mediterranean regions of the world (Daane et al. 2012). VMB honeydew excretion, which supports sooty mold growth, can compromise both harvest quality and quantity leading to substantial economic losses for producers (Daane et al. 2008). Also serving also a key vector of grapevine leaf roll viruses, VMB can be an economically damaging pest even at low population densities (Tsai et al. 2010, Daane et al. 2012). Conventional insecticides used for the control of VMB are frequently inadequate, drive the development of genetic resistance to pesticides, disrupt the actions of natural enemies, and pose a significant risk to non-target organisms and human health (Barron et al. 1995, Daane et al. 2006, Bostanian et al. 2012, Horton et al. 2012).

Anagyrus pseudococci (Giralt) (Hymenoptera: Encritidae) and *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Cocccinellidae) are two of the most effective, widely distributed and commercially available natural enemies of VMB, holding much potential for enhancing biological control in vineyards (Daane et al. 2006, Daane et al. 2012, Walton et al. 2012).

The 2-year study measured the impact of FRP, PBMD and one augmentative release of mealybug destroyer (*C. montrouzieri*) on biological control of VMB in order to evaluate their potential for use in managing VMB in California and other Mediterranean climate vineyards. Previous laboratory studies demonstrated that *A. pseudococci* can feed on a variety of plant nectar sources and that exposure to some FRP species increased survival probability (Miles et al. in preparation). The objective of the study was to measure the impact of FRP, pheromone-based mating disruption and inoculative releases of *C. montrouzieri* on VMB density, parasitism rate and crop damage at harvest time. The research aims to advance scientific knowledge of key ecological factors influencing population dynamics at the field level, while evaluating practical strategies for biological control of VMB in Mediterranean-climate vineyards.

2. Methods and materials:

Research site: All research was conducted over a 2-year period (2009-2010) at the UC Kearney Agriculture Research Station in Parlier, California, San Joaquin Valley (36° 36' 42" N / 119° 31' 34" W). The region is characterized by a Mediterranean climate with hot, dry summers (mean July temperature of 37°C) and mild winters. Average annual precipitation is 269 mm, falling largely between October-April. The research vineyard was a 50-year-old, 1.2-ha block of *Vitis vinifera* L. (cultivar Thompson Seedless) planted in a Hanford sandy loam soil. Vine spacing was 3.6 m between rows and 2.4 m within each row. Treatments were organized in a randomized block design with all treatment and control plots separated by a 1-vine buffer (no spray, mowed resident weedy vegetation). Individual treatment (FRP) and control plots (no spray, mowed resident weedy vegetation) were comprised of 7 vine rows x 40 vines long each totaling 280 vines. There were a total of 3 replicates (see Appendix D. for diagrams).

Sources and methods for plant materials: Bishop's weed (Apiaceae*: Ammi majus*) was purchased from Le Ballister's Seed and Fertilizers, Santa Rosa, CA. Lacey phacelia (Boraginaceae: *Phacelia tanacetifolia*), was purchased from Turtle Tree Seed Company,□Copake, New York. Wild carrot (Apiaceae*: Daucus carota*) was wild collected from Napa County, Ca. In preparation for seeding, the vineyard was irrigated, disked and rototilled. Seed was sown at the rate of 2.25 kg/ha (Phacelia and Ammi) and .55 kg/ha (Daucus) using broadcast seeder. Seed was covered using a mechanical ring roller. Vineyard alleyway were flood irrigated every 10 days. The sequence and bloom period for flowing plants listed above were the following: lacey phacelia (February 15th – April), bishop's weed (May 15th - July 15th) and wild carrot (July 15th – September). (See Appendix E. and F. for illustrations.)

Sources of the insect predator *Cryptolaemus montrouzieri*: *C. montrouzieri* used for inoculative release were purchased from Sterling Insectary, Delano CA. *Cryptolaemus montrouzieri* were released on July 15th at a rate of 250 individual per treatment and control area (500 per replicate x 3 replicates = 1500 total). The release of *Cryptolaemus montrouzieri* coincided with wild carrot reaching 75% of full bloom (see Appendix for image of species phenology).

Sources and methods for sentinel VMB parasitism study: Colonies of VMB were raised under controlled laboratory conditions on acorn squash, *Cucurbita pepo* (Cucurbitaceae) at the UC Kearney Agriculture Research Station. Squash and potatoes were cleaned in a 0.5% bleach solution to reduce mold growth, and then triple rinsed in water. Squash were inoculated with ~100 1st-2nd instar VMB and held at a constant temperature of 27 ± 2 °C, 40% relative humidity, and 16:8 photoperiod until introduce into field. 8 squash, each inoculated with approximately 100 2-3rd instar VMB, were placed in lidless, white plastic buckets (20 OD x 15cm) with two screened ventilation holes (5cm) and individually suspended from the interior of 8 randomly selected vines (n=48) in each treatment and control plot for 7 days (168 hr) of exposure to parasitism by *A. pseudococci.* After the 7 day exposure period (June 16th- June 23), VMB-infested squash were collected and incubated for an additional 20 days under controlled conditions (27 ± 2 °C, 40% relative humidity, and 16:8 photoperiod), at which time they were removed, frozen to -18 °C to arrest development, and the number of parasitized VMB tabulated. VMB were examined under the dissecting scope where tallies of parasitized and non-parasitized VMB were made. The parasitism experiment was conducted once per year.

Protocols for natural enemy observation study: To qualify and quantify predation and parasitism events by natural enemies of VMB, a 24-hr natural enemy observation study was conducted during the VMB parasitism study (described above). 30-second observations of all 8 buckets containing the VMB-inoculated acorn squash per plot were conducted every 3 hours for a 24-hour period. During the 30-second observation, all predators and parasitoids found ovipositing, actively feeding on, actively inspecting, or searching nearby the sentinel VMB were identified and tabulated. There were a total of 5 replicates.

Sampling and processing for measuring VMB density and crop damage at harvest: Using a 1-minute visual categorical rating protocol outlined by Walton et al. (2006), 30 vines per block (15 treatment: 15 control) were selected at random and sampled for one-minute (counting everywhere on the vine). VMB were identified using field diagnostic characters developed by Godfrey et al. (2002). Crop damage was assessed at harvest time (September 1) following the categorical VMB density and grape cluster damage rating protocols outlined by Geiger and Daane (2001).

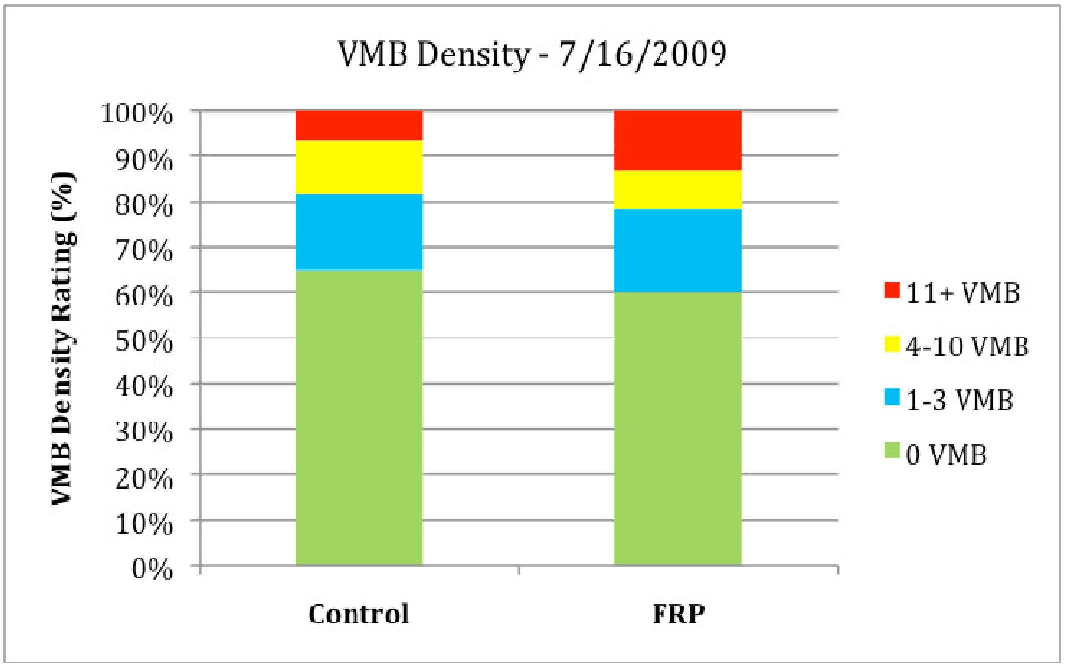
Sampling and processing: pheromone-based mating disruption: In each of the three blocks, twelve 3-vine sub-plots were established (6 in each treatment and 6 in each control plot) with half of the sub-plots receiving a single microencapsulated pheromone dispenser (Suterra Crop Protection, Bend, Ore) and half not (control). To assess density and parasitism rates of VMB by *A. pseudococci*, 6 leaves per 3-vine plot were sampled, taking 2 basal leaves from each vine in July, August, September and November. Leaf density of VMB were tabulated in the field. Sampled leaves were harvested and VMB were examined under the dissecting scope where tallies of parasitized and non-parasitized VMB were made.

Experimental design: FRP research was organized in a randomized block design with all treatment and control plots separated by a 1-vine buffer (no spray, mowed resident weedy vegetation). Individual treatment and control plots were comprised of 7 vine rows x 40 vines long each consisting of 280 vines (total = 1,680 vines). There were a total of 3 replicates (see Appendix). The PBMD study was set in a randomized block design, with a split plot to block against individual plots in each row being too close to each other.

Statistical analysis: *A. pseudococci* parasitism rates of sentinel VMB were evaluated using ANOVA. Parasitism rates were arcsine-square-root transformed prior to analysis. Treatment influence on VMB density, as measured by the rating scale, was summarized in a 2 x 2 contingency table and Fisher's exact test was used to determine differences in VMB density levels. Treatment influence on grape cluster damage at harvest, as measured by a damage rating scale (Walton et al. 2006), were compared in a 2 x 2 contingency table. Fisher's Exact Test was used to determine differences in mean crop damage levels. To analyze data from the pheromone-based mating disruption study, a general linear model was used with date ('j-day') set as a categorical variable. Data was square root transformed prior to analysis.

3. Results:

Vine mealy bug density was assessed on two separate dates in 2009 (July 16th and October 21st) to determine the density of VMB in treatment (FRP) and control plots through time. Our analysis showed that VMB densities were not significantly different between FRP and Control plots on either 7/16/2009 ($P = 0.6984$) or 10/22/2009 ($P = 1$) (Fig. 1).



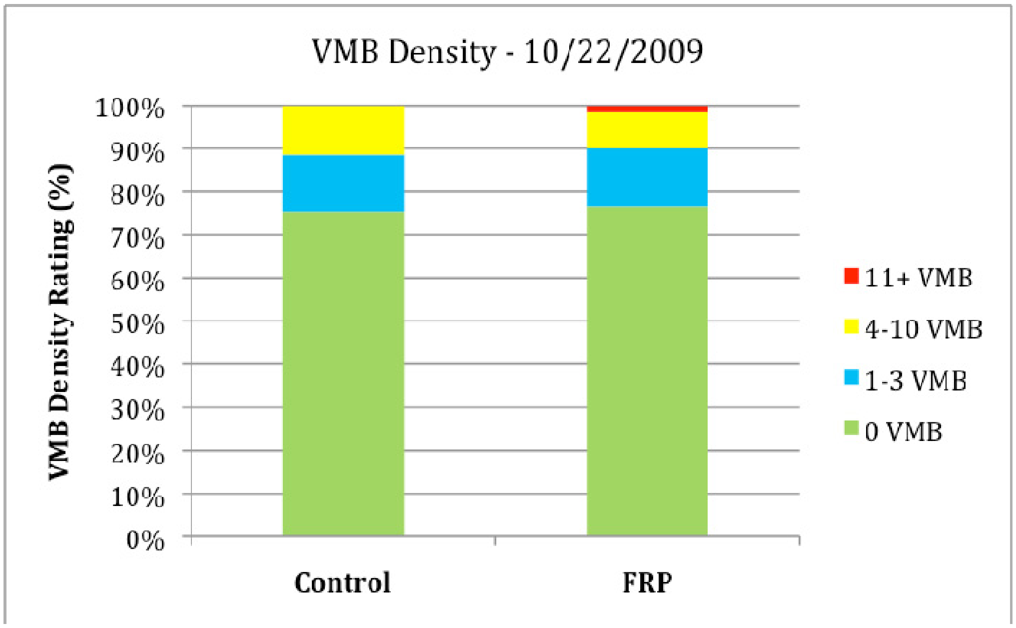


Figure 1. Vine mealybug density in treatment (FRP) and control plots (July and Oct. 2009) as measured by a categorical rating scale.

Parasitism rates of sentinel VMB by *A. pseudococci* (2009): Sentinel VMB were used to measure the impact of FRP on the rate the parasitism by *A. pseudococci* and thus the potential to enhance biological control of VMB through on-farm habitat management techniques. Results of the experiment indicate that there was no significant difference in parasitism rates of VMB by *A. pseudococci* between treatment (FRP) and control plots in 2009 (F = 0.007, $DF_{1,8}$ P = 0.933) (Fig. 2, Appendix Table 1).

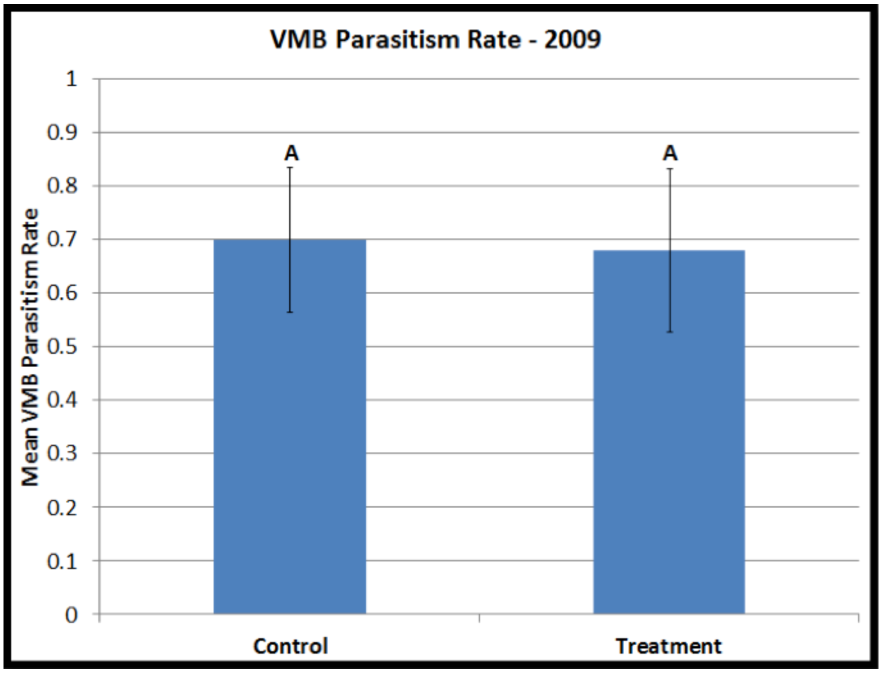


Figure 2: Data from 2009: Parasitism of sentinel VMB by *A. pseudococci* (2009). Results from the study indicate that there was no significant difference in parasitism rates between treatment (FRP) and control plots (F = 0.007, $DF_{1,8}$ P = 0.933).

Natural enemy observation study: The 24-hour observation study resulted in 95 total observations of natural enemies ovipositing, actively feeding on, actively inspecting, or nearby/searching the sentinel VMB. Of 95 observations of natural enemies, 86.32% were female *A. pseudococci,* 5.26% were other parasitoids, and 6.32% were spiders. 2.10% were male *A. pseudococci* (see Appendix Table 2).

Leaf density and parasitism rate of VMB by *A. pseudococci:* FRP and pheromone-based mating disruption (2010): The findings of the study show that leaf densities of VMB were significantly lower in FRP plots than the control at harvest time (September) (Fig. 2 a.). However, when contrasted to the control, a significantly lower mean rate of parasitism of VMB was measured in the FRP plots in late summer (August) (Fig. 2 a.). Pheromone-based mating disruption had no significant impact on either VMB densities or parasitism rate when contrasted with the control (Fig. 2 b.). FRP plots were shown to have lower mean harvest period (September) VMB leaf densities when contrasted with the PBMD plots (Fig. 3).

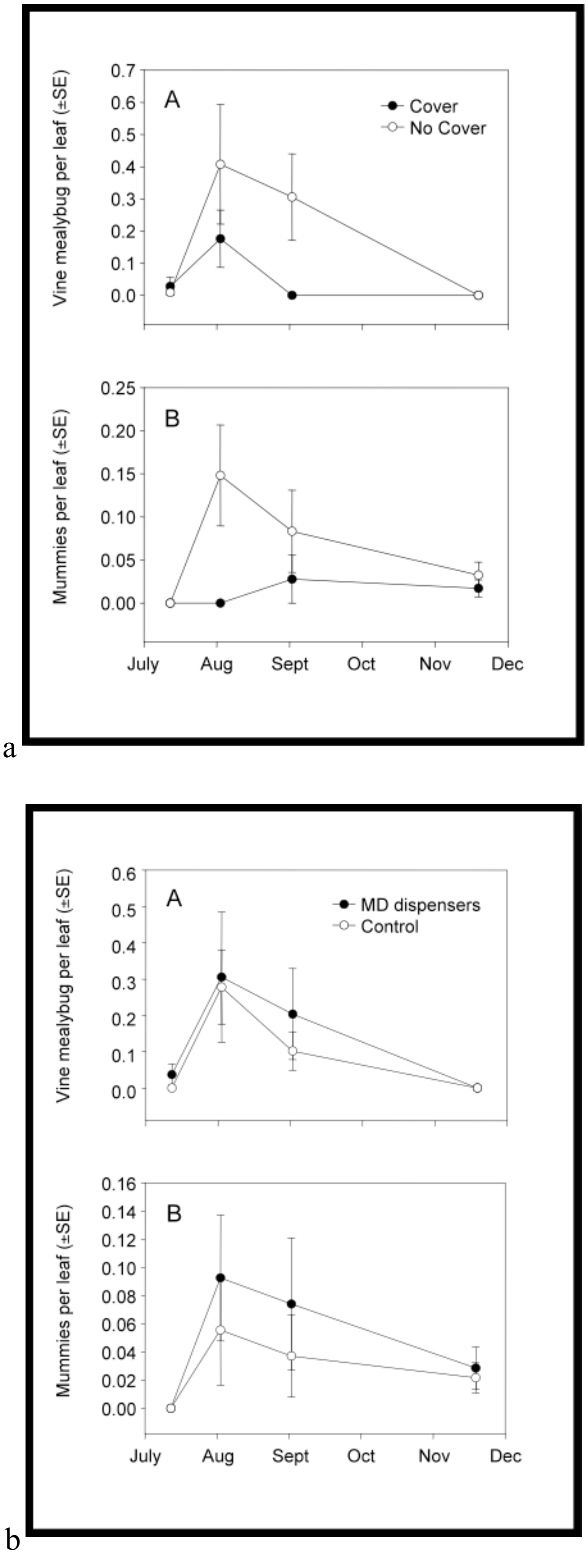


Figure 3: Data from 2010: (a) Vine mealybug leaf density and parasitism rates by *A. pseudococci* in FRP (cover) and control plots. (b) Vine mealybug leaf density and parasitism rate by *A. pseudococci* in pheromone-based mating disruption and control plots.

VMB crop damage assessments at harvest: Comparisons of harvest period VMB density and crop damage were conducted in September 17th, 2009 and September 29th, 2010 using the categorical VMB density and grape cluster damage rating protocols outlined by Geiger and Daane (2001). Treatment influence on grape cluster damage at harvest were compared in a 2 x 2 contingency table. Fisher's Exact Test was used to determine differences in mean crop damage levels. VMB crop damage levels were not significantly different between FRP and control plots in either 2009 ($P = 0.5808$) or 2010 ($P = 1$) (Fig. 4).

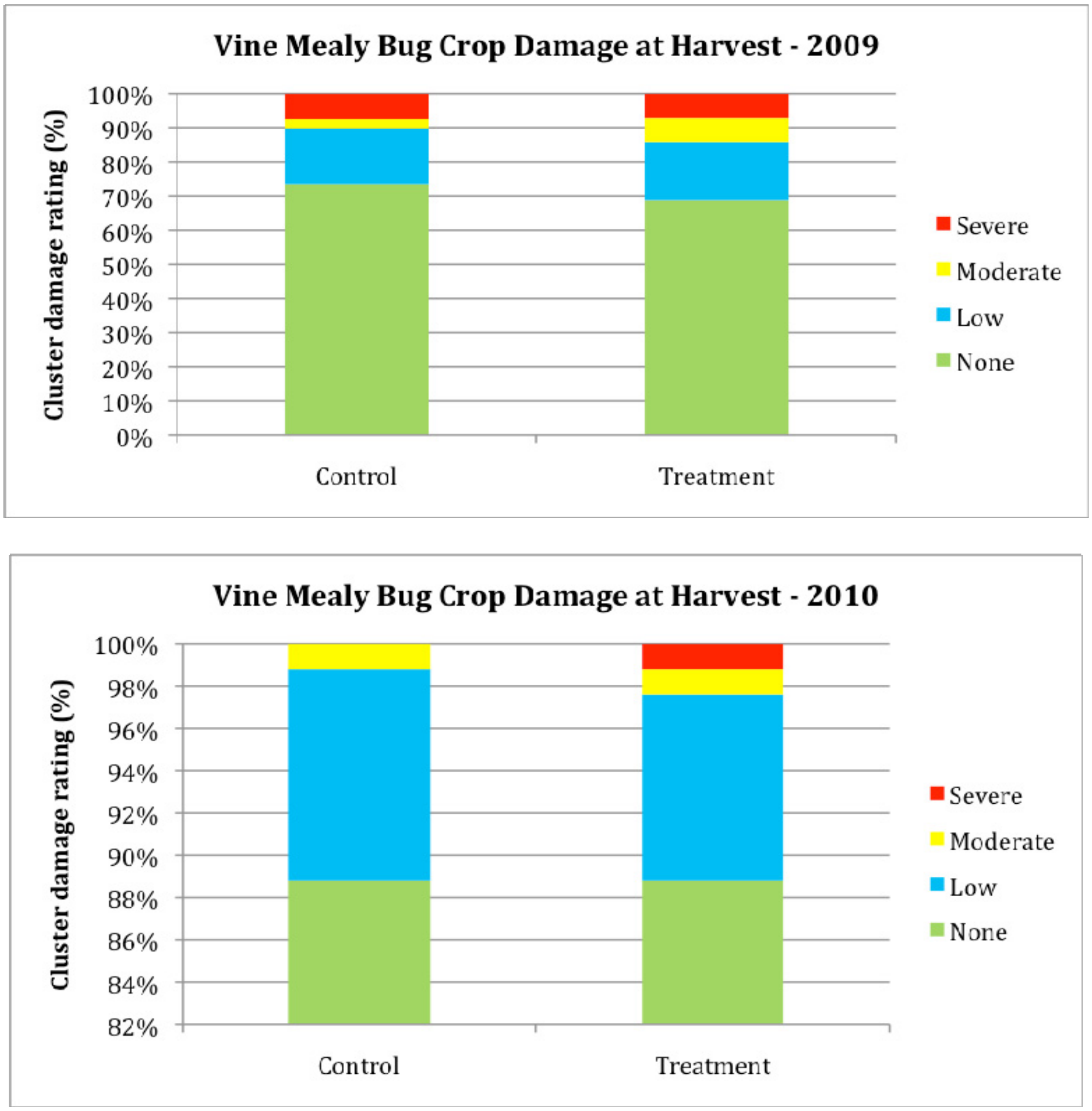


Figure 4: Vine mealybug crop damage at harvest (2009-2010): Vine mealy bug damage levels were not significantly different between FRP and control plots in 2009 ($P = 0.5808$) or 2010 ($P = 1$).

4. Discussion:

The present study provides evidence that FRP using lacey phacelia, bishop's weed and wild carrot can reduce harvest-period (September) VMB densities in San Joaquin Valley vineyards. However, given the low levels of parasitism by *A. pseudococci* observed in the treatment plots, the mechanism by which VMD density was reduced is not clear. Prior work on the VMB-predator system indicates that predation by *C. montrouzieri* or spiders may be, in part, responsible for lower VMB densities in FRP plots (Daane et al. 2008, Gutierrez et al. 2008). This conclusion, however, was not supported by a 24-hr predator observation study, finding no *C. montrouzieri*, a specialized predator of VMB. Spiders were found at very low density (6.32% of all natural enemies observed) and are assumed to play a secondary role behind that of specialist predators and parasitoids (Daane et al. 2008, Miles and Wilson unpublished data).

The study showed that FRP reduced late summer vine mealybug density over that of pheromone-based mating disruption, indicating that habitat management through FRP could be a potentially important complementary biological control strategy for VMB (Walton et al. 2012).

As no significant differences in crop damage at harvest time were detected in either 2009 or 2010, the hypothesis that FRP would lead to significantly lower levels of crop damage through enhanced biological control of VMB was not statistically supported in this study. These findings are consistent with the larger body of research showing that biological control of vine mealybug is only partially successful and that additional conservation biological control research is necessary to identify more effective natural enemies and enhance their control potential (Gutierrez et al. 2008, Daane et al. 2012, Wäckers and van Rijn 2012, Walton et al. 2012).

Acknowledgements:

The authors would like to sincerely thank the following individuals and institutions for their generous support of this research: The California Department of Food and Agriculture (CDFA); the Organic Farming Research Foundation (OFRF); The United States Environmental Protection Agency (EPA); the many UC Berkeley undergraduate research interns; collaborating growers and their firms; California Sustainable Wine Growers Group; UC Kearney Agriculture Research Center staff and researchers, including Glenn Yokota and Dale Pattigan; staff and research assistants with the UC Berkeley Natural resources lab, Korry Kassir and John Hutchins; and the Robert van den Bosch Scholarship for providing research scholarships for Albie Miles.

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Appendix:

A. Example of floral resources provisioning treatment using wild carrot (*Daucus carota*), University of California Kearney Agriculture Research and Extension Center 2010.

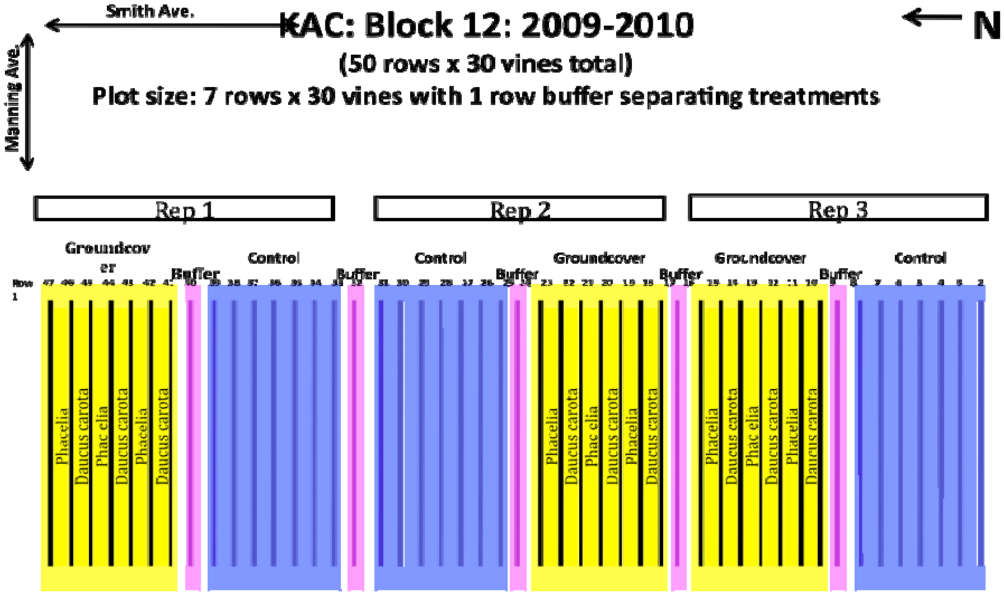
B. Table 1: Data from 2009: Mean parasitism rate of sentinel VMB by *A. pseudococci*. Results of the study indicate that there were no significant difference in parasitism rates between treatment and control plots (F = 0.007, $DF_{1,8}$ P = 0.933).

Plot	**n**	**Avg. Parasitism Rate ±SE**
Treatment	5	0.68 ±0.15 a
Control	5	0.70 ±0.14 a

C. Table 2: Data from 2009: 24-hr natural enemy observation study, UC Kearny Agriculture Research and Extension Center (July 23-24, 2009):

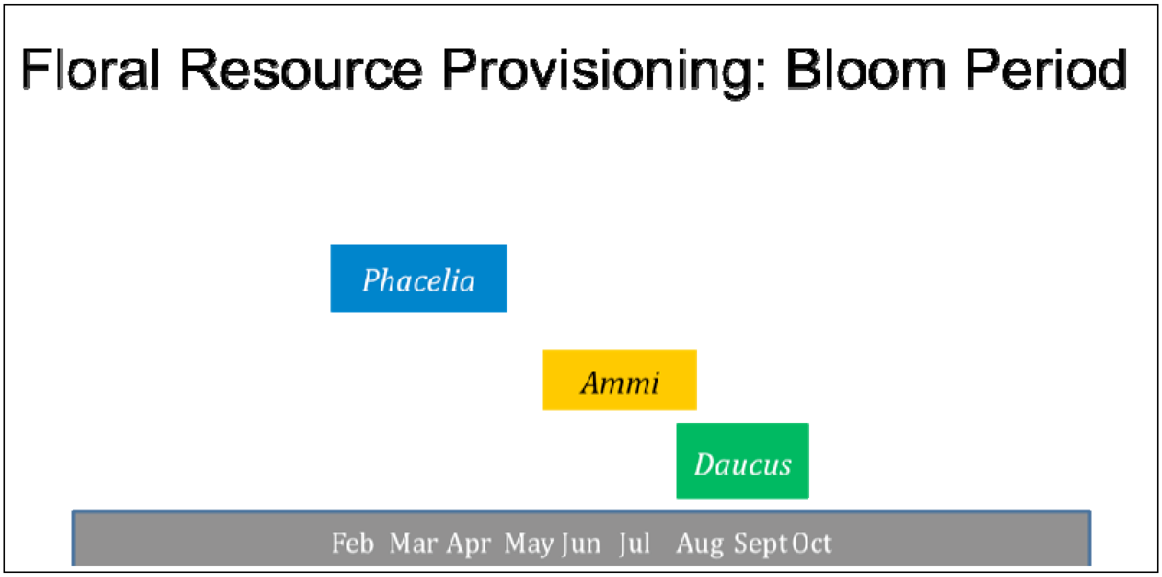
Natural Enemy	Count	Percentage
Female *A. pseudococci*	82	86.32%
Male *A. pseudococci*	2	2.10%
Other parasitoids	5	5.26%
Spiders	6	6.32%
Total	95	100.00%

D. Figure: Diagram of research design used in the study of floral resource provisioning at the University of California Kearney Agriculture Research and Extension Center 2009-2010. Plots were randomized each year.

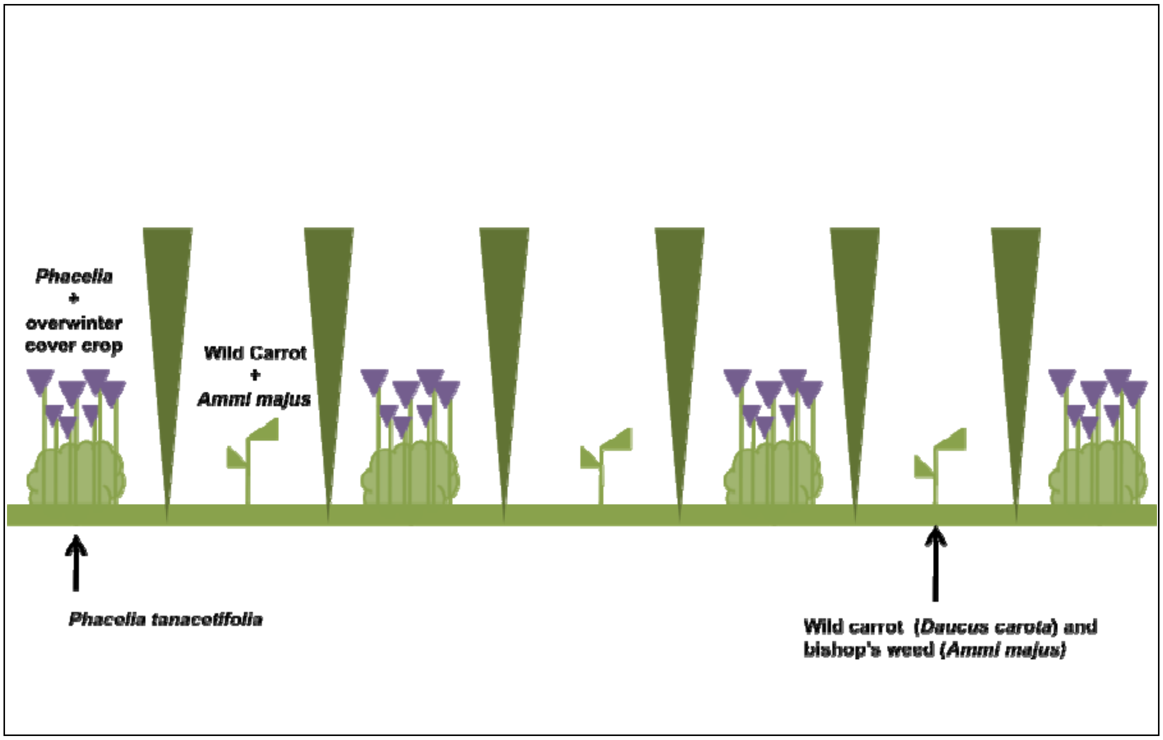


**In "Groundcover" treatment , cane prunings should only be thrown into row-middles where *Daucus carota* was sown.

E. Figure: Floral Resource Provisioning: species phenology 2009-2010:



F. Figure: Floral Resource Provisioning: species placement in plots 2009-2010:



Biodiversity and Ecosystem Services in Agriculture: Evaluating the Influence of Floral Resource Provisioning on Biological Control of *Erythroneura* Leafhoppers (Hemiptera: Cicadellidae) and *Planococcus* Mealybug (Hemiptera: Pseudococcidae) in California Vineyards

Chapter 4:

'Attract and Reward': Measuring the Impact of Floral Resource Provisioning and Methyl Salicylate (MeSA) on Biological Control of *Erythroneura* Leafhoppers in California Vineyards

Abstract:

Conservation biological control aims to enhance arthropod pest regulation through managing environmental conditions that maximize the effectiveness of natural enemies such as parasitoids and predators. The study sought to evaluate the impact of an "attract and reward" strategy for biological control of *Erythroneura* leafhoppers in California vineyards. In large-scale field trials, PredaLure ®, a methyl salicylate (MeSA) attractant, was combined with floral resource provisioning (FRP) to test the synergistic impacts on biological control. It is theorized that attract and reward systems enhance biological control through increased rates of predation or parasitism resulting from increased residency, enhanced abundance or diversity of natural enemies, and/or improved natural enemy fitness. Separate 2-year field studies were conducted between 2009-2011. The first experiment, conducted in 2009 – 2010, measured the impact of PredaLure ® as a sole treatment on the abundance of a key parasitoid natural enemy, *Anagrus spp.*, and *Erythroneura* leafhopper densities. The second experiment conducted in 2010 - 2011 combined PredaLure ® with a fall-planted ground cover of Lacey phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*) and wild carrot (*Daucus carota*) providing season-long floral resources. Key questions we sought to answer were the following: Does MeSA treatments alone impact *Anagrus spp.* abundance and leafhopper density? Does 'attract and reward' impact the density and parasitism rates of leafhoppers by *Anagrus* wasps leading to reduce leafhoppers densities? Results from the Experiment # 1 indicate that there was no significant impact of the PredaLure ® (MeSA) treatment alone on biological control of *Erythroneura* leafhoppers. Our analysis found no evidence supporting Predalure has any significant and meaningful impact on female *Anagrus spp.,* leafhopper nymphs, or adult leafhopper densities at harvest time in either 2009 or 2010. The overall findings of Experiment #2, the "attract and reward" study, indicate that the combination of floral resource provisioning (FRP) and PredaLure ® (MeSA) had no significant impact on western grape leafhopper nymph density or leafhopper egg parasitism rate by *Anagrus spp.* in 2010 or 2011. The relevance of these results for conservation biological control of *Erythroneura* leafhoppers in California vineyards is discussed.

Keywords:

Attract and reward; floral resource provisioning; methyl salicylate; conservation biological control; herbivore-induced plant volatiles; pest management; vineyards; *Anagrus* wasps; *Erythroneura* leafhoppers; *Ammi majus; Dacus carota; Phacelia tanecetifolia.*

1. Introduction:

Conservation biological control aims to enhance natural pest regulation through managing environmental conditions that enhance the fitness and effectiveness of arthropod natural enemies (Barbosa 1998, Altieri and Nicholls 2004, Wäckers and van Rijn 2012). Biological diversification of farming systems, including the provisioning of plant-based foods such as pollen or nectar, is considered an important strategy for enhancing natural pest control while potentially enhancing a range of ecosystem services to and from agriculture, including the conservation of pollinators, soil quality enhancement and carbon sequestration, among others (Lewis et al. 1997, Gurr et al. 2003, Power 2010, Kremen and Miles 2012, Tomkins et al. 2012).

Many species of natural enemies of arthropod pests are known to feed on non-host food, including floral resources such as nectar and pollen (Kehrli and Bacher 2008, Carrié et al. 2012, Harvey et al. 2012). Nectar feeding has been shown to enhance the reproductive success of natural enemies such as parasitoids and hover flies through enhanced rate of egg maturation, prolonged reproductive longevity, or both (Wäckers et al. 2005, Lee and Heimpel 2008, Sivinski et al. 2011). Habitat management via floral resource provisioning (FRP) has been shown to enhance the diversity and abundance of arthropod natural enemies (Landis et al. 2000, Bennet et al. 2013), increase rates of predation and parasitism (Geneau et al. 2012), and decrease pest densities and crop damage in multiple cropping systems (Jacometti et al. 2010, Johnson et al. 2010, Letourneau et al. 2011, Hogg et al. 2011). However, given the mixed results from field trials of floral resource provisioning studies, additional research is necessary to better define and manage non-crop vegetation that serves to consistently reduce crop damage (Lewis et al. 1997, Gurr et al. 2000, Lavendero et al. 2006, Jonnson et al. 2010, Winkler et al. 2010, Carrié et al. 2012).

Many crop plants have been recorded to emit a range of semiochemicals that attract natural enemies in response to herbivore damage (Khan et al. 2008, Simpson et al. 2011a, Simpson et al. 2013, Meiners and Peri 2013). Herbivore induced plant volatile compounds (HIPVs) are a form of induced plant defense that can influence the abundance, diversity and behavior of both natural enemies and herbivore pests (Orre et al. 2010, 2012). The synthesis and deployment of semiochemicals (HIPVs) is considered a promising new strategy for attracting or retaining natural enemies and enhancing natural pest regulation in cropping systems (Gurr and Kvedaras 2010, Colazza and Wajnburg 2013, Colazza and Cusumano 2013).

Research on the effect of MeSA on biological control potential in vineyards has demonstrated that grapevines baited with controlled release dispensers of MeSA (PredaLure ®) served to recruit significantly larger populations of *Anagrus spp.* wasps, a key natural enemy of *Erythroneura* leafhoppers (James and Grassmitz 2005). Further, a 2011 meta-analysis of 14 prior studies (91 observations) measuring the impact of MeSA (PredaLure ®) on natural enemies concluded that there was no systematic difference in the magnitude of attraction to MeSA when comparing predators and parasitoid groups (Rodriguez-Saona et al. 2011). Despite strong evidence that insect predators and parasitoids are attracted to MeSA in agroecosystems, how best to deploy this strategy to successfully enhance biological control in specific cropping systems remains unclear (Rodriguez-Saona et al. 2011, Kaplan 2012).

The combining of semiochemicals and floral resource provisioning (i.e. "attract and reward") has been theorized as an effective way to enhance biological control in many cropping systems (Jonsson et al. 2008, Khan et al. 2008, Simpson et al. 2011b). Density dependence and the resultant asynchronous colonization of agroecosystems by pest and beneficial insects are considered a key ecological reason for why biological control of arthropod pest is difficult to achieve in some cropping systems (Kaplan 2012). In "attract and reward" systems, natural enemies of arthropod pest are theorized to be attracted to the cropping system earlier, or in greater abundance or diversity, in response to the release of anthropogenic semiochemicals that mimic the chemical signals of crops damaged by herbivores (Gadino et al. 2012). It is further theorized that once attracted to the system, the residence time of predators and parasitoids is increased as a result of the presence of a floral reward (Orr et al. 2010). The potential combination of increased early abundance, diversity, fitness and residence time of natural enemies is thought to result in higher rates of predation and parasitism, thus increased biological control of pests (Orre et al. 2012, Simpson and Gurr 2013, Simpson et al. 2013).

The present study assessed the impact of a specific 'attract and reward' strategy for enhancing biological control of the western grape leafhopper, *Erythroneura elegantula* Osborn (Hemiptera: Cicadellidae). *Erythroneura* leafhoppers are important herbivores of *Vitis spp.,* including both wild and cultivated grapes in California (Segoli and Rosenheim 2013). Two separate 2-year field studies were conducted between 2009-2011. The first study measured the impact of PredaLure ® (AgBio Inc., Westminster, CO, USA), a MeSA-based attractant on the abundance of the parasitoid *Anagrus spp.* (Hymenoptera: Mymaridae) and leafhoppers. The second study combined PredaLure ® with a fall-planted ground cover of Lacey phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*) and wild carrot (*Daucus carota*), providing both an attractant and season-long floral resource "reward". Key questions we sought to answer through the study were the following: does PredaLure ® alone impact key natural enemy abundance and leafhopper density? Does 'attract and reward' impact the density and parasitism rates of leafhoppers by *Anagrus* wasps leading to reduced leafhoppers densities?

2. Methods and materials:

Research sites: Two separate research sites were used in the study. Experiment #1, the PredaLure ® only research, was conducted over a 2-year period (2009-2010) in Napa, California (38° 23' 40" N / 122° 19' 33" W). The region is characterized by a Mediterranean climate with hot, dry summers (mean July temperature of 28.3°C) and mild, wet winters. Average annual precipitation is 70.9 cm, falling largely between October-April. The research vineyard was a 12-year-old, 8.3-ha block of *Vitis vinifera* L. (cultivar Cabernet Sauvignon) planted in a Bale clay loam soil. Vine spacing was 1.8 m between rows and 1.5 m within each row. Treatments were organized in a randomized block design with all treatment and control plots separated by a 24-row (45 m) buffer (no spray, tilled resident weedy vegetation). Individual treatment (PredaLure ®/MeSA) and control plots (no spray, tilled resident weedy vegetation) were 12 vine rows wide x 125 vines long totaling 1,452 vines. 90-day PredaLure ® (5 gm dispensers, AgBio Inc., Westminster, CO, USA) were equidistantly distributed in the middle 62 vines of the treatment plots at a rate of 75 lures/acre (185 lures/ha). PredaLure ® dispensers were suspended from the head of each vine at a height of ~1 m. PredaLure ® was placed in the vine canopy at bud break on April 15th and were replaced with new lures after 90 days on July 15th. The sampling area was isolated to the center 62 vines and 5 middle vine rows (.20 ac). The application rate of 185 lures/ha is 2.5 times the recommended application rate of ~74.1 lures/ha. There were a total of 3 replicates (Appendix B.).

Experiment #2, the "attract and reward" research, was conducted over a 2-year period in Acampo (2010) and Elk Grove (2011), California. The respective coordinates for the 2 research site are the following: 38° 11' 26" N/121° 9' 6" W; 38° 25' 27" N/122° 15' 41" W. The region is characterized by a Mediterranean climate with hot, dry summers (mean July temperature of 33.8°C) and mild, wet winters. Average annual precipitation is 51.8 cm, falling largely between October-April. In 2009, the research vineyard was comprised of 36-year old, 16.9-ha block of *Vitis vinifera* L (cultivar Zinfandel) planted in a Ramoth sandy loam soil. Vines were planted at 11'x 7' spacing (566 vines/acre). In 2010, the research vineyard was comprised of a 12-year-old, 9.1-ha block of *Vitis vinifera* L. (cultivar Sauvignon Blanc) planted in a Columbia sandy loam soil. Treatments were organized in a randomized block design with all treatment and control plots separated by a 5-vine buffer (no spray, tilled resident weedy vegetation). Individual treatment and control plots (no spray, tilled resident weedy vegetation) were comprised of 12 vine rows x 93 vines long, each totaling 1,116 vines. Vines were planted at 11'x 7' spacing (566 vines/acre). There were a total of 4 replicates each year.

Plant material for Experiment #2: Bishop's weed (Apiaceae*: Ammi majus*) was purchased from Le Ballister's Seed and Fertilizers, Santa Rosa, CA. Lacey phacelia (Boraginaceae: *Phacelia tanacetifolia*), was purchased from Turtle Tree Seed Co.,Copake, New York. Wild carrot (Apiaceae*: Daucus carota*) was wild collected from Napa County, Ca. In preparation for seeding in the fall, the vineyard was disked and rototilled. Seed was sown at the rate of 2.25 kg/ha (Phacelia and Ammi) and .55 kg/ha (Daucus) using broadcast seeder into the middle 1 m of each alley way. Seed was covered using a mechanical ring roller. No supplemental irrigation was provided. All flowering ground cover plants were flail mowed following senescence.

Measuring leafhopper nymph densities (Experiments #1 and #2): *Erythroneura* leafhopper nymph densities were assessed by counting the total number of nymphs found on leaf samples throughout the year. 60 large grape leaves per plot were sampled approximately every 3 weeks on multiple dates each year, including before, near and after the anticipated peak density of each leafhopper generation. Leaves were randomly sampled from each plot selecting leaves from nodes 1-3 and 4-6 in the first and second leafhopper generation, respectively following protocols outlined by Costello and Daane (2003).

Measuring adult leafhopper, seasonal female *Anagrus spp.* and natural enemy density (Experiments #1 and #2): Adult leafhopper, female *Anagrus spp.* and natural enemy densities were sampled approximately every 3 weeks throughout the season using 5 randomly placed yellow sticky traps (Seabright Laboratories, Emeryville, Ca) per plot (10 per block). Traps were suspended in the interior of the vine canopy for 14 days. Yellow sticky traps were then collected and the insects were identified and tallied using dissecting microscopes and visual identification characteristics described for western grape leafhoppers by UC IPM (2013), and *Anagrus spp.* described by Triapitsyn et al. (2010). There were 5-9 separate sample dates per season.

Sampling and processing for PredaLure ® sub-plots (Experiment #2): In each of the 4 blocks, two 30-vine sub-plots (3 rows x 10 vines) were established (1 in each treatment and control plot). Each PredaLure ® sub-plot received 1 PredaLure ® (90-day 5 gm dispensers, AgBio Inc., Westminster, CO, USA). PredaLures ® were suspended from the head of each vine at a height of ~1 m on approximately July 10th of each year for 90 days. Each PredaLure subplot was 1,540 square feet (143 m^2) in size. Recommended application rate of PredaLure ® = 1/7,500 square feet (1/696 m^2). Actual application rate in sample areas was equal to 4.86 times the recommended application rate. Leafhopper and natural enemy sampling protocols were identical to those described above. There were a total of 4 replicates.

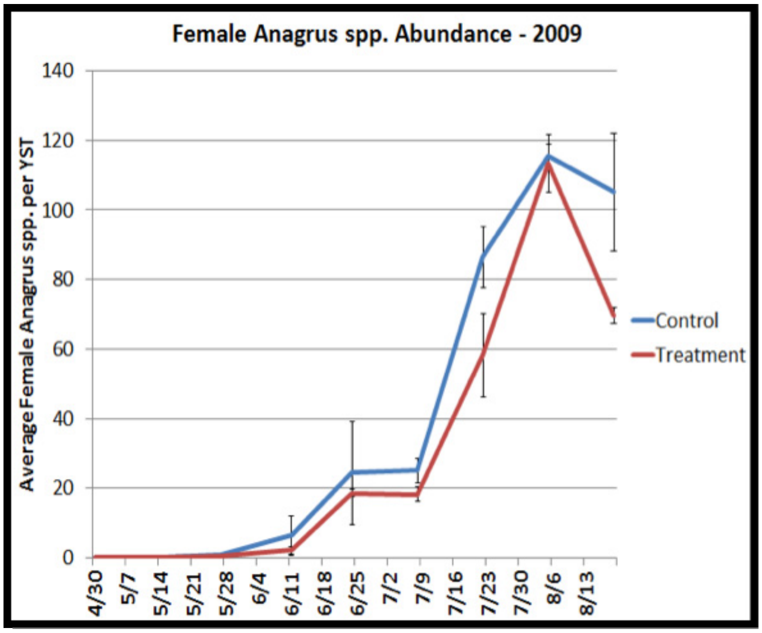
Parasitism rates by *Anagrus* wasps with and without natural enemy exclusion: To measure the rate of parasitism between FRP and control plots and estimate the magnitude of pest regulation by *Anagrus spp.,*exclusion cages (Trimaco #11313/25 5-Gallon Paint Strainer, Kelly-Moore Paints) were placed over 15 randomly selected vine shoots at bud break (approximately April 15th) in each treatment and control plot (n = 120). Five replicates of each of three types of exclusion cage were used in all plots: 5 "Exclusion", 5 "No Exclusion" and 5 "Sham Cage". Exclusion cages were secured to the vine spur and sealed off from natural enemy entry using two 12" produce twist ties (SAVEties Riverside, CA). The tops of exclusion cages were later cut and resealed using 12" produce twist ties on approximately May 15th to allow uninterrupted vine growth and to isolate 3-5 basal leaves inside each exclusion cage. 10 adult leafhoppers were introduced into each cage on approximately June 20th to lay eggs on leaves of selected shoots. On approximately Aug. 12th, of the 15 exclusion cages, 5 were conserved ("Exclusion"), and 5 were fully removed ("No exclusion") allowing full access to the vine by natural enemies. The remaining 5 were cut along 4 sides to allow access by natural enemies ("Sham cage"), were used to determine any impact of the exclusion cage itself on vine growth and population dynamics. On approximately August 20, basal leaves of the 15 selected vines were removed from lower 4 nodes, taken to lab and assessed for the density of *Erythroneura* leafhopper eggs and the rate of parasitism by *Anagrus spp*. Insects were identified and tallied using dissecting microscopes with visual identification characteristics described for western grape leafhoppers and egg parasitism by UC IPM (2013).

Statistical analysis: Experiment #1: PredaLure ® only trial: For both the 2009 and 2010 data, we tested the main impact of the treatment (PredaLure ®) and sample date, as well as interactions between these factors, on the abundance of female *Anagrus spp.*, other parasitoids, and the generalist predators *Chrysoperla spp.* and spiders using a generalized linear model with Poisson error distribution and log link function in 'R'. A two-way ANOVA with a repeated measures factor was used to evaluate differences in mean leafhopper nymph densities through time. One-way ANOVA was used to analyze data on mean peak leafhopper nymph density and mean adult leafhopper density at harvest time between treatment (PredaLure ®/MeSA) and control groups. Experiment #2: "Attract and Reward": To analyze mean *Erythroneura* leafhopper nymph data, we tested the impact of treatment and sample date, and interactions between these factors, on the abundance of leafhopper nymphs and natural enemies using a generalized linear model (GLM) with Poisson error distribution and log link function. Parasitism data from the natural enemy exclusion study was arcsine-square-root transformed prior to analysis. Parasitism rates were evaluated using ANOVA. Means were separated using Tukey's honestly significant difference (HSD) test. WGLH adults at harvest were analyzed using ANOVA. When the assumptions for

ANOVA were not met the WGLH abundance data was log-transformed prior to analysis. The calculation of cumulative means for analyzing arthropod abundance was conducted using methods outlined by (Altieri et al. 2005).

3. Results:

Experiment #1: Measuring the impact of PredaLure ® only on *Anagrus spp.* wasp density: We first measured the ability of PredaLure ® to attract female *Anagrus spp.*, a key parasitoid natural enemy of *Erythroneura* leafhoppers (Daane and Costello 2000, Triapitsyn et al. 2010). Results from the generalized linear model (GLM) indicated that there was no significant effect of the PredaLure ® treatment on female *Anagrus spp.* abundance in either 2009 or 2010 (Fig. 1). There were significant effects observed related to sample date, but this pattern is expected as *Anagrus spp.* populations develop fluctuate over the growing season (Costello and Daane 2003, English-Loeb et al. 2003). The calculation of the cumulative abundance of *Anagrus spp.* showed that there was a greater abundance of the *Anagrus spp.* parasitoid in control plots in 2009. No differences in *Anagrus spp* abundance were found between treatment and control plots in 2010 (Fig. 2). With the exception of a greater number of non-*Anagrus spp.* parasitoids found in the PredaLure plots in 2009, the calculation of a cumulative mean for other parasitoids, spiders and *Chrysoperla spp.* found no meaningful differences in abundance between treatment and control plots (Fig. 3-5).



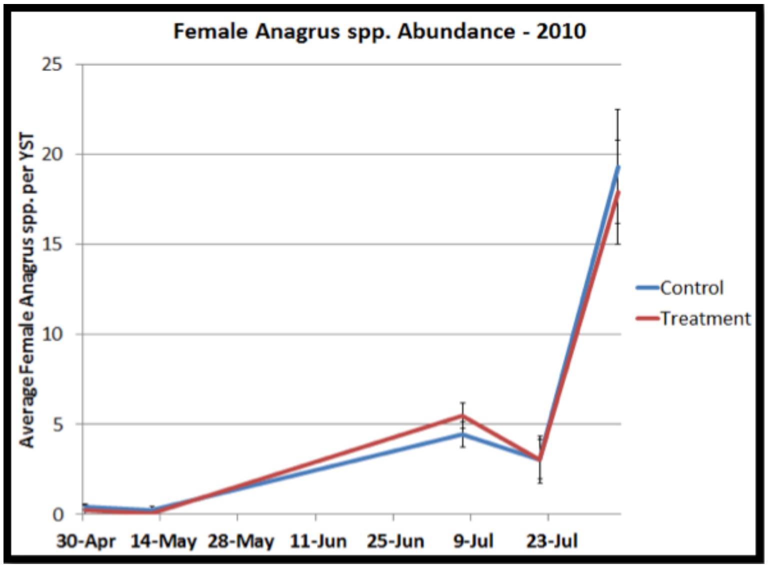
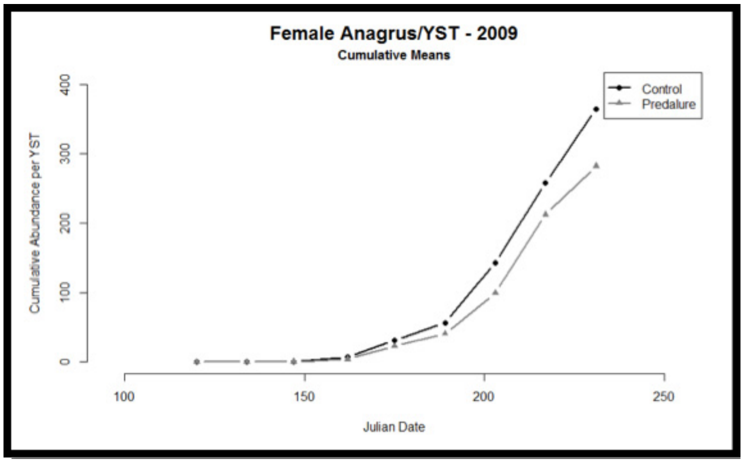


Figure 1: Mean seasonal abundance of female *Anagrus spp.* wasps per yellow sticky trap in PredaLure ® (MeSA) and control plots (2009-2010). Results from the GLM indicated that there was no significant effect of the PredaLure ® only treatment on female *Anagrus spp.* abundance in either 2009 or 2010. *PredaLure ® placement dates were April 15th and July 15th.



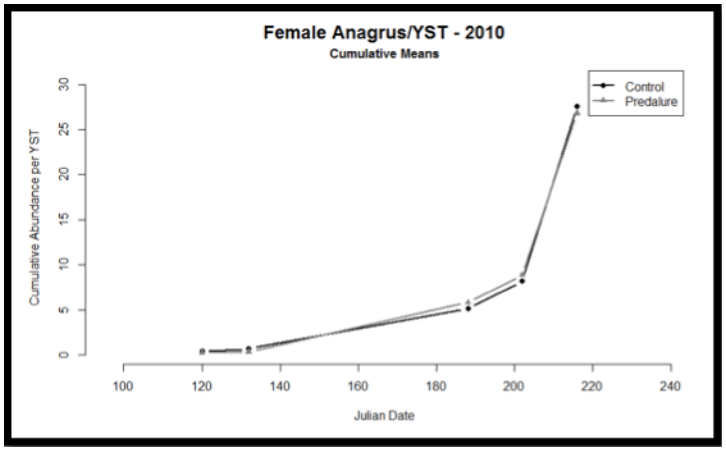


Figure 2: Cumulative mean of female *Anagrus spp.* found in yellow sticky traps placed in MESA/PredaLure ® and control plots 2009-2010.

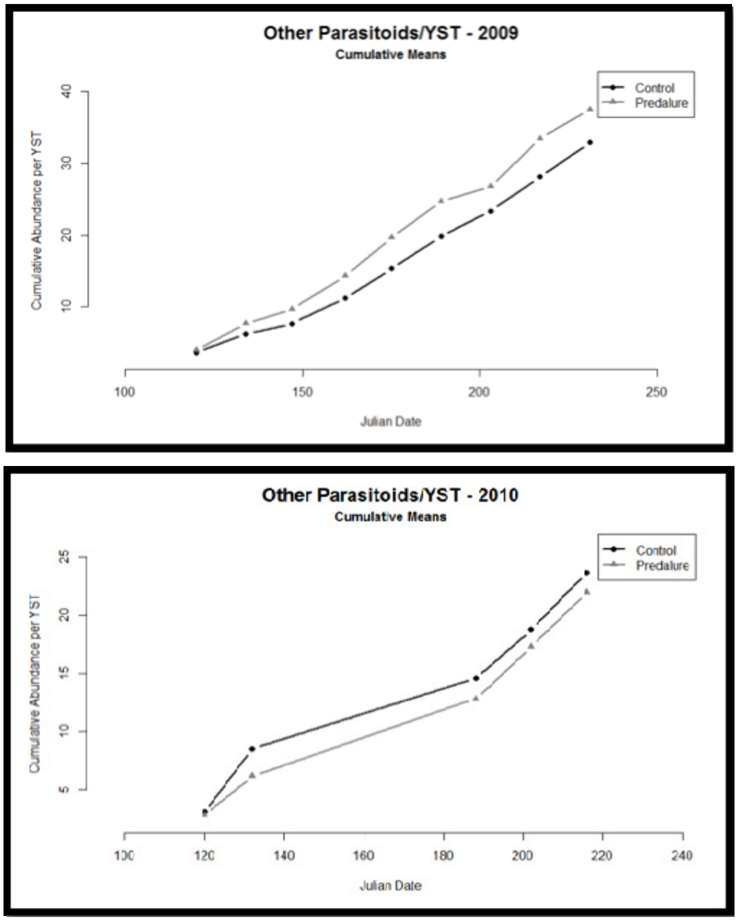


Figure 3: Cumulative mean of other parasitoids found in yellow sticky traps placed in MESA/PredaLure ® and control plots 2009-2010.

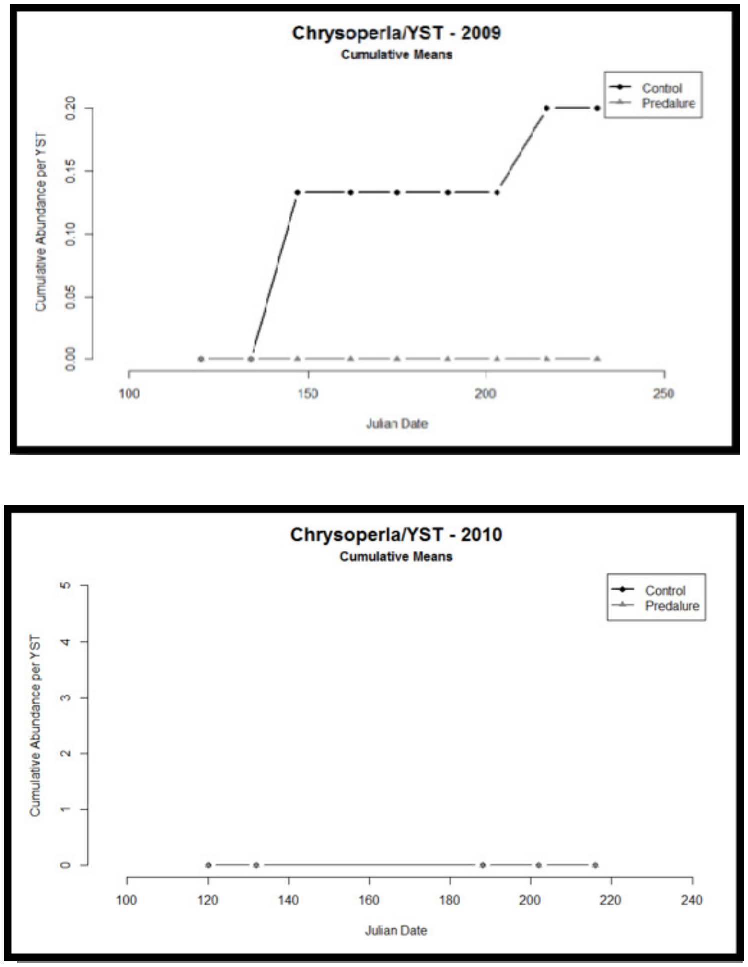
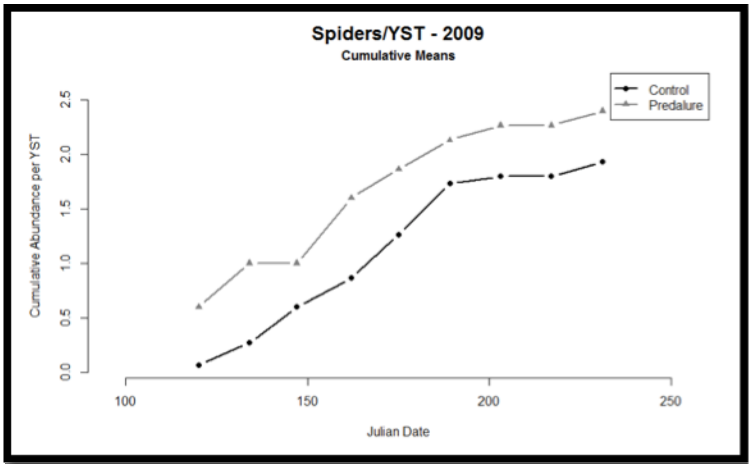


Figure 4: Cumulative mean of *Chrysoperla spp.* found in yellow sticky traps placed in MESA/PredaLure ® and control plots 2009-2010.



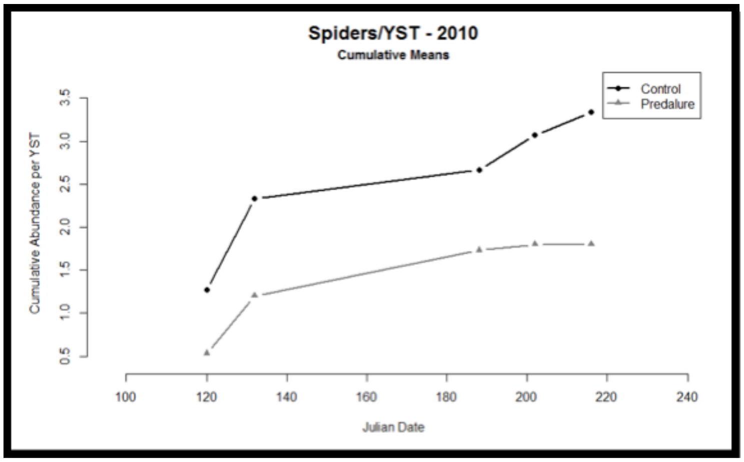


Figure 5: Cumulative mean of spiders found in yellow sticky traps placed in MESA/PredaLure ® and control plots 2009-2010.

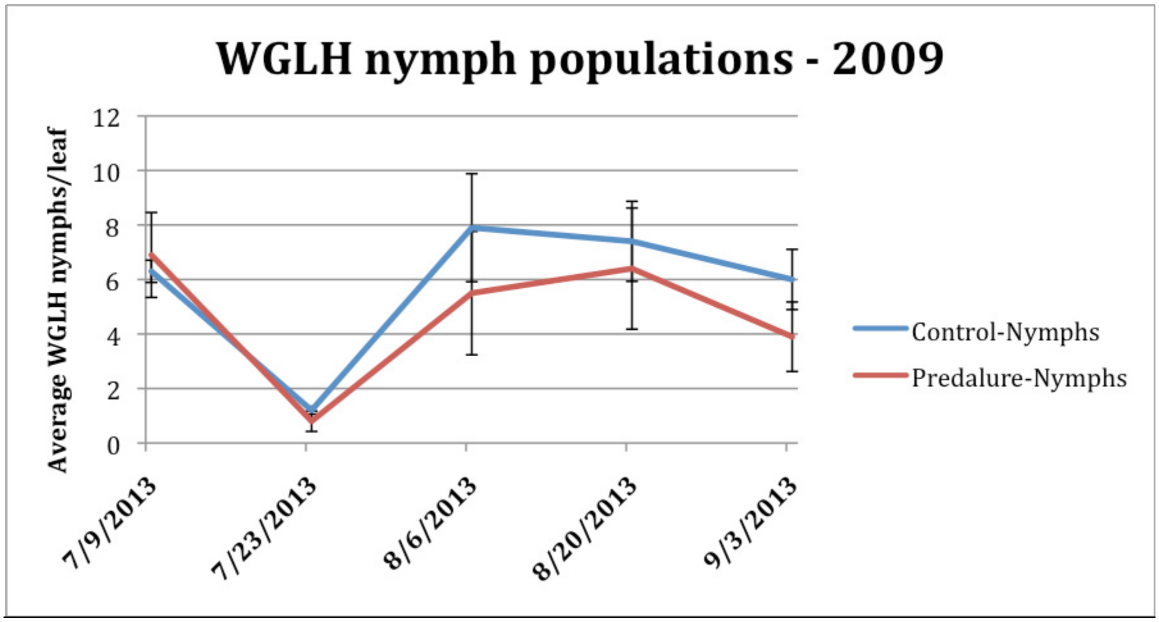
Experiment #1: Measuring the impact of PredaLure ® on the density of generalist predators and other parasitoids: For both the 2009 and 2010 data, we tested the main effect of treatment and sample date on the abundance of key natural enemies (other parasitoids, *Chrysoperla spp.* and spiders) using a generalized linear model with Poisson error distribution and log link function. The results from the generalized linear model indicated that there was no significant effect of the PredaLure ® treatment on natural enemy abundance in either 2009 or 2010 (Table 1).

Table 1: Results of a generalized linear model testing the effect of treatment (PredaLure ®) and sample date on the abundance of key natural enemies.

Year	Organism	z-value	p-value
2009	Other parasitoids	0.935	0.3497
	Chrysoperla	-0.001	0.999
	Spiders	0.388	0.698
2010	Other parasitoids	-0.428	0.66886
	Chrysoperla	0	1
	Spiders	-1.154	0.249

Experiment #1: Measuring the impact of PredaLure ® on seasonal and peak *Erythroneura* leafhopper nymph density: We measured the impact of PredaLure ® only on seasonal and peak *Erythroneura* leafhopper nymph density to determine if the MeSA treatment alone would produce meaningful reductions in pest abundance. A two-way ANOVA with a repeated measures factor indicated no significant effect of treatment in 2009 (F(1,4)=1.9008, p = 0.18) and 2010 (F(1,4)=0.0667, p = 0.80). There was a significant change in nymph densities over time in 2009 (F(1,4) = 8.5363, p < 0.001) and 2010 (F(1,4) = 54.3347, p < 0.001). This is an expected pattern, however, as nymph densities seasonally rise and fall as the pest population develops between leafhopper generations (English-loeb et al. 2003). Finally, there was no significant interaction between treatment and sample date in 2009 (F(1,4) = 0.5177, p = 0.772) or

2010 (F(1,4) = 0.2014, p = 0.99) (Fig. 6, Table 2). The calculation of the cumulative abundance of *Erythroneura* leafhopper nymphs show that there was no meaningful difference leafhopper abundance in either 2009 in 2010 (Fig. 7).



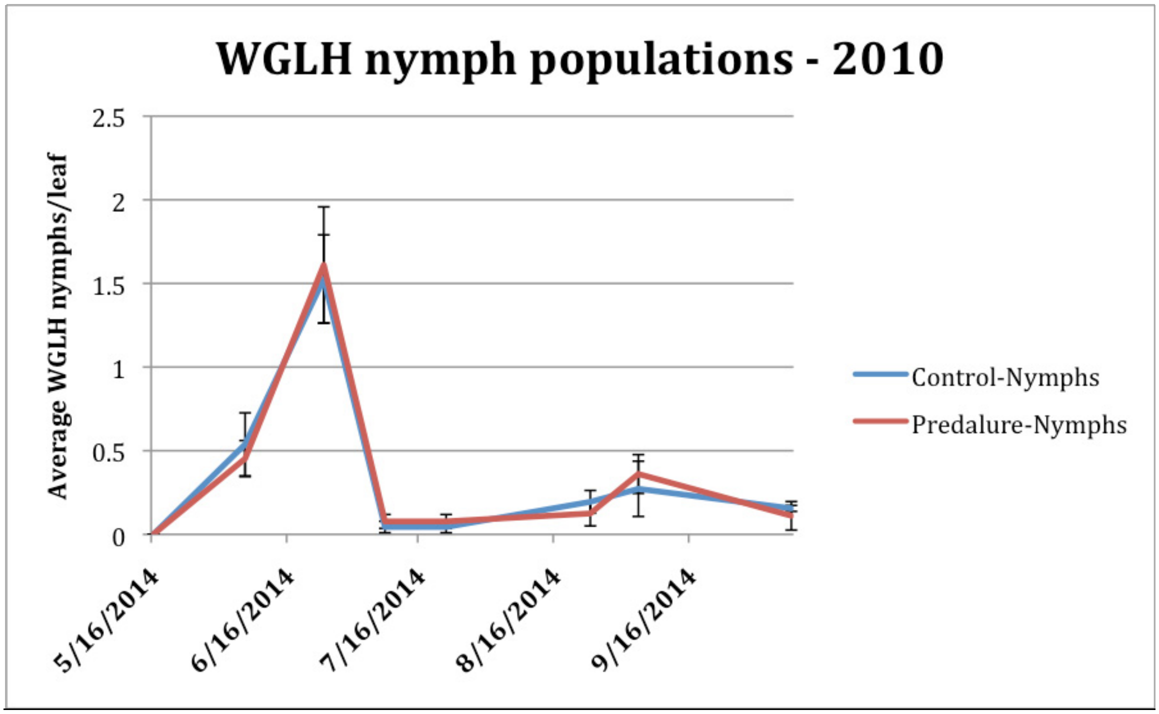
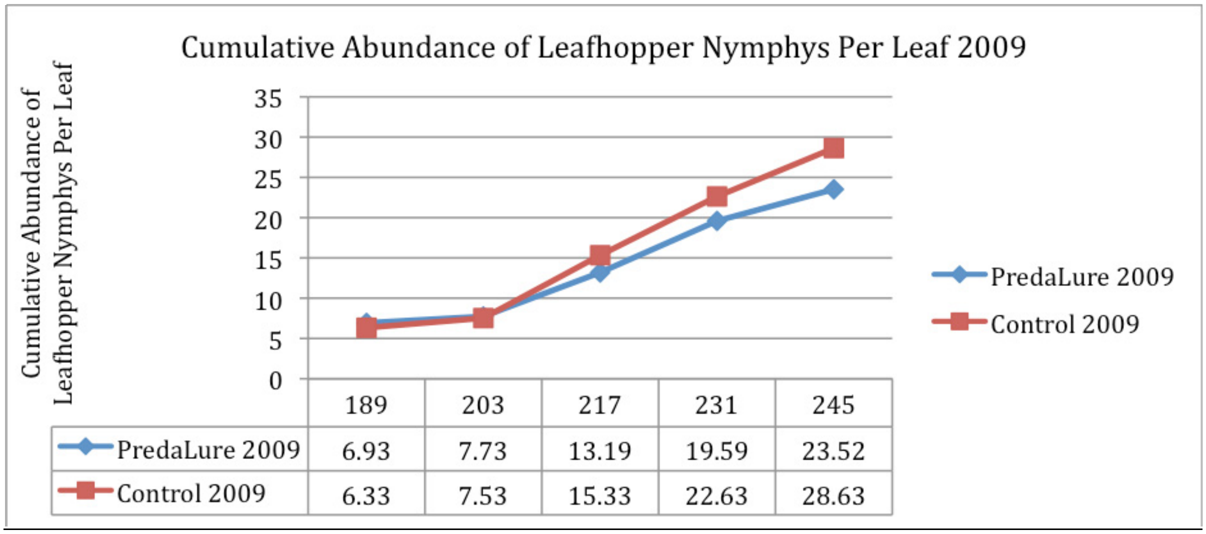
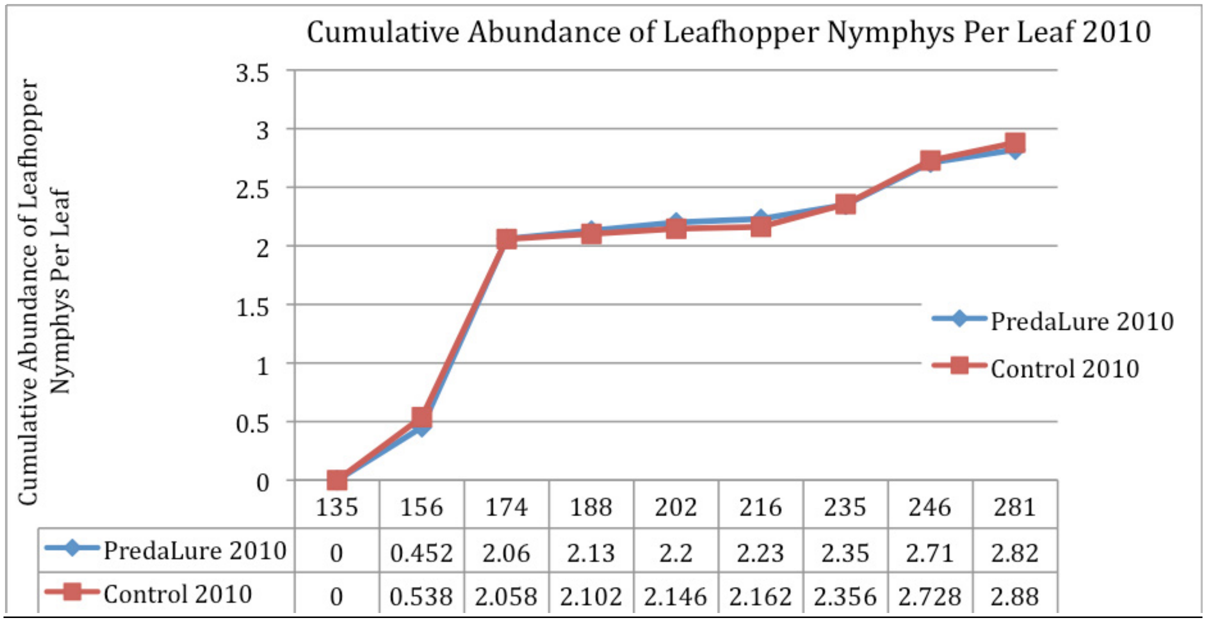


Figure 6: Mean abundance of *Erythroneura* leafhopper nymphs per leaf (2009-2010), PredaLure ® only trial. Two-way ANOVA with a repeated measures factor indicated that there was significant effect of treatment in 2009 (F(1,4)=1.9008, p = 0.18) and 2010 (F(1,4)=0.0667, p = 0.80).



	189	203	217	231	245
PredaLure 2009	6.93	7.73	13.19	19.59	23.52
Control 2009	6.33	7.53	15.33	22.63	28.63



	135	156	174	188	202	216	235	246	281
PredaLure 2010	0	0.452	2.06	2.13	2.2	2.23	2.35	2.71	2.82
Control 2010	0	0.538	2.058	2.102	2.146	2.162	2.356	2.728	2.88

Figure 7: Cumulative mean abundance of *Erythroneura* leafhopper nymphs per leaf (2009-2010), PredaLure ® only trial. Top row in each table refers to the Julian calendar day,

Experiment #1: Measuring the impact of PredaLure ® only on peak *Erythroneura* leafhopper nymph density: As *Erythroneura* leafhopper nymph densities are known to fluctuate significantly through the season, peak *Erythroneura* leafhopper nymph densities were also compared to isolate the potential impact of PredaLure ® on biological control. One-way ANOVA was used to analyze data on peak nymph density of leafhoppers between treatment (PredaLure ®/MeSA) and control groups. In both years there were 2 distinct leafhopper generations: early July and late August in 2009, and late June and early September in 2010 (Fig. 8). One-way ANOVA was used to evaluate differences in peak leafhopper nymph populations in both years. Findings indicate that there were no signiicant differences in peak leafhopper nymph densities between treatment and control plots in either 2009 (1^{st} generation $DF_{1,4}$, F=0.208, p=0.672; 2^{nd} generation $DF_{1,4}$, F= 0.954, p=0.384) or 2010 (1^{st} generation $DF_{1,4}$, F=0.053, p = 0.829; 2^{nd} generation $DF_{1,4}$, F= 0.005, p=0.948) (Fig. 8).

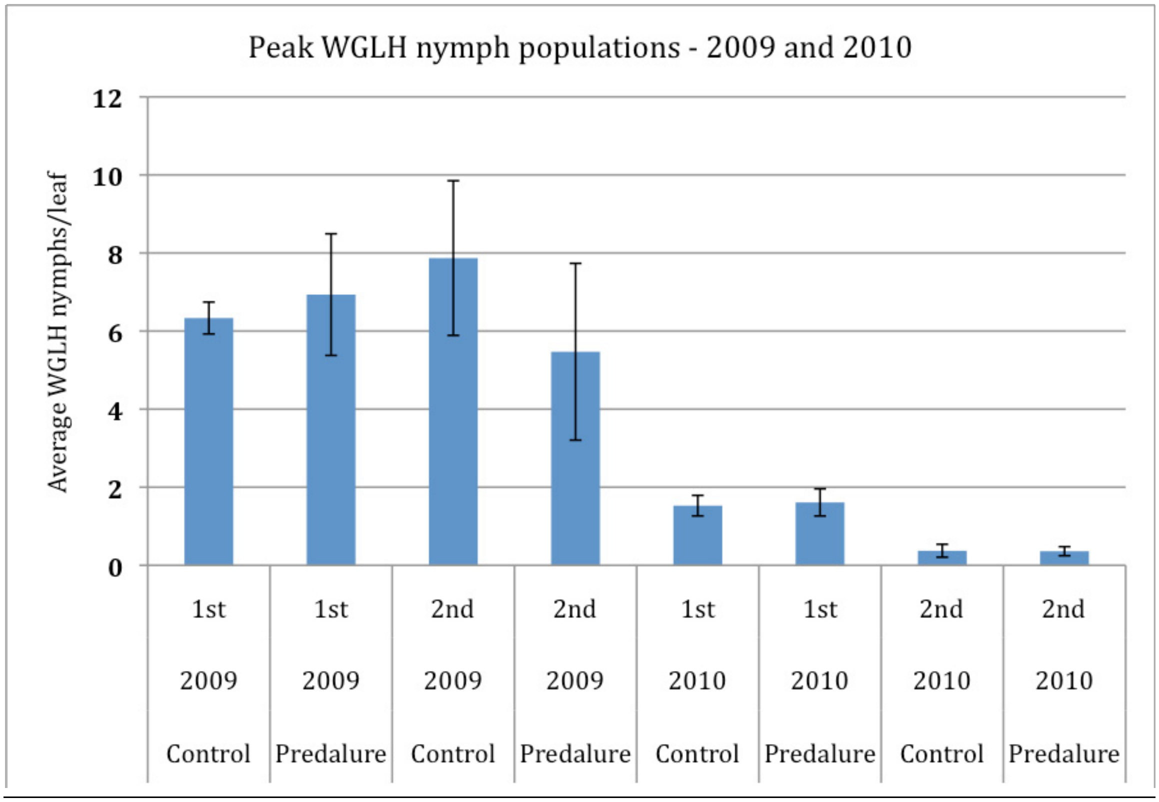
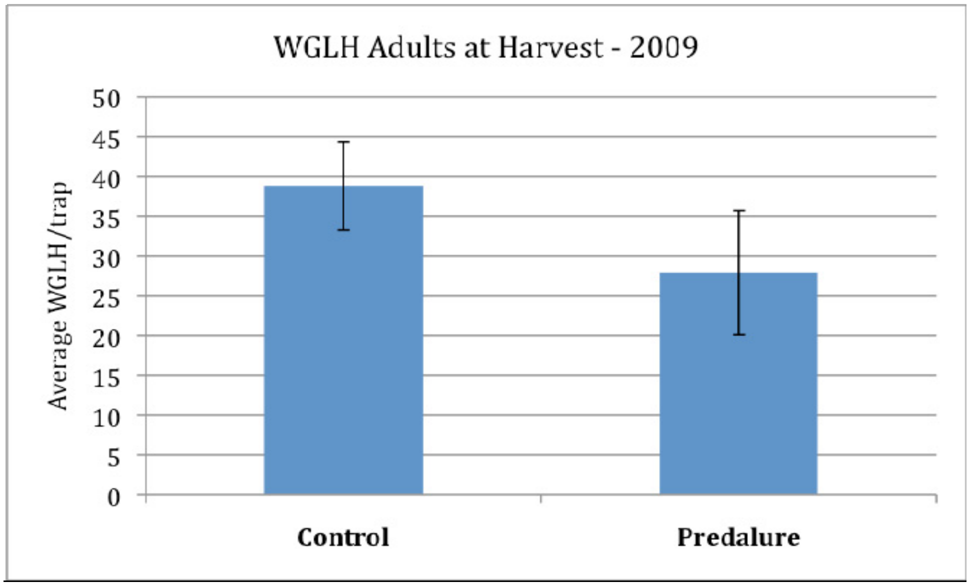


Figure 8: Mean peak *Erythroneura* leafhopper nymph density per leaf (2009-2010), PredaLure ® only trial. One-way ANOVA indicated that there were no significant differences in peak leafhopper nymph densities in either 2009 (1^{st} generation $DF_{1,4}$, F=0.208, p=0.672; 2^{nd} generation $DF_{1,4}$, F= 0.954, p=0.384) or 2010 (1^{st} generation $DF_{1,4}$, F=0.053, p = 0.829; 2^{nd} generation $DF_{1,4}$, F= 0.005, p=0.948).

Experiment #1: Measuring the impact of PredaLure ® only on *Erythroneura* leafhopper (WGLH) adult density at harvest time: In much of the marine influenced parts of California's wine growing regions, high *Erythroneura* leafhopper densities do not result in significant damage to the crop or vine, but serve as nuisance pest for harvest crews as they reach peak adult densities in late summer and early fall (Daane and Costello 2000). Here we analyzed the impact of PredaLure ® as the sole treatment on adult *Erythroneura* leafhopper densities at harvest time to determine its impact on biological control of leafhoppers as a nuisance pest. ANOVA indicated no significant difference in end-of-season *Erythroneura* leafhoppers adult populations in both 2009 (F = 1.96, $DF_{1,4}$, P = 0.234) and 2010 (F = 5.468, $DF_{1,4}$, P = 0.0795) (Fig. 9).



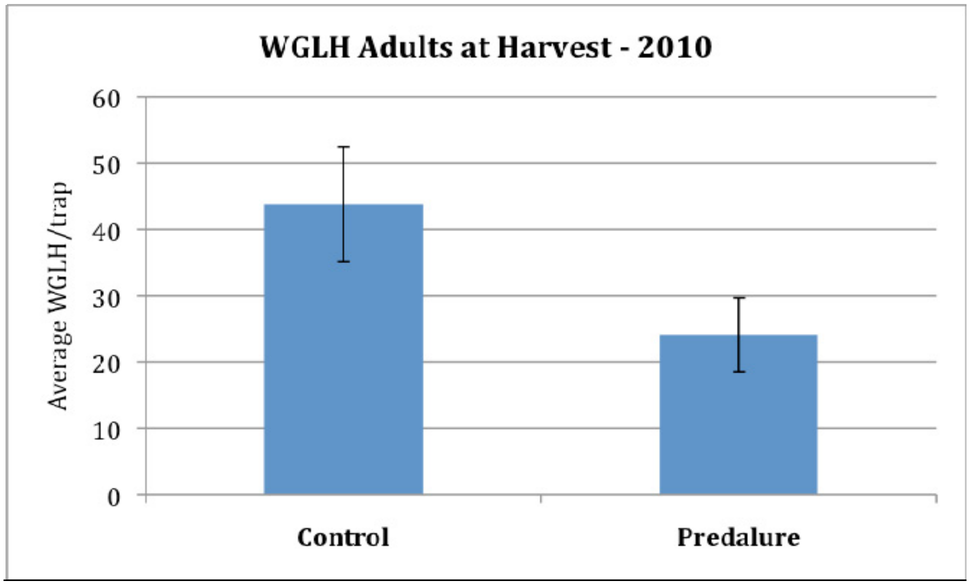


Figure 9: Mean *Erythroneura* leafhopper adult density at harvest time, PredaLure ® only (2009-2010). ANOVA indicated no significant difference in end-of-season *Erythroneura* leafhoppers adult populations in both 2009 (F = 1.96, $DF_{1,4}$, P = 0.234) and 2010 (F = 5.468, $DF_{1,4}$, P = 0.0795).

Experiment #2: Measuring the impact of “Attract and Reward” on *Erythroneura* leafhopper nymph densities: We tested the main effects of treatment and sample date, as well as interactions between these factors, on the abundance of leafhopper nymph density using a generalized linear model (GLM) with Poisson error distribution and log link function. Results from the GLM indicated that there was no significant effect of the treatments (FRP, PredaLure, and FRP + PredaLure ®) or sample date on leafhopper nymph abundance in 2010 or 2011 (Fig. 10).

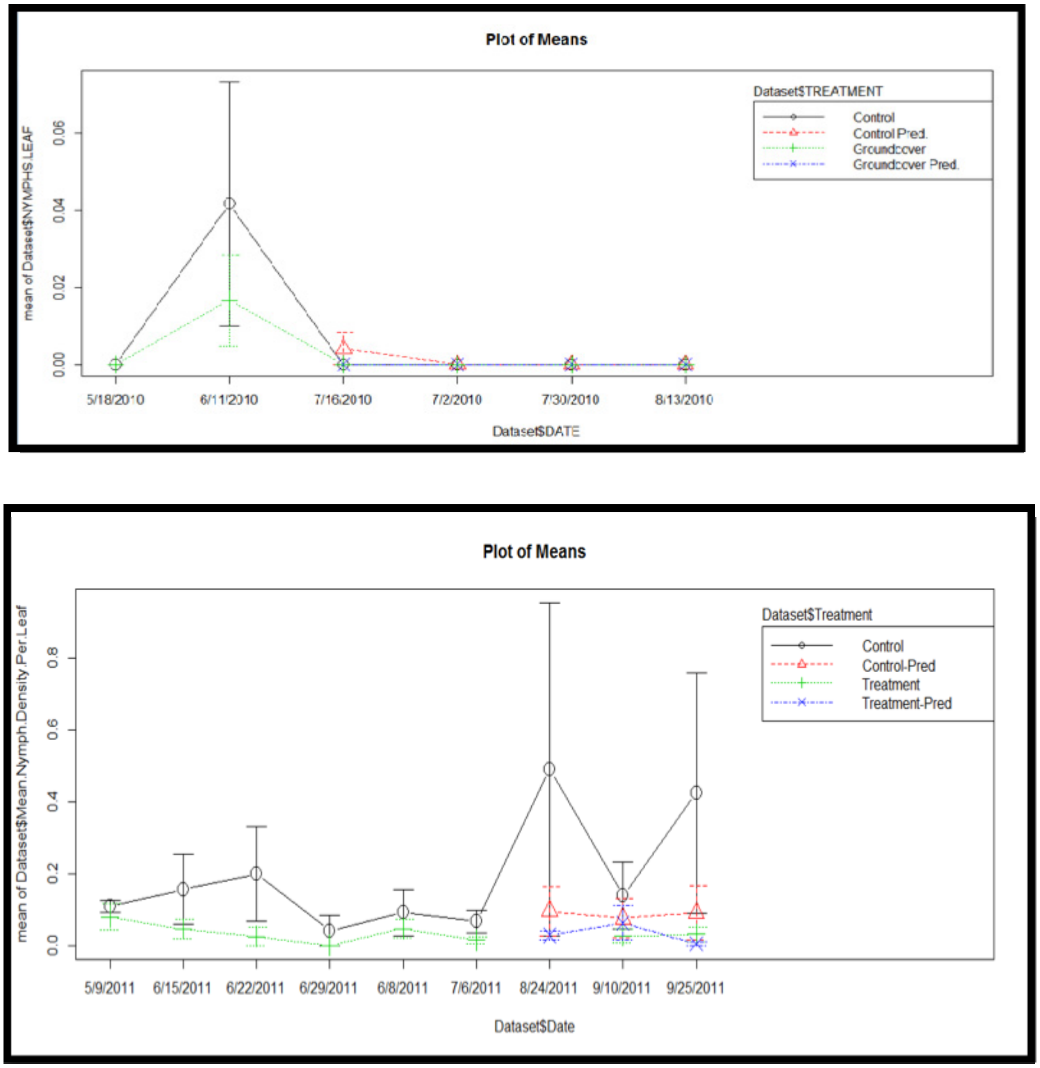


Figure 10: Mean seasonal *Erythroneura* leafhopper nymph density per leaf in "attract and reward" and control plots (2010-2011). Results of the GLM indicate no significant influence of either treatment (FRP, PredaLure ® or FRP + Predalure ®) or sample date on mean leafhopper nymph abundance.

Experiment #2: Measuring the impact of floral resource provisioning (FRP) with and without PredaLure on key natural enemy abundance: Results from the generalized linear model indicated that in both years of the study (20010-2011) there was no significant interaction between treatment and sample date for *Anagrus* spp., *Chrysoperla* spp. and spiders. "Other parasitoids" were found to be significantly more abundant on 3 sample dates in July of 2010 (Fig. 11, Appendix B. Table 3).

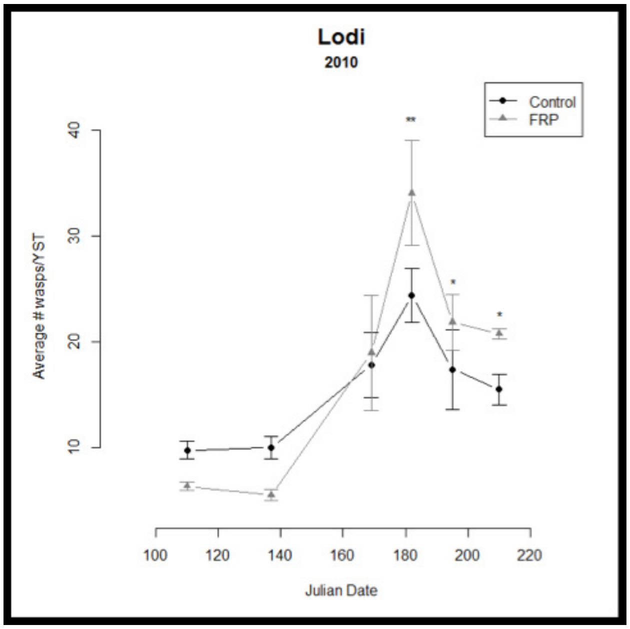


Figure 11: Mean seasonal abundance of "other parasitoids" (excluding *Anagrus spp.*) in 2010 as measured by yellow sticky trap sampling. Results from the GLM indicated that "other parasitoids" were found to be significantly more abundant in FRP plots on 3 sample dates in July of 2010.

Experiment #2: Measuring the impact of floral resource provisioning (FRP) on parasitism rates of *Erythroneura* leafhopper eggs by *Anagrus spp.* with and without natural enemy exclusion: We evaluated the impact of FRP on parasitism rates of *Erythroneura* leafhopper eggs by *Anagrus spp.*, with and without natural enemy exclusion, to determine its impact on biological control. ANOVA indicated that there were significant differences in parasitism rates between the "No Exclusion" vines (allowing access by *Anagrus spp.*) in control plots and the "Exclusion" vines (excluding *Anagrus spp.*) in both the treatment/FRP and control plots ($DF_{5,18}$, F=7.162, p <0.001). Parasitism rates were 0% in both treatment/FRP and control plots where exclusion cages were present. No significant differences in parasitism rated were found between the "No Exclusion" treatment/FRP and control plots, indicating that FRP had no meaningful impact on parasitism of leafhopper eggs by *Anagrus spp.* Our analysis of natural enemy exclusion showed that *Anagrus spp.* was responsible for an estimated 20% parasitism rate of *Erythroneura* leafhoppers in both treatment and control plots (Fig. 12).

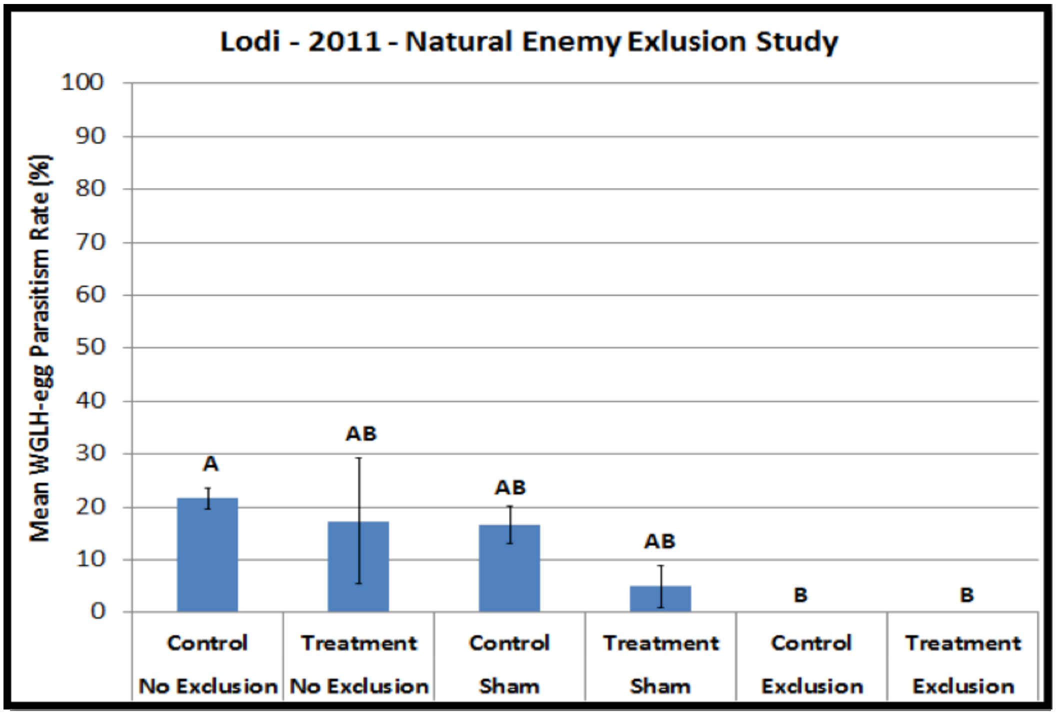


Figure 12: Mean rate of *Erythroneura* leafhopper egg parasitism in 3 separate treatments of a natural enemy exclusion study. Significant differences in parasitism rates were found between the “No Exclusion” vines in control plots and the “Exclusion” vines in both the treatment/FRP and control plots ($DF_{5,18}$, F=7.162, p <0.001). No significant differences in parasitism rated were found between the “No Exclusion” treatment/FRP and control plots.

Experiment #2: Measuring the impact of FRP with and without PredaLure ® on adult *Erythroneura* leafhopper adult density at harvest time: ANOVA of mean indicated no significant difference in end-of-season adult leafhopper abundance in both 2010 (F = 0, $DF_{1,6}$, P = 1.0) and 2011 (F = 0.767, $DF_{3,12}$, P =0.534) (Fig 13).

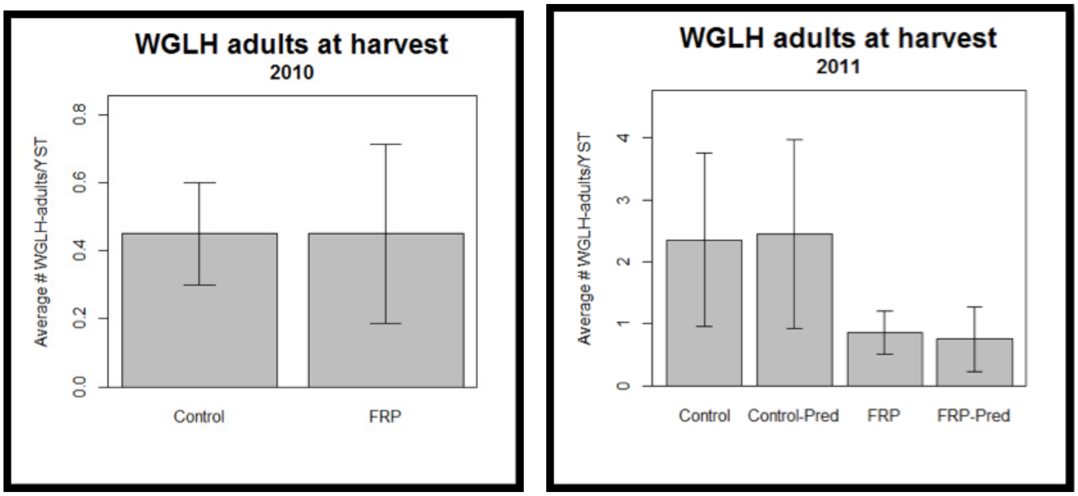


Figure 13: Mean abundance of adult leafhopper at harvest 2010-2011.

4. Discussion:

Results from the Experiment # 1: The overall findings indicate that there was no significant impact of the PredaLure ® (MeSA) treatment alone on biological control of *Erythroneura* leafhoppers. Our analysis found no evidence supporting Predalure had any significant or meaningful impact on female *Anagrus spp.,* leafhopper nymphs, or adult leafhopper densities at harvest time in either 2009 or 2010. The calculation of the cumulative mean of *Anagrus spp.* showed, however, that there was a greater abundance of *Anagrus spp.* parasitoids in control plots in 2009 only. With the exception of a greater number of non-*Anagrus spp.* parasitoids found in the PredaLure plots in 2009, the calculation of a cumulative mean for other key natural enemies found no meaningful differences in abundance between treatment and control plots in either 2009 or 2010.

The finding of no significant difference in natural enemy and pest abundance in between PredaLure ® only and control plots is consistent with prior research showing no or conflicting results of the impact of MeSA treatments on *Anagrus spp.,* other natural enemies and pest regulation (Lee 2010, Rodriguez-Saona et al. 2011, Gadino et al. 2012, Kaplan 2012). The lack of supporting statistical evidence that PredaLure ® alone had any significantly impact on natural enemies or pest abundance may also be attributed to plot size. As there is little scientific knowledge on the distribution of HIPVs in the field, the plot size and buffer areas assigned to the study may have been too small to prevent the MeSA signal emitted from the PredaLures being equally distributed throughout the research plots resulting in the elimination of an effective control (Kaplan 2012). This issue may be compounded by the phenomenon of synthetic HIPVs stimulating plant-produced HIPVs in neighboring plants (James et al. 2012).

Results from Experiment #2: The overall findings of the "attract and reward" study indicate that the combination of floral resource provisioning (FRP) and PredaLure ® (MeSA) had no significant impact on seasonal western grape leafhopper nymph abundance or leafhopper egg parasitism rate by *Anagrus spp.* With the exception of a significantly greater abundance of non-*Anagrus spp.* found in FRP plots on 3 dates in 2010, our analysis showed that floral resource provisioning alone, Predalure alone, nor combination of floral resource provisioning + PredaLure ® (i.e. "attract and reward") had any significant effect on key natural enemy abundance.

The results of our study finding no significant difference in *Erythroneura* leafhopper nymph densities between FRP and control plots in the "attract and reward" research may be, in part, attributed to the low background population densities of leafhoppers documented in 2010-2011. These findings of are consistent with prior cover crop research by Daane and Costello (1998) finding no significant effect of a barley and vetch cover crop on either key natural enemies or *Erythroneura* leafhopper abundance when mean leafhopper densities were less than 10 nymphs/leaf. Their study did find an estimated 15-20 % reduction of leafhopper nymphs only at higher mean densities of ~20-30 nymphs/leaf, a non-economic control level. No effective control was found at nymph densities above ~ 30 nymphs/leaf. Importantly, the mechanism of control was found to be most strongly correlated with the cover crop's impact on vine vigor/plant host quality and only secondarily to predation by spiders (Costello and Daane 2003). Further, the finding of no significant effect of FRP on leafhopper nymph abundance is consistent with prior work showing no reduction in leafhopper nymph abundance with FRP (English-Loeb et al. 2003).

The lack of significant differences in key natural enemy abundance found in FRP v. control plots in the "attract and reward" study may have resulted, in part, from small plot size resulting in natural enemies being relatively evenly distributed throughout treatment and control plots. The specific finding of FRP having no significant impact on *Anagrus spp.* abundance is consistent with prior work showing no FRP with buckwheat, clover and sod had no consistent impact on adult *Anagrus spp.* abundance (English-Loeb et al. 2003).

The finding of no significant effect of FRP on parasitism rates of leafhopper eggs by *Anagrus spp.* wasps is consistent with prior work by Nicholls et al. (2000) showing FRP with sunflower (*Helianthus annuus*), and annual buckwheat (*Fagopyrum esculentum*) had no significant impact on parasitism by *Anagrus spp.* These findings however are inconsistent with the work of English-Loeb et al. (2003) who found that, under controlled conditions, *Anagrus spp.* longevity was significantly increased when parasitoids were provided with honey or sugar water, and parasitism of leafhopper eggs were greater when *Anagrus spp.* had access to flowering buckwheat flowers.

The absence of supporting evidence that FRP + PredaLure ® had any significant impact on natural enemy or pest abundance may also be attributed to low background density of leafhoppers and plot size, as discussed above. Lastly, as the area, diversity, species composition and distance from non-crop habitats are all know play an important role in determining the abundance and diversity of both pest and natural enemies in agroecosystems (Tscharntke et al. 2007) landscape factors my be overwhelming any localized treatments of FRP and/or MeSA (Gámez-Virués et al. 2012)

As many ecological factors influence natural enemy abundance and pest regulation in vineyards, including landscape heterogeneity (Tylianakis et al. 2010, Chaplin-Kramer et al. 2011, Miles et al. 2012, Segoli and Rosenheim 2012, Martin et al. 2013), vineyard age and cultural practices such as fertilization and irrigation (Costello and Daane 2003), much more applied ecological research will be necessary to develop a comprehensive scientific and practical understanding of how to manage both the biotic and physical environmental conditions in vineyards to consistently reduce *Erythroneura* leafhoppers.

Acknowledgements: The authors would like to sincerely thank the following individuals and institutions for their generous support of this research: The California Department of Food and Agriculture (CDFA); the Organic Farming Research Foundation (OFRF); The United States Environmental Protection Agency (EPA); the many UC Berkeley undergraduate research interns; collaborating growers and their firms, Chris Storm and Vino Farms; the California Sustainable Wine Growers Group; and the Robert van den Bosch Scholarship for providing research scholarships for Albie Miles.

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Appendix:

A. Table 2: Mean Western Grape Leafhopper Nymph Density (2009-2010), PredaLure ® only study, Napa, California (Experiment #1).

Treatment	jday	Mean	SEM		Treatment	jday	Mean	SEM
Control	189	6.333	1.021		Predalure	189	6.933	0.992
Control	203	1.2	0.242		Predalure	203	0.8	0.326
Control	217	7.866	1.351		Predalure	217	5.466	1.116
Control	231	7.538	1.470		Predalure	231	6.4	1.094
Control	245	6	0.856		Predalure	245	3.933	0.720
Control	500	0	0		Predalure	500	0	0
Control	521	0.538	0.066		Predalure	521	0.469	0.070
Control	539	1.527	0.123		Predalure	539	1.605	0.125
Control	553	0.044	0.015		Predalure	553	0.077	0.022
Control	567	0.044	0.015		Predalure	567	0.077	0.022
Control	581	0.016	0.012		Predalure	581	0.022	0.011
Control	600	0.194	0.036		Predalure	600	0.138	0.038
Control	611	0.372	0.054		Predalure	611	0.361	0.059
Control	646	0.155	0.030		Predalure	646	0.111	0.023

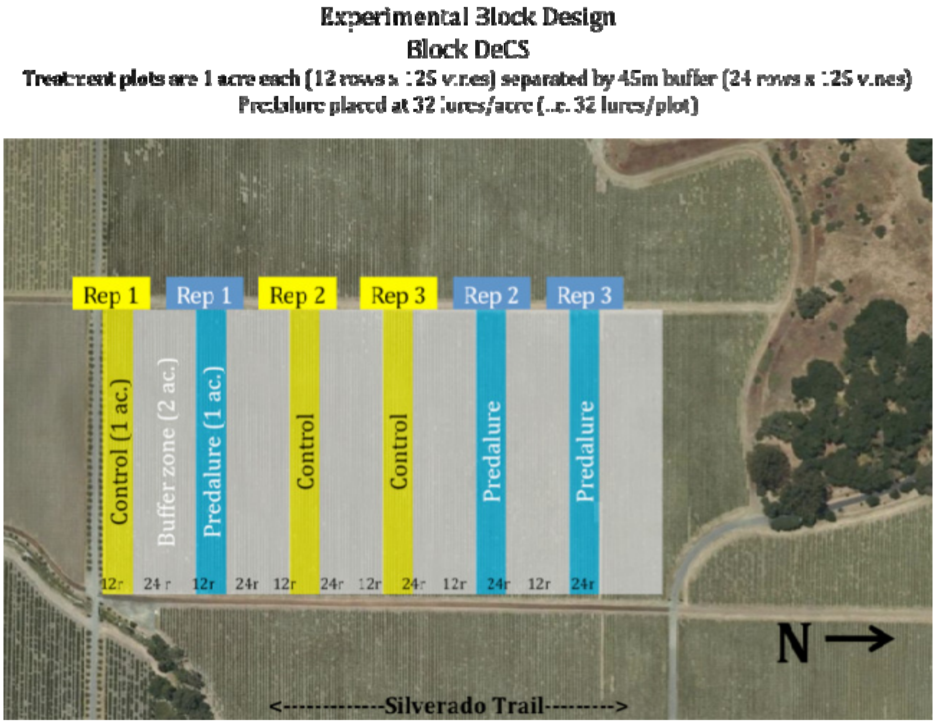
B. Table 3: Mean natural enemy abundance in FRP field plots with and without PredaLure (2010-2011) in Elk Grove and Acampo, Ca (Experiment #2). GLM indiacated that there was no significant interaction between treatment and sample date for *Anagrus* spp., *Chrysoperla* spp. and spiders. "Other parasitoids" were found to be significantly more abundant in FRP plots on 3 sample dates in July, 2010.

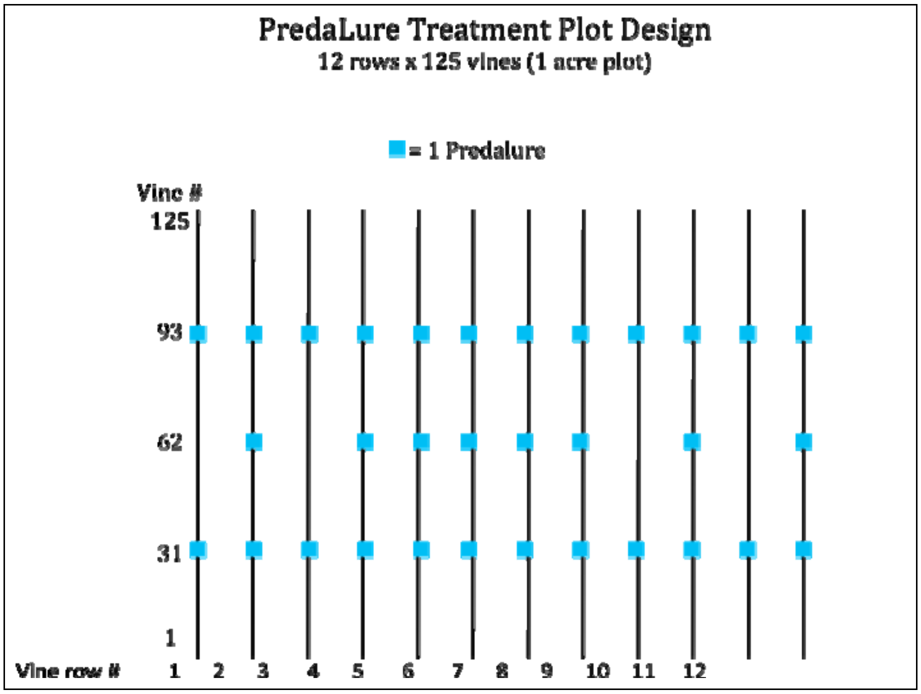
Year	Response	Predictor	Treatment	Date	Z value	P value
2010	Anagrus.Fem	Date*Treatment	FRP	5/17/2010	0.001	0.99
			FRP	6/18/2010	0.0	1.0
			FRP	7/1/2010	0.0	1.0
			FRP	7/14/2010	0.0	1.0
			FRP	7/29/2010	0.0	1.0
	Chrysoperla	Date*Treatment	FRP	5/17/2010	0.681	0.496
			FRP	6/18/2010	0.002	0.999
			FRP	7/1/2010	0.0	1.0
			FRP	7/14/2010	0.0	1.0
			FRP	7/29/2010	0.0	1.0
	Wasps	Date*Treatment	FRP	5/17/2010	-0.459	0.64607
			FRP	6/18/2010	1.609	0.10760
			FRP	7/1/2010	2.652	0.00801**

			FRP	7/14/2010	2.179	0.02936*
			FRP	7/29/2010	2.36	0.01826*
	Spiders	Date*Treatment	FRP	5/17/2010	0.530	0.596
			FRP	6/18/2010	0.610	0.542
			FRP	7/1/2010	0.753	0.451
			FRP	7/14/2010	0.109	0914
			FRP	7/29/2010	0.014	0.989
2011	Anagrus.Fem	Date*Treatment	FRP	5/9/2011	0.015	0.9883
			FRP	6/8/2011	0.552	0.5811
			FRP	7/6/2011	0.465	0.6419
			Control-Pred	8/11/2011	-0.160	0.8725
			FRP	8/11/2011	0.501	0.6164
			FRP-Pred	8/11/2011	-0.083	0.9341
			Control-Pred	9/9/2011	NA	NA
			FRP	9/9/2011	0.489	0.6246
			FRP-Pred	9/9/2011	NA	NA
	Chrysoperla	Date*Treatment	FRP	5/9/2011	0.002	0.999
			FRP	6/8/2011	0.002	0.999
			FRP	7/6/2011	0.001	0.999
			Control-Pred	8/11/2011	0.0	1.0
			FRP	8/11/2011	0.0	1.0
			FRP-Pred	8/11/2011	-0.001	0.999
			Control-Pred	9/9/2011	NA	NA
			FRP	9/9/2011	0.0	1.0
			FRP-Pred	9/9/2011	NA	NA
	Wasps	Date*Treatment	FRP	5/9/2011	0.557	0.57741
			FRP	6/8/2011	1.547	0.12190
			FRP	7/6/2011	1.038	0.29947
			Control-Pred	8/11/2011	-1.644	0.10026
			FRP	8/11/2011	-0.062	0.95089
			FRP-Pred	8/11/2011	-1.154	0.24868
			Control-Pred	9/9/2011	NA	NA
			FRP	9/9/2011	0.559	0.57609
			FRP-Pred	9/9/2011	NA	NA
	Spiders	Date*Treatment	FRP	5/9/2011	0.724	0.469

			FRP	6/8/2011	1.103	0.270
			FRP	7/6/2011	1.149	0.250
			Control-Pred	8/11/2011	-0.091	0.927
			FRP	8/11/2011	0.596	0.551
			FRP-Pred	8/11/2011	0.123	0.902
			Control-Pred	9/9/2011	NA	NA
			FRP	9/9/2011	-0.004	0.996
			FRP-Pred	9/9/2011	NA	NA

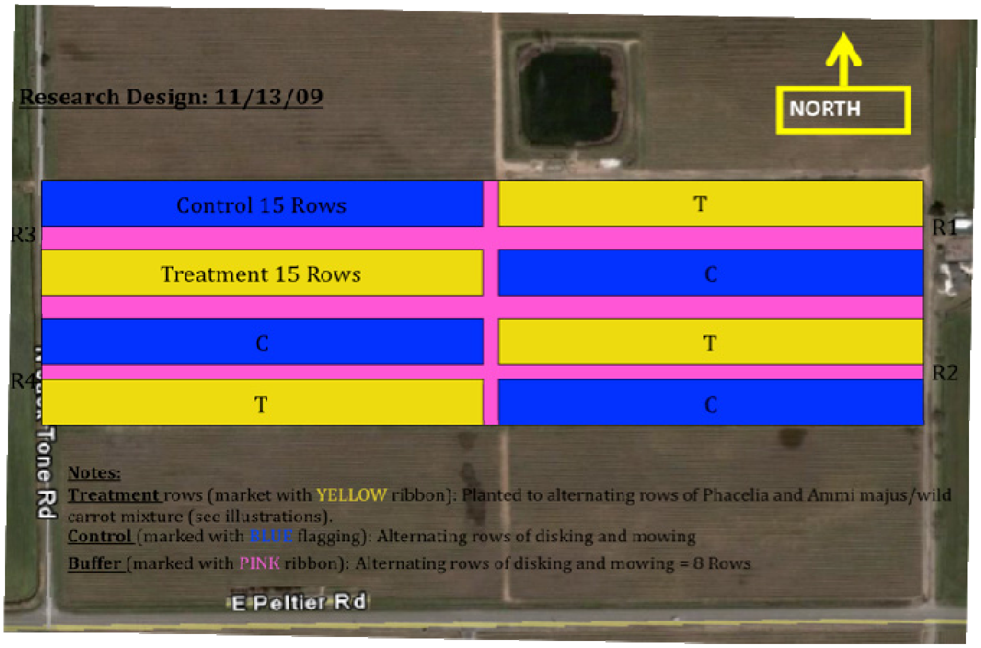
C. Research Design: Experiment #1: Wappo Hill 'PredaLure ®' Study, Napa, California, 2010





D. Research Design: Experiment #2: Vino Farms, Ranch 5, 2010

Vino Farms: Ranch 5 2010



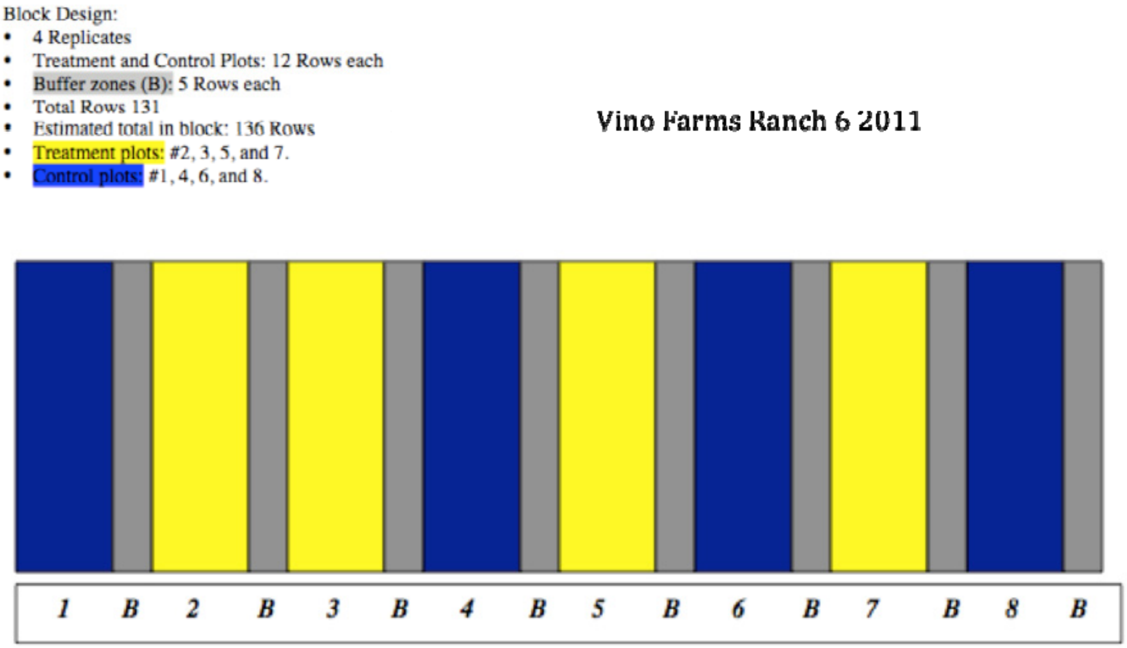
Notes:

Treatment rows (market with YELLOW ribbon): Planted to alternating rows of Phacelia and Ammi majus/wild carrot mixture (see illustrations).

Control (marked with BLUE flagging): Alternating rows of disking and mowing

Buffer (marked with PINK ribbon): Alternating rows of disking and mowing = 8 Rows

E. Research Design: Vino Farms Ranch 6, 2011



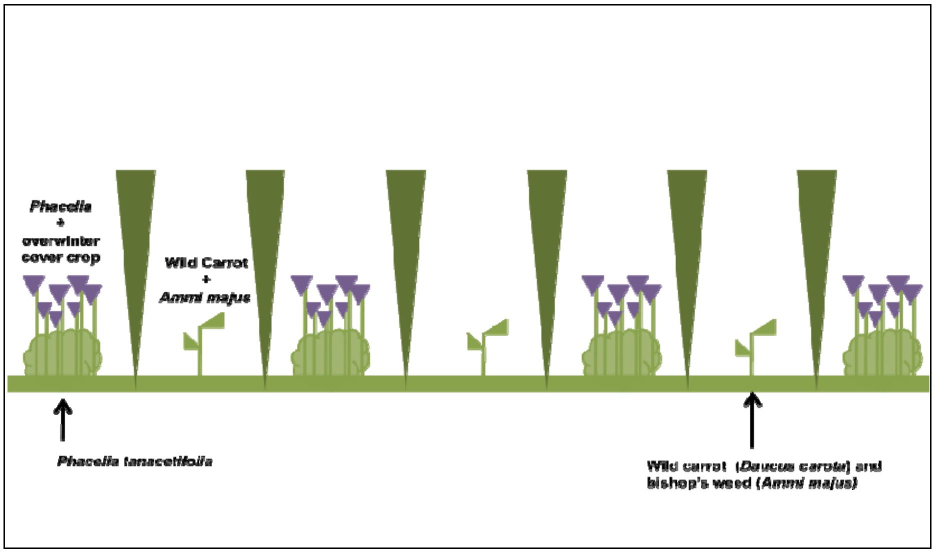
Block Design:

- 4 Replicates
- Treatment and Control Plots: 12 Rows each
- Buffer zones (B): 5 Rows each
- Total Rows 131
- Estimated total in block: 136 Rows
- Treatment plots: #2, 3, 5, and 7.
- Control plots: #1, 4, 6, and 8.

Vino Farms Ranch 6 2011

1 B 2 B 3 B 4 B 5 B 6 B 7 B 8 B

F. Figure: Floral Resource Provisioning: species placement in plots Ranch 5 and 6 (2010-2011)



Conclusions

Many species of natural enemies (predators and parasitoids) of agricultural pests feed on non-host food, including floral resources (Kehrli and Bacher 2008, Gurr et al. 2012). It has been demonstrated that nectar feeding is key to the survival of many adult parasitoids and can have a positive impact on fitness through an enhanced rate of egg maturation, prolonged reproductive longevity, or both (Heimpel and Jervis 2005, Lee and Heimpel 2008, Harvey et al. 2012).

A leading hypotheses in biological control science explaining decreased pest densities in some biologically diversified farming systems is the 'natural enemies hypothesis.' The hypothesis posits that non-crop plant abundance and diversity in agroecosystems enhances the fitness and effectiveness of natural enemies of arthropod pests through habitat provisioning resulting in increased natural pest regulation (Russell 1989, Letourneau et al. 2011, Landis et al. 2012, Bennett and Gratton 2013). It is further theorized that increased flowering plant abundance in agroecosystems provides predators and parasitoids of pests with otherwise limiting resources such as refuge, favorable microclimates, alternative hosts or prey, or plant-based foods such as pollen and nectar, thus increasing natural enemy fitness, herbivore mortality and thus increased pest regulation (Altieri and Nicholls 2004, New 2005). Farming system diversification with functional biological diversity is increasingly identified as an important management strategy for both enhancing biological control of arthropod pests and improving the multi-functionality and ecological sustainability of agroecosystems (Altieri 1999, Fiedler et al. 2008, Crowder et al. 2010, Power 2010, Kremen and Miles 2012).

The objectives of the study were to evaluate the influence of floral resource provisioning – intercropping with selected flowering plant species - and complementary chemical ecology strategies on biological control of *Erythroneura* Leafhoppers (Hemiptera: Cicadellidae) and *Planococcus* Mealybug (Hemiptera: Pseudococcidae) in California vineyards. The aim of the research was to both test the natural enemies hypothesis while contributing to the development of cost-effective pest management strategies that meet or exceed USDA National Organic Program production standards.

The study evaluated the effect of floral resource provisioning on selected natural enemy fitness, reduced crop damage, pest regulation and natural enemy abundance, all theorized to be enhanced through the addition of flowering plants to monoculture agroecosystems. The study measured the impact of 3 flowering ground covers, lacy phacelia (*Phacelia tanacetifolia*), bishop's weed (*Ammi majus*), and common carrot (*Daucus carota*) on biological control of leafhoppers and vine mealybug by the parasitoids *Anagrus spp.* (Hymenoptera: Mymaridae) and *Anagyrus pseudococci* (Hymenoptera: Encyrtidae). The research project included three large-scale and fully replicated research designs located in the central San Joaquin, the northern San Joaquin, and the Napa Valley of California, all important grape-growing regions of California.

Controlled laboratory studies quantified the impacts of floral resource provisioning on the fitness of *Anagyrus pseudococci*, a key parasitoid natural enemy of the vine mealybug, a globally important vineyard pest. The central San Joaquin Valley field study measured the impact of FRP and pheromone based mating disruption on biological control of vine mealybug. The northern

San Joaquin Valley field study measured the impact of FRP and methyl salicylate on natural enemy abundance and biological control of *Erythroneura* leafhoppers. The Napa Valley field study measured the effect of methyl salicylate (MeSA) alone on natural enemy abundance and biological control of *Erythroneura* leafhoppers.

The overall findings of the study were that FRP alone (and in combination with chemical ecology strategies) had little significant impact on natural enemy fitness, abundance, parasitism rates or pest abundance. The controlled laboratory studies found that, despite evidence of nectar feeding on the FRP species, the enhancement of *A. pseudococci* longevity correlated only with exposure to a honey solution and *Ammi majus*. Mean longevity of *A. pseudococci* was found to be significantly negatively impacted by exposure to phacelia. Survivorship analysis showed that survival probability of *A. pseudococci* was significantly greater in the honey solution, buckwheat, wild carrot and bishop's weed treatments. Exposure to Phacelia, however, was found to significantly reduce the survivorship probability of *A. pseudococci*. None of the FRP treatments were found to have any significant impact on the parasitism rate of vine mealybug (VMB), with the exception of *Ammi majus,* which exhibited a significant negative impact on parasitism. None of the FRP treatments had any significant effect on second-generation sex ratios of *A. pseudococci*.

The central San Joaquin Valley field study showed that FRP alone resulted in no significant differences on parasitism rates of sentinel vine mealybug (VMB), overall VMB densities or crop damage in 2009. In 2010, leaf densities of VMB were found to be significantly lower in FRP plots at harvest time (September) when compared to control plots. FRP was shown to reduce late summer (August) VMB density over that of pheromone-based mating disruption. However, a lower mean rate of parasitism was measured in the FRP treatment plots in late summer of 2010, indicating a control mechanism other than parasitism by *A. pseudococci*. No significant difference in crop damage levels were found between FRP and control plots in either 2009 or 2010.

The Napa Valley field study found no significant impact of the PredaLure ® (MeSA) alone on biological control of *Erythroneura* leafhoppers. With the exception of a greater cumulative mean abundance of non-*Anagrus spp.* parasitoids found in the MeSA plots in 2009, and a statistically marginal ($P = 0.0795$) reduction in adult leafhopper densities at harvest time in 2010 only, no evidence was found supporting the conclusion that MeSA had any significant impact on seasonal or peak *Erythroneura* leafhopper nymph density, key natural enemy abundance or adult leafhopper densities at harvest time in either 2009 or 2010.

The northern San Joaquin Valley "attract and reward" field study found that, other than enhancing the abundance of non-*Anagrus spp.* parasitoids on 3 sample dates in 2010, neither floral resource provisioning alone nor the combination of FRP + PredaLure ® (MeSA) had any significant impact on key natural enemy abundance, *Erythroneura* leafhopper nymph density or leafhopper egg parasitism rate by *Anagrus spp.* in 2010 or 2011.

Theoretical explanations of key findings of research:
Chapter 2: Quantifying the impact of floral resource provisioning on the fitness of *Anagyrus pseudococci*, a key natural enemy of the vine mealy bug, *Planococcus ficus.* Key theoretical

reasons why longevity was extended only by the honey solution and *Ammi majus* (and not other FRP treatments) may be due to the quantity or quality of the sugars provided being not suitable or accessible to *A. pseudococci*. It has been postulated that sugar concentration, sugar ratios (glucose, fructose and sucrose) as well as flower morphology (thus accessibility of available nectar) may have a significant impact on longevity and parasitoid fitness (Araj et al 2008, Tompkins et al. 2010, Carrié and Wäckers 2012, Suma et al. 2013). The finding that *A. pseudococci* longevity was significantly decreased in the presence of phacelia is consistent with prior parasitoid nectar feeding studies where it was hypothesized that corolla depth may be incompatible with the preference of the parasitoid (Vattala et al. 2006). This hypothesis, however, is inconsistent with our research findings showing that *A. pseudococci* derived significant amounts of fructose and sucrose from phacelia. This finding raises the further question as to the possible toxicity of phacelia nectar to *A. pseudococci*. Our findings also show that, with the exception of *Ammi majus*, which increased mean longevity while reducing the mean parasitism by *A. pseudococci*, exposure to all other FRP species resulted in no significant differences in parasitism rates. This finding is consistent with other studies of nectar feeding showing that natural enemies exhibit significant inter-specific variation in their preferences and physiological responses to different species of flowering plants (Araj et al 2008; Luo et al. 2010; Sivinski et al. 2011). The finding that FRP treatments resulted in no statistically significantly effect on the sex ratios of offspring of *A. pseudococci* is consistent with the scientific literature showing sex ratio being dependent on the host instar stage and temperature over other environmental conditions such as carbohydrate feeding (Suma et al. 2013).

Chapter 3: Evaluating the influence of floral resource provisioning and pheromone-based mating disruption on biological control of *planococcus* mealybug. The central San Joaquin Valley field study showed that FRP alone resulted in no significant differences on parasitism rates of sentinel vine mealybug (VMB), overall VMB densities or crop damage at harvest time in 2009. These findings are consistent with the laboratory results of this research showing that, despite evidence of nectar feeding, FRP had no significant impact on VMB parasitism rates by *A. pseudococci.* In 2010 leaf densities of VMB were found to be significantly lower in FRP plots at harvest time (September) when compared to control plots. FRP was also shown to reduce late summer (August) VMB leaf density over that of pheromone-based mating disruption. A lower mean rate of parasitism was measured, however, in the FRP treatment plots in late summer of 2010, indicating a control mechanism other than parasitism by *A. pseudococci*. Prior work on the VMB-predator system indicates that predation by *C. montrouzieri* (“mealybug destroyer”) or spiders may be, in part, responsible for lower VMB densities in FRP plots (Daane et al. 2008, Gutierrez et al. 2008). This conclusion, however, was not supported by a 24-hr predator observation study, finding no *C. montrouzieri* in treatment or control plots. In the same predator observation study, spiders were found to constitute only 6.32% of all natural enemies observed and are assumed to play a secondary role behind that of the specialist predators and parasitoids, *C. montrouzieri* and *A. pseudococci*, respectively (Daane et al. 2008, Miles and Wilson unpublished data). As no significant differences in crop damage at harvest time were detected in either 2009 or 2010, the hypothesis that FRP would lead to significantly lower levels of crop damage through enhanced biological control of VMB was not statistically supported in this study. These findings are consistent with the laboratory experiment showing no significant differences in parasitism with exposure to FRP species, and the larger body of research showing that biological control of vine mealybug by *A. pseudococci* is consistently only partially successful (Daane et al. 2012, Walton et al. 2012).

Chapter 4 (Experiment #1): measuring the impact of floral resource provisioning and methyl salicylate on biological control of *Erythroneura* leafhoppers in California vineyards. The finding of no significant difference in natural enemy and pest abundance in between PredaLure ® only and control plots is consistent with prior research showing no or conflicting results of the impact of MeSA treatments on *Anagrus spp.,* other natural enemies and pest regulation (Lee 2010, Rodriguez-Saona et al. 2011, Gadino et al. 2012, Kaplan 2012). The lack of supporting statistical evidence that PredaLure ® alone had any significantly impact on natural enemies or pest abundance may also be attributed to plot size. As there is little scientific knowledge on the distribution of HIPVs in the field, the plot size and buffer areas assigned to the study may have been too small to prevent the MeSA signal emitted from the PredaLures being equally distributed throughout the research plots resulting in the elimination of an effective control (Kaplan 2012). This issue may be compounded by the phenomenon of synthetic HIPVs stimulating plant-produced HIPVs in neighboring plants (James et al. 2012).

Chapter 4 (Experiment #2): The results of our study finding no significant difference in *Erythroneura* leafhopper nymph densities between FRP and control plots in the "attract and reward" research may be, in part, attributed to the low background population densities of leafhoppers documented in 2010-2011. These findings of are consistent with prior cover crop research by Daane and Costello (1998) finding no significant effect of a barley and vetch cover crop on either key natural enemies or *Erythroneura* leafhopper abundance when mean leafhopper densities were less than 10 nymphs/leaf. Their study did find an estimated 15-20 % reduction of leafhopper nymphs only at higher mean densities of ~20-30 nymphs/leaf, a non-economic control level. No effective control was found at nymph densities above ~ 30 nymphs/leaf. Importantly, the mechanism of control was found to be most strongly correlated with the cover crop's impact on vine vigor/plant host quality and only secondarily to predation by spiders (Costello and Daane 2003). Further, the finding of no significant effect of FRP on leafhopper nymph abundance is consistent with prior work showing no reduction in leafhopper nymph abundance with FRP (English-Loeb et al. 2003).

The lack of significant differences in key natural enemy abundance found in FRP v. control plots in the "attract and reward" study may have resulted, in part, from small plot size resulting in natural enemies being relatively evenly distributed throughout treatment and control plots. The specific finding of FRP having no significant impact on *Anagrus spp.* abundance is consistent with prior work showing no FRP with buckwheat, clover and sod had no consistent impact on adult *Anagrus spp.* abundance (English-Loeb et al. 2003).

The finding of no significant effect of FRP on parasitism rates of leafhopper eggs by *Anagrus spp.* wasps is consistent with prior work by Nicholls et al. (2000) showing FRP with sunflower (*Helianthus annuus*), and annual buckwheat (*Fagopyrum esculentum*) had no significant impact on parasitism by *Anagrus spp.* These findings however are inconsistent with the work of English-Loeb et al. (2003) who found that, under controlled conditions, *Anagrus spp.* longevity was significantly increased when parasitoids were provided with honey or sugar water, and parasitism of leafhopper eggs were greater when *Anagrus spp.* had access to flowering buckwheat flowers.

The absence of supporting evidence that FRP + PredaLure ® had any significant impact on natural enemy or pest abundance may also be attributed to low background density of leafhoppers and plot size, as discussed above. Lastly, as the area, diversity, species composition and distance from non-crop habitats are all know play an important role in determining the abundance and diversity of both pest and natural enemies in agroecosystems (Tscharntke et al. 2007) landscape factors my be overwhelming any localized treatments of FRP and/or MeSA (Gámez-Virués et al. 2012).

Though floral resource provisioning may serve to convey a range of other important ecosystem services to and from agroecosystems, including the enhancement of soil quality and the provisioning of nectar to pollinator species, among others, the findings of this research indicate that the integration of the selected species of flowering plants into large-scale vineyard monocultres is not a reliable strategy for effectively managing either *Erythroneura* leafhoppers or *Planococcus* mealybug. Although enhanced pest regulation may occur at higher densities of the pest, consistent economic control of *Erythroneura* leafhoppers or *Planococcus* mealybug has not been established using FRP alone or in combination with other chemical ecology strategies in California vineyards.

As many ecological factors influence natural enemy abundance and pest regulation in vineyards, including landscape heterogeneity (Chaplin-Kramer et al. 2011), vineyard age and cultural practices such as fertilization and irrigation (Costello and Daane 2003), much more applied ecological research will be necessary to develop a comprehensive scientific and practical understanding of how to manage both the biotic and physical environmental conditions in vineyards to consistently reduce *Erythroneura* leafhoppers and *Planococcus* mealybug.

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