ORGANIC FARMING RESEARCH FOUNDATION

FINAL RESEARCH REPORT

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Project Title: Integrating Biological Control with Trap Crop Management in California Organic Strawberries

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<u>Project Summary</u>:

This research project sought evidence that a selective, imported biological control agent can persist in alfalfa trap crops that are managed with tractor-mounted vacuums for key pest control in an organic strawberry system in coastal Central California. This project was built upon previous OFRF-sponsored research conducted in Monterey County that demonstrated reduced damage of organic strawberries and improved net returns when using tractor-mounted vacuums to remove the key pest lygus bug (*Lygus hesperus*) from inter-planted alfalfa trap crops. With the release and conservation of the selective endoparasitoid *Peristenus relictus* (a nymphal parasitoid of the lygus bug) in organic strawberries, a more balanced systems-management approach to key pest control has been achieved. Despite the disturbances created by tractor-mounted vacuums, there were no statistically significant differences in lygus bug parasitism between vacuumed and un-vacuumed alfalfa and strawberries ($P > 0.05$). Both *P. relictus* larval abundance and percent parasitism were similar between treatments in 2007, 2008 and 2009. From the results of this study, we can conclude that a biological control program using the imported *P. relictus* can be successfully integrated with a vacuum-managed trap crop program in California organic strawberries.

The long-term conservation of *P. relictus* in this alfalfa trap cropping system also requires attention to alternate (low-disturbance) habitats and distribution patterns. Lygus nymphs collected from a floral refuge and insectary plant borders adjacent to organic strawberries demonstrated parasitism on a variety of host plants. Parasitism was detected on native California buckwheat and yarrow in 2007 and 2008. Sweet alyssum also served as a good alternate host site for *P. relictus* parasitism of lygus bugs during all three years. In 2009, alyssum demonstrated the greatest potential as an alternate host site for *P. relictus* by generating a 27% lygus nymph parasitism rate.

In a replicated transect experiment, we did not detect any positive spatial effects regarding increased biological control of lygus bugs with increased proximity to these floral resources. Future attempts to document a distance effect should utilize more precise methods, such as mark-capture techniques that will specify the exact scale of insect movement from floral sources into fields. All these future experimental efforts will help to continue our work in establishing a unique systems-management approach to lygus bug control in organic strawberries, offering a combination of physical suppression (vacuuming), selective parasitism (introduced biological control), and alternative floral resources for conservation biological control.

Research Issue Statement

Lygus hesperus Knight (Hemiptera: Miridae), commonly known as the lygus bug, is a key pest of strawberries on the California Central Coast. The “catfacing” damage caused by lygus bug feeding is often the most costly arthropod pest problem in organic strawberries. The lack of effective and compliant spray materials for lygus bug control makes this pest particularly challenging to organic strawberry growers. Our 2002 OFRF research grant report documented improved, organically compliant, non-chemical suppression of lygus bugs with a combination of alfalfa trap cropping and tractor-mounted vacuums (Swezey *et al*., 2007). This trap cropping system reduces lygus bug-induced damage in strawberry rows adjacent to vacuumed trap crops when compared with the more typical approach of vacuuming strawberry plants without a trap crop (Fig. 1). We subsequently successfully introduced the exotic nymphal parasitoid *Peristenus relictus* (Hymenoptera: Braconidae), which is now abundant on our organic research site (Pickett *et al*. 2009).

However, little was known about the effects of trap crop management on native or introduced biological control agents attacking lygus bugs. A more balanced systems-management approach to lygus bug control in organic strawberries would incorporate imported parasitoids and establish undisturbed floral resources to complement the already successful physical suppression methods. This integrated approach to lygus bug management strives to further diminish the cosmetic damage caused by lygus bug and improve fresh market net yields for organic growers.

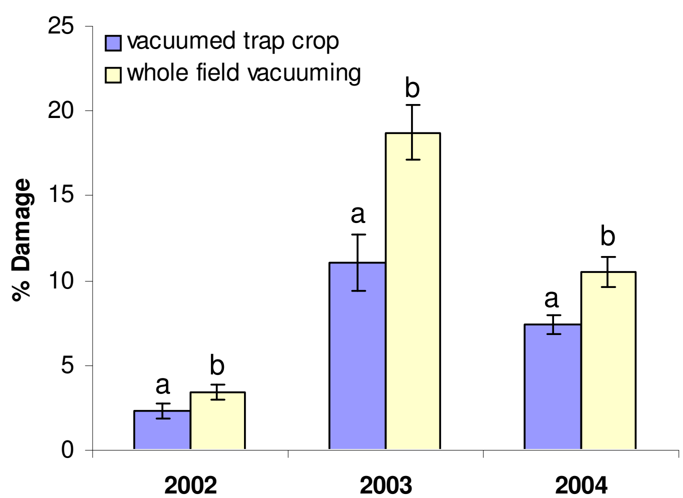

Figure 1. Average lygus bug-induced damage in June treatments of organic strawberries at Eagle Tree Farm, Prunedale. Treatment means followed by a different letter are significantly different within a season; ANOVA ($p<0.05$).

Beginning in 2004, Pacific Gold Farms collaborated with our research team to release the lygus bug nymphal parasitoid *P. relictus* on an organic strawberry farm in Prunedale, CA. Since that time, Pacific Gold has planted and maintained flowering vegetation with low disturbance levels and high resource availability to ensure the establishment and proliferation of these imported parasitoids. As a result, *P. relictus* increased parasitism of lygus bug nymphs in unvacuumed vegetation. However, it was unclear to what degree these parasitoids could be integrated into a more intensely disturbed system such as vacuumed alfalfa or strawberries.

If it were demonstrated that *P. relictus* could reliably persist in an intensively managed environment, they would provide valuable biological control without compromising the lygus bug reductions already achieved through vacuuming. If successful, a more integrated, cultural/biological systems-management approach to lygus bug control in organic strawberries would be achieved through alfalfa trap crop establishment, selective/proactive physical suppression (vacuuming), and the introduction and colonization of host-specific biological control agents.

Pacific Gold Farms has been an integral part of our lygus bug management research for seven years. Grower collaborator Larry Eddings has contributed innovative and progressive ideas based on nearly three decades of growing experience. This collaboration has generated practical results that have changed organic farming practices and improved profitability. This research project includes protocols designed to reduce the impact of lygus bugs in a manner that is feasible and advantageous to the grower-collaborator.

Literature

Strawberries are damaged by the western tarnished plant bug (WTPB) or lygus bug, *Lygus hesperus* Knight (Hemiptera: Miridae) in California (Allen and Gaede 1963). This insect is a native species and it feeds on a broad range of winter broadleaved weeds in coastal central California, including wild radish, mustards, chickweed, common groundsel, lupine and other legumes, and knotweed (Strand, 1994; Barlow et al. 1999). More than 100 species in 24 plant families have been listed as WTPB hosts (Scott, 1977; Barlow et al. 1999). Leguminous cover crops (vetches, clover, and especially alfalfa, etc.) can also host WTPB in spring and summer. Alfalfa is consistently listed in these host inventories as a preferred host. Only the adult form of WTPB can fly from one plant to another, and they appear to move from one host plant to another as each plant begins to flower. In coastal central California, *L. hesperus* overwinters as an adult in winter broadleaved hosts. In the spring, when the rains cease and the weeds desiccate, the adults rapidly colonize flowering crops, such as strawberry. Strawberries are not a preferred host of WTPB in California, but the absence of other more attractive host plants in early summer stimulates their colonization. Based on heat unit accumulations typical of the central coast, two to three generations of WTPB develop between April and November in strawberries.

WTPB adults are about 6 mm. long and variable in color. They are characterized by a conspicuous yellow or pale green 'v' on the scutellum. Females insert eggs into host plant tissues. Often, only the operculum or tip of the egg is visible externally; thus lygus eggs are not easily detectable. The first and second lygus instars are tiny and pale green with a distinct red terminal antennal segment. The third through fifth instars are larger and have five dorsal black dots (Sorensen 1939).

Feeding by all five nymphal stages and adults causes distortion of the berries, known as "catfacing," rendering the fruits unacceptable for fresh market sale. Distortion of the berries occurs when feeding by WTPB destroys developing embryos in achenes (seeds) during early fruit development, preventing growth of the fruit tissue beneath and surrounding the damaged achenes (Handley and Pollard 1993). Damage can also be centered on the apical area of the berry and, for that reason, is sometimes referred to as "apical seediness." Allen and Gaede (1963) first showed that WTPB damage was not due to a toxic action but rather due to feeding inside the achenes and the subsequent destruction of the enclosed embryo and endosperm. Hormones responsible for receptacle enlargement are produced in the achenes and then translocated to the surrounding tissue. When WTPB feeds on the achenes, hormone synthesis and translocation are disrupted.

Several studies indicate that WTPB feed on strawberry achenes from the time that the flower opens until the beginning of enlargement, when the achenes become too hard for their stylets (mouthparts) to penetrate. Handley and Pollard (1993) conducted electron microscope observations of WTPB feeding and damage and found that the holes in damaged achenes were consistent with the size of WTPB stylets. They also found that after selecting a feeding site, WTPB punctures the achene several times, causing considerable damage that results in a hollow achene. Damaged achenes range in size from completely undeveloped to fully developed. Allen and Gaede (1963) hypothesized that because WTPB bugs are able to feed on the achenes from 4 days before pollination to approximately 10 days after pollination, there should be more large hollow achenes than small achenes on a damaged strawberry. Both WTPB adults and nymphs feed on developing achenes, but because nymphs appear to be quicker to select a site and are unable to fly, they tend to stay on a single seed/plant longer and thus cause more damage than adults.

In their electron microscope observations, Handley and Pollard (1993) found that after achenes become too hard for WTPB to penetrate, they begin to feed on the receptacle near the achenes. WTPB then targets the vessel conducting nutrients to the developing achene. Digestive enzymes from WTPB saliva can cause necrosis, deformation, and fruit abscission. Udayagiri and Welter (2000) observed that WTPB preferred to oviposit on the strawberry receptacle because of the complexity of its surface and the small distance between achenes on an enlarging berry. WTPB oviposition into the receptacle alone can cause damage because of the wound created. WTPB adults that oviposit in strawberry may also simultaneously feed, resulting in additional structural fruit damage.

Insufficient pollination can also be responsible for the non-enlargement of some achenes. However, these achenes are small with their embryo and endosperm intact. Riggs (1990) found that non-pollination alone did not have a significant effect on fruit deformity but that the interaction between WTPB feeding and pollination was highly significant. He also demonstrated that, while WTPB feeding at the early stages of achene development caused fruit deformity, after 16-17 days of development WTPB feeding mostly affected only fruit weight. These sources suggest that the main criteria for distinguishing WTPB damage from other types of damage in strawberries are: (1) the emptiness of the damaged achenes, (2) the non or partial enlargement of the surrounding receptacle, and (3) the color of the achenes: green (possibly damaged) or brown (damaged).

WTPB nymphal densities in strawberry fields can be consistently and most economically estimated by beating plants onto a clean enclosed surface, such as a white pan or white sheet of paper or cloth. Since adults are very mobile, their numbers are more accurately estimated by vacuuming plants using a leaf blower modified to act as a vacuum (Zalom et al. 1993). Economic thresholds are estimated to be exceeded at densities of 1 or 2 WTPB per 20 strawberry plants sampled (Strand 1994).

WTPB densities should be monitored throughout the season in organic strawberries. Organically acceptable insecticides are not very effective against the early instars; hence preventive control measures must be undertaken. These preventive measures must be timed especially to coincide with periods soon after egg hatch in each generation. Since WTPB adults migrate to strawberries from weeds in the spring, when strawberry flowering commences, plants need to be monitored in spring to determine the first appearance of WTPB adults. Egg hatch can be estimated using a degree-day (DDF) model (Pickel et al. 1990). Using a base temperature of 54°F, egg hatch is estimated to occur at 252° DDF. On the central coast in California, this translates to approximately 3 - 4 weeks after the first appearance of an adult in a trap crop, weeds, or strawberries under cool spring temperatures. The second hatch of nymphs is predicted to occur 779° DDF after the first nymphs are found in surrounding weedy host plants. The third hatch of nymphs is predicted to occur 799° DDF after the first adults are found in the strawberries (Strand 1994).

Naturally occurring native predators of WTPB eggs and nymphs include big-eyed bugs, *Geocoris spp*.; minute pirate bugs, *Orius spp*.; green and brown lacewings, *Chrysoperla* and *Hemerobius spp;,* damsel bugs, *Nabis spp.;* and several species of spiders (Clancy and Pierce 1966). Monitoring or scouting should take the abundance of these beneficial insects into account. While these predators feed on WTPB eggs and nymphs, they often do not keep summer populations below the economic injury level. *L. hesperus* is also parasitized by the egg parasitoid *Anaphes iole* Girault (Hymenoptera: Mymaridae) (Norton and Welter 1996) and the nymphal parasitoid *P. relictus* (Pickett et al. 2009).

Efforts to suppress WTPB populations using tractor mounted vacuum devices, including the "bug vac" technology (originating on the California central coast), were originally reported to be successful in lowering adult and nymphal densities and reducing damage (Vincent and LaChance, 1993; Pickel *et al*. 1994). Depending on suction force generated and operational method, vacuum machines are an important and common tool for suppression of WTPB in organic strawberries in coastal Central California. Since most vacuum machines remove larger instars and adults, but may have limited impact on the early instars, weekly use of the vacuum is necessary for effective control. Given that WTPB adults are highly mobile, growers should routinely check the vacuuming airflow and tractor speeds of operation. If necessary, mechanical adjustments and refinements to vacuuming protocols should be made to ensure a machine is effective in reducing WTPB densities and damage. Vacuuming alfalfa should begin as soon as fields are dry in the spring and can be timed to coincide with intervals in which nymphs and adults are abundant according to degree-day unit calculations mentioned above.

A WTPB suppression strategy that had been tested in cotton in the San Joaquin Valley of California (Stern et al. 1964, 1969; Sevacherian and Stern 1974; Godfrey and Leigh 1994) is the establishment of alfalfa trap crops adjacent to cotton. Since alfalfa is a preferred host for WTPB, cotton is protected by close proximity to alfalfa when properly managed. We showed that this relationship is also true for an alfalfa/strawberry association for organic strawberry production (Swezey et al., 2007). Alfalfa trap crops are inter-planted within strawberry fields to attract WTPB. Once this pest is concentrated in the trap crop, it can be controlled by vacuuming with a tractor-mounted vacuum machine, which generates a suction force sufficient to remove these insects from the trap crop and killing them in the fan housing. This strategy also seeks to avoid vacuuming the adjacent strawberry rows as much as possible, since the vacuum machines are non-selective and also remove beneficial insects from the strawberry crop (Vincent and LaChance 1993). Repeated in-field vacuuming may also increase problems with strawberry pollination and mold and mildew by spreading pathogenic spores (Strand 1994). Any control measures (vacuuming, release of beneficial insects and pathogens, etc.) would be concentrated in the trap crop vegetation. Easterbrook and Tooley (1999) reported that trap crops of alfalfa and plants in the family Asteraceae did *not* consistently reduce *Lygus rugulipennis* in strawberries in England. However, they did not incorporate vacuum treatments of the trap crop into their experiment.

Methods

OBJECTIVE 1: Determine parasitism levels of host lygus bugs by *P. relictus* while exposed to the growing season disturbances created by tractor-mounted vacuums.

This experiment was conducted on the 120-acre Pacific Gold organic strawberry farm in Prunedale, CA from 2007-2009. Alfalfa trap crops were inter-planted every 40-50 strawberry rows, amounting to approximately 2-3% of total acreage. To test the effects of vacuuming on *P. relictus* activity in alfalfa, four 50 m. alfalfa trap crop replicates were vacuumed weekly (2007) or biweekly (2008-2009) from July-October, and were compared with four similar un-vacuumed replicates. An insect sample in these replicates consisted of 200 one-second suctions using a hand held insect vacuum (reversed leaf blower) taken randomly from each replicate. Once collected, lygus bug nymphs were separated from other insects on a beating sheet using an aspirator and immediately preserved in alcohol. These preserved samples were mailed to the CDFA Biological Control laboratory where they were dissected to detect the presence of developing parasitoids. Nymphs found with *Peristenus* eggs or larvae inside were recorded as parasitized.

OBJECTIVE 2: Measure the parasitism levels of lygus bug nymphs by *P. relictus* in floral refuges adjacent to strawberry production areas.

To determine which flowering plant might be best suited for *Peristenus* in an adjacent floral refuge, samples were taken from a two-acre refuge that was set aside for experimentation, containing 16 managed rows, each 350 ft. long, and separated by 5 ft. Among the plants included in these rows are sweet alyssum, buckwheat and yarrow. Each planting was replicated four times in 50 ft strips randomly within each row. Samples consisted of 100 one-second suctions using the hand-held vacuum and were collected monthly from July-September. Once collected, lygus bug nymphs were processed as previously described above. Samples were also taken from four adjacent 50 m. "insectary plant" rows (clover, buckwheat, alyssum, yarrow, and other annuals), sampling only from the buckwheat, alyssum, and yarrow plants in the row.

OBJECTIVE 3: Identify spatial correlation between *P. relictus* parasitism of lygus bugs and mixed floral insectary rows.

Insectary borders or the floral refuge (depending on the experimental year) were used as the starting point for all distance transects. The first replicate of vacuumed and un-vacuumed alfalfa and strawberries was approximately 40 rows away from this border. The second set of replicates was 80 rows away from the border, the third 120 rows distant, etc. Data that were collected for the vacuuming experiment were analyzed separately based on their spatial relationship to these floral resources.

All statistical analyses of these experiments were performed with the ANOVA program of STATISTICA software, by Stat Soft Inc., Tulsa, OK at an alpha level = 0.05. Insect counts were transformed by the log transformation if necessary before statistical analysis.

Results and Discussion

OBJECTIVE 1: Determine parasitism levels of host lygus bugs by *P. relictus* while exposed to the growing season disturbances created by tractor-mounted vacuums.

In 2007, *P. relictus* parasitism persisted in highly disturbed (vacuumed) environments (Figs. 2 and 3). Percent parasitism rates in both vacuumed alfalfa and strawberries were comparable to rates in controlled (unvacuumed) treatments, particularly in October.

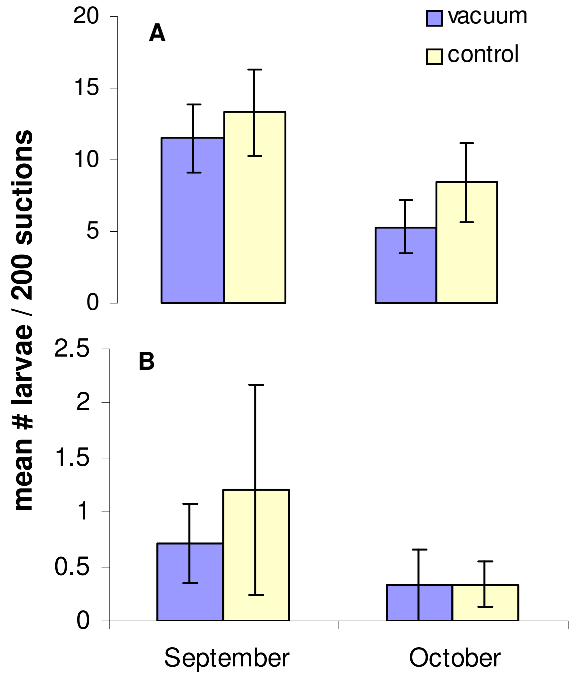

Figure 2. Average *P. relictus* larvae abundance from dissected lygus bug nymphs in vacuumed and control (un-vacuumed) alfalfa (A) and strawberries (B). Data collected from Eagle Tree Farm, Prunedale, CA (2007).

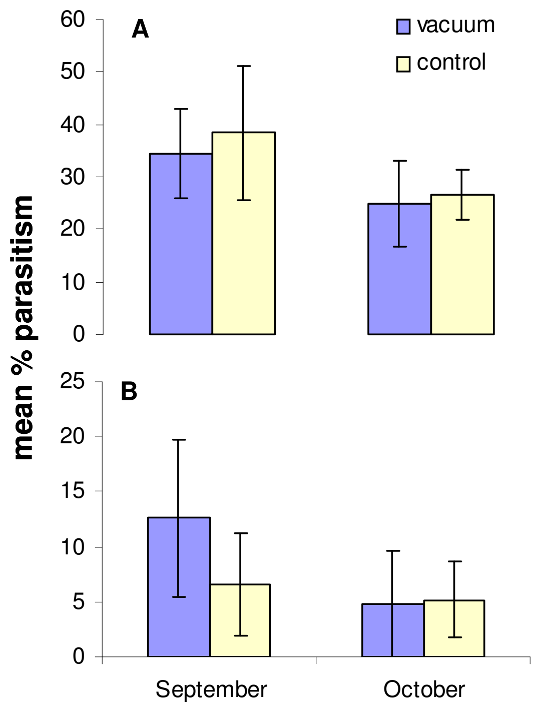

Figure 3. Average percent *P. relictus* parasitism of lygus bug nymphs in vacuumed and control (un-vacuumed) alfalfa (A) and strawberries (B). Data collected from Eagle Tree Farm, Prunedale, CA (2007).

In July-September of 2008, mean *P. relictus* larval parasitoid abundance (Fig. 4) and percent parasitism (Fig. 5) were again comparable (no statistical differences) in vacuumed and un-vacuumed alfalfa. In 2008, parasitism of lygus nymphs in alfalfa peaked at 16%.

In September of 2008, mean *P. relictus* larval abundance and percent parasitism was compared in vacuumed and un-vacuumed strawberries on three sample dates (data not shown). No significant differences in parasitism were detected between these treatments: mean larval abundance was 0 and (0.06 ± 0.04) per 200 suctions in un-vacuumed and vacuumed strawberries, respectively. Mean percent parasitism was 0 and (2.0 ± 1.4) in un-vacuumed and vacuumed strawberries, respectively.

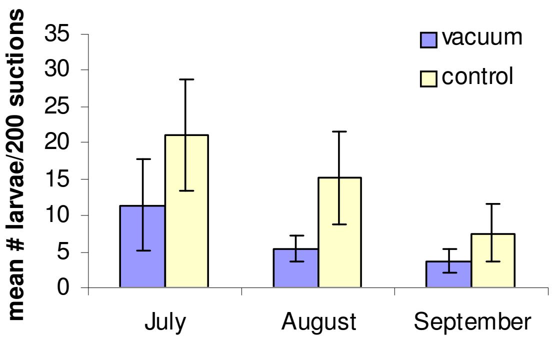

Figure 4. Average *P. relictus* larvae abundance from dissected lygus bug nymphs in vacuumed and control (un-vacuumed) alfalfa. Data collected from Eagle Tree Farm, Prunedale, CA (2008).

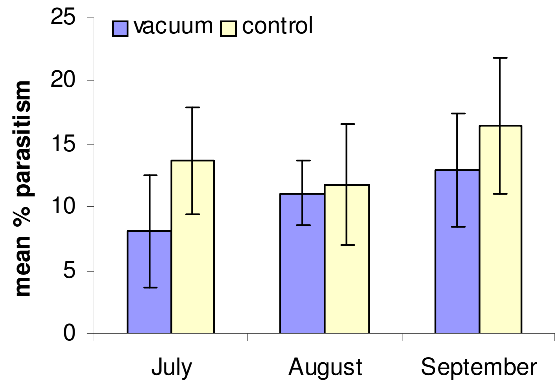

Figure 5. Average percent *P. relictus* parasitism of lygus bug nymphs in vacuumed and control (un-vacuumed) alfalfa. Data collected from Eagle Tree Farm, Prunedale, CA (2008).

In 2009, parasitism of lygus nymphs in alfalfa was again similar between vacuuming treatments (Figs. 6 and 7). In August of 2009, parasitism of collected nymphs reached 52% in un-vacuumed alfalfa, which exceeded recorded rates in either 2007 or 2008. Both treatments were above 40% parasitism in August and September of 2009.

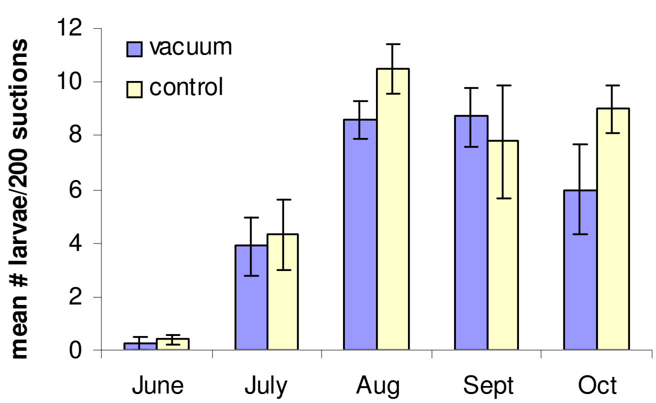

Figure 6. Average *P. relictus* larvae abundance from dissected lygus bug nymphs in vacuumed and control (un-vacuumed) alfalfa. Data collected from Eagle Tree Farm, Prunedale, CA (2009).

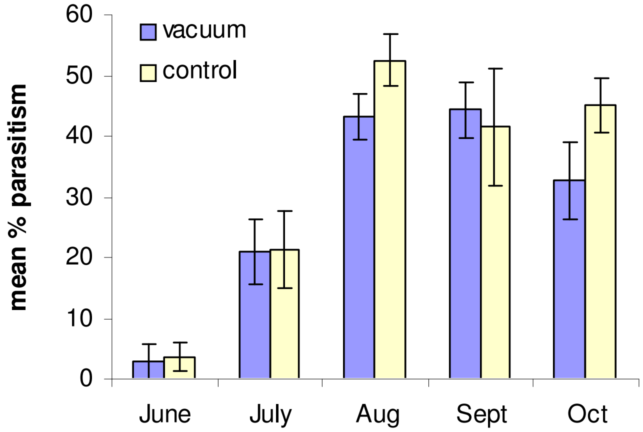

Figure 7. Average percent *P. relictus* parasitism of lygus bug nymphs in vacuumed and control (un-vacuumed) alfalfa. Data collected from Eagle Tree Farm, Prunedale, CA (2009).

There were no statistical differences in parasitism of lygus bugs between vacuumed and un-vacuumed alfalfa and strawberries. Both *P. relictus* larval abundance and percent parasitism were similar between treatments in 2007, 2008, and 2009 in spite of the disturbances caused by tractor-mounted vacuums. This indicates that *P. relictus* adults can avoid these large, slow-moving tractors, either by movement or establishment in un-vacuumed field margins. Future studies could test these hypotheses using sticky cards and/or marker proteins in treated and untreated areas. Also, as trap crops in this experiment were vacuumed once-weekly, there were extended periods of time with relatively low levels of disturbance that likely facilitated re-invasion, host detection and egg-laying by *P. relictus*.

Future studies should investigate how more frequent passes of the tractor-mounted vacuums (disturbances) are related to parasitism. It appears that current vacuuming protocols (one pass per week) do not significantly interfere with the biological control of lygus bugs by *P. relictus* in organic strawberries. From the results of this study, we can conclude that a biological control program using the imported *P. relictus* can be successfully integrated into a vacuum-managed trap crop program in California organic strawberries.

OBJECTIVE 2: Measure the parasitism of lygus bug nymphs by *P. relictus* in floral refuges adjacent to strawberry production areas.

Due to floral architecture, *P. relictus* may not be able access nectar from either strawberry or alfalfa flowers. Providing additional flower types in floral refuges may be important to increasing parasitism levels. While this experiment didn't observe adult *P. relictus* feeding, it did document *P. relictus* parasitism on other plants that potentially offer accessible feeding sites. Other than alfalfa and strawberries, *P. relictus* was able to locate and parasitize lygus bugs on sweet alyssum, buckwheat and yarrow. This result indicates that this parasitoid is dynamic in its ability to parasitize lygus nymphs.

In the experimental two-acre floral refuge in the interior of this organic strawberry farm, parasitism of lygus nymphs was high on buckwheat, alyssum, and yarrow in 2008 (Fig. 8). However, in 2009, strawberries were rotated out of production in the areas surrounding this refuge. Consequently, both lygus bug and *P. relictus* parasitism were almost completely absent during all of 2009 in the refuge despite the many different suitable feeding hosts available to lygus bugs. In 2010, strawberries will again be planted adjacent to this refuge and we expect parasitoid activity to again be elevated. If this is the case, it may be useful to think of this refuge not as an ecological island, but rather an important interactive component of nearby organic strawberry production.

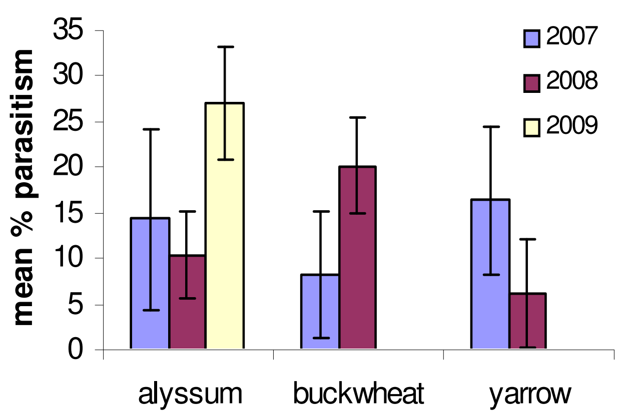

Figure 8. Parasitism of lygus bug nymphs by *P. relictus*. Nymphs collected from annual and perennial plants in a floral refuge and field-edge insectary habitats. Data collected from Eagle Tree Farm, Prunedale, CA.

P. relictus was also active in field-edge insectary habitats, particularly in sweet alyssum. In 2009, nymphal parasitism reached 27% in alyssum. Parasitism on sweet alyssum is especially important due to its easily accessible nectar, particularly for braconid parasitoids such as *P. relictus*. Sweet alyssum has the potential to provide *P. relictus* with ample nutritional resources and hosts simultaneously. Future studies should focus on the interactions between alternate flowering vegetation, host detection, parasitoid feeding, and potential biological control benefits in strawberries.

OBJECTIVE 3: Identify any spatial correlation between *P. relictus* parasitism of lygus bugs and mixed floral insectary border rows.

We did not detect any significant spatial effects regarding the increased biological control of lygus bugs with reference to the proximity of the mixed insectary plant border in 2007 (Fig. 9) or the floral refuge in 2008 (Fig. 10). Lygus bug nymphs in all cases were more abundant in alfalfa trap crop rows than in mixed insectary plant borders.

Future attempts to document the effects of border insectary flower plantings should utilize more precise sampling methods, such as mark-recapture techniques, that will specify exact insect movement distances out of these plantings. A much smaller sampling universe may also be helpful, as the scale of this experiment may have been too large to detect spatial differences in lygus bug abundance and parasitism.

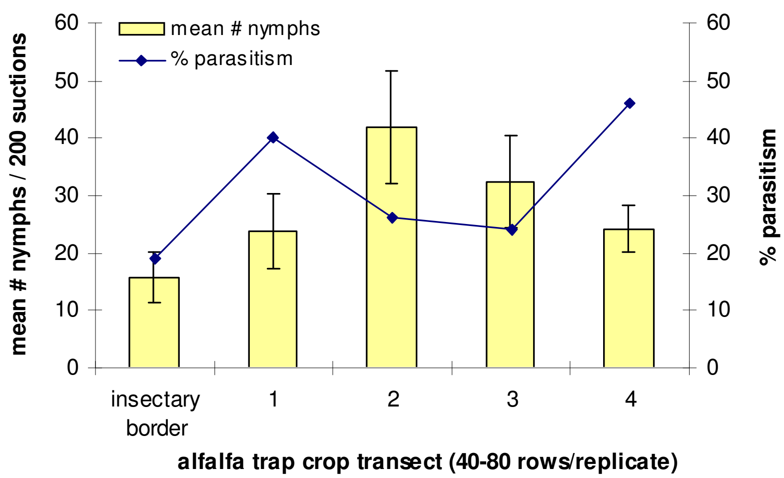

Figure 9. Average lygus bug nymph abundance and *P. relictus* percent parasitism from an insectary border and in-field alfalfa trap crops. Each trap crop (1-4) is separated by 40 strawberry rows. Data collected from Eagle Tree Farm, Prunedale, CA (2007).

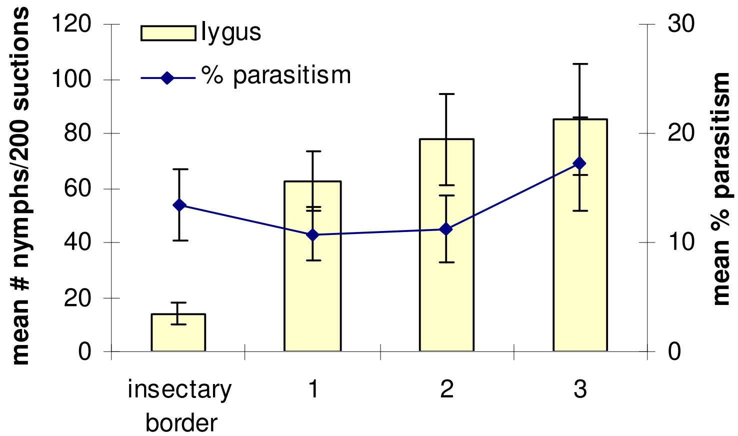

Figure 10. Average lygus bug nymph abundance and *P. relictus* percent parasitism from an insectary border and in-field alfalfa trap crops. Each trap crop (1-3) is separated by 40 strawberry rows. Data collected from Eagle Tree Farm, Prunedale, CA (2008).

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