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Influences of genetic population structure, landscape composition and herbivore phenology on parasitism of sunflower moth, *Homoeosoma electellum*, in California

By

Caterina Nerney Meyers

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Committee in charge:

Professor Stephen C. Welter, Chair
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Professor George Roderick

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Abstract

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Doctor of Philosophy in Environmental Science, Policy and Management

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This research examines influences upon the parasitoid guild of sunflower moth, *Homoeosoma electellum* Hulst (Lepidoptera: Pyralidae), a North American native specialist herbivore and pest of crop sunflower, *Helianthus annuus var. macrocarpus* (L.). I approach the system from the genetic population, landscape, and habitat scales to determine the relevance of different variables towards future efforts in conservation biological control of sunflower moth.
At the population level, I used a set of 9 microsatellites, or polymorphic neutral genetic markers, to examine the population genetic structure and level of gene flow within the most important specialist parasitoid of sunflower moth, *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae). I found that gene flow rates in this species are high, and that there are no signals of population substructure at the level of wild versus agricultural settings or local- regional geography. At the continental scale the population structure is detectable but gene flow still occurs. This points to a high degree of genetic mixing within this species in North America and indicates that genetic specialization towards wild or agricultural sunflower habitats is very unlikely. At the landscape scale I performed a field survey of sunflower moth larvae at 60 agricultural sunflower sites throughout the Central Valley of California during three summer growing seasons in 2003-2005. I then compared total parasitism rate, parasitoid species richness and relative proportions of generalist parasitoids to the proportion of habitat in the 1km radius circular area around each field in annual crops, orchards, riparian habitat and wild sunflower habitat. To follow this, I set up a sentinel larvae experiment to test the effects of surrounding habitat type upon the parasitism parameters. In the survey, I found a significant positive relationship between parasitism rate and the two other parasitoid guild parameters: parasitoid species richness and proportion of parasitism due to generalist parasitoids. The proportion of the area surrounding the field in orchard habitat was positively correlated with parasitism, parasitoid species richness and relative impact by generalists compared to the specialist parasitoid. While not statistically significant, parasitoid guild parameters in the sentinel larvae experiment generally followed the trends found in the survey. At the flower and

habitat scale I examined herbivore phenology in self seeding sunflower, *Helianthus annuus var. annuus* (L.) in 5 different patches during three peak bloom summer seasons to determine the relative abundance and within flower-head co-ocurrence rates of the major sunflower herbivores and their parasitoids. In addition to sunflower moth, *H. electellum,* I found several other common flower consuming herbivores in the wild sunflower habitat in California. One in particular, *Plagiomimicus spumosum* (Lepidoptera: Noctuidae), exhibited an earlier peak in and higher overall abundance relative to *H. electellum* and was present in high densities at all of the sites surveyed. This herbivore was rarely found in flowers with conspecifics or other florivore species and was statistically negatively correlated with other species at the flower-head level. This indicates that this species may be exerting competitive pressure within the flower consuming guild in wild settings. Observed larval parasitism of *P. spumosum* averaged 12.2%, compared to 33.3% parasitism of *H. electellum.* Two parasitoid species were found to attack both *H. electelluma* and *P. spumosum*: *Bracon nuperus* and *Erynnia tortricis*. Given that the co-occurrence of the two most abundant herbivores in flowers is significantly lower than that expected by chance and they share at least two parasitoid species, further experimental investigation of the interaction between these herbivores and the consequences for community composition is warranted.

In summary, this research demonstrates that while the current population structure of the specialist parasitoid of sunflower moth does not show genetic substructure that could lead towards improved control of sunflower moth in agricultural fields, the role of generalist parasitoids and in particular those associated with nearby orchard habitat could be very important in biological control. In addition, complex interactions amongst herbivores in the wild sunflower habitats may contribute to the differences observed in parasitism rate between the wild and agricultural sunflower settings. These results show that using the tools and theories of population genetics, landscape and community ecology, we can contribute valuable information towards efforts in conservation biological control and sustainable agricultural practices.

This dissertation is dedicated to my grandmother, Encarnación Cabré de Moran, who taught me to appreciate nature, science and family.

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Chapter 1.

Geographic population structure and potential for conservation biological control by a native specialist parasitoid: do wild habitats prevent local adaptation in agriculture?

Abstract

Gene flow between managed and unmanaged or "wild" habitats has important evolutionary and conservation consequences for the degree of genetic differentiation among local populations. Conservation Biological Control (CBC) emphasizes the use of land management practices to maintain or increase the effectiveness of existing natural enemies, such as parasitoids, in agricultural settings. Analysis of geographic population structure as it relates to the potential for gene flow to impact habitat specialization by parasitoids has yet to be integrated into this approach.

This work explores the geographic population structure of *Dolichogenidea homoeosomae*, a native specialist parasitoid of sunflower moth *Homoeosomae electellum*, an important pest in California, where self-seeding wild sunflower and agricultural sunflower fields are both common. Using nine microsatellite markers to examine genetic connectivity, we assessed population structure at the habitat (wild vs. agricultural), regional, and continental scale.

Overall, we found the degree of gene flow among populations to be high relative to other parasitoids examined in similar studies. Measures of genetic differentiation, F_{ST}, between Cwild and agricultural sunflower sites within California ranged from 0.000 to 0.037. Populations were significantly more differentiated between California and the Central States (Kansas and Texas) at 0.013 to 0.065 ($t= -6.13$ Bonferroni adjusted $p < 0.001$).

Mantel tests and a reduced major axis regression slope of 0.000187 demonstrate a small significant isolation by distance signal, but only at the continental scale.

Results suggest that the high level of gene flow amongst sites sampled for the specialist native parasitoid *D. homoeosomae*, compounded with previous knowledge of the natural history and ecology of this species, preclude habitat specialization within agricultural fields of its host. From a biological control perspective, this is limits the usefulness of this species in the long term as natural enemy of *H. electellum*. This finding underscores the need for CBC practitioners to consider the adaptive implications of the geographic population structure of targeted specialist parasitoids and how gene flow from unmanaged to managed habitats may affect a natural enemy's ability to control native pests in agricultural settings

Introduction

Context: Landscape level management, Conservation Biological Control and population structure

Concerns about the impacts of human mediated gene flow upon local adaptation have been highlighted in restoration ecology (for a review see McKay et al. 2005), yet much work remains to be done to create guidelines about how adaptive variation is ideally distributed among populations (managed or unmanaged) and when less than optimal adaptive scenarios (Crespi 2000) are likely to occur from a species conservation, ecosystem service, or agricultural production perspective. Successful landscape level management of ecosystem services in mixed agricultural-wildland settings requires consideration of both positive (ie: increased pollinator provisioning (Martins and Johnson 2009; Klein et al. 2003; Kremen et al. 2004; Steffan-Dewenter et al. 2006) and negative (ie: prevention of local adaptation) flux between habitat types

Insect pests are a tremendous economic problem for agriculture, and increasingly the methods used to combat their impact have become a human and environmental health concern (Bale et al. 2008; Margni et al. 2002; Weisenburger et al. 1993; Sagar 1991). A promising trend in pest management is an emphasis on Conservation Biological Control (CBC), an approach that uses habitat and landscape level management practices to maximize the ecosystem service of pest regulation by existing natural enemies (Pickett and Bugg 1998; Barbosa 1998). Several of the important agricultural pests in North America are native to the bioregion where they cause crop losses (Marino et al 2006). The respective natural enemies of these herbivores, notably parasitoids, have failed to provide economically significant levels of regulation in agricultural settings while their impact in nearby "wild" or unmanaged environments is much greater (Chen and Welter 2007; Bianchi et al. 2006; Gounou et al. 2009; Wang et al. 2009). Population structure, and in particular gene flow, may play a role in this observed pattern (Nuismer et al. 1999; Roderick and Navajas 2003).

As emerging genetic technologies allow for a more detailed understanding of how pest and natural enemy populations change in time and space relative to one another, both the potential for improvement and the assessment of risk in biological control practices has been revolutionized (Roderick and Navajas 2003; Holderegger and Wagner 2006; de Leon et al. 2006; Bouyer et al. 2007; Anton et al. 2007). Within this research agenda, a befitting yet understudied topic is the role of geographic population structure in influencing the adaptive potential of native parasitoids of agronomic significance. In contrast to exotic parasitoids that are introduced into a system for biological control purposes, native parasitoids presumably share an evolutionary history with their hosts within the host's home range. The change in habitat and host population structure that follows the introduction of a domesticated plant with an indigenous ancestor, such as planting fields of sunflowers in the Western U.S., implies a new adaptive scenario for these specialist natural enemies. Habitat specialization in the form of local adaptation could be considered of great benefit to agriculture if in fact the natural enemies are likely to, over time, provide acceptable regulation of the native pest species.

While neutral genetic markers such as microsatellites are not effective for defining scales of local adaptation because they are influenced by selection (McKay and Latta 2002), they do reflect historical gene flow and genetic drift, which is a good starting point for understanding the degree to which gene flow among populations could be swamping local adaptations (King and Lawson 1995; Storfer 1999). Restoration ecologists, agronomists, and conservation biologists alike stand to benefit from a growing body of work on the potentially negative effects that human caused gene flow could have for locally adapted populations (Storfer 1999).

Population structure in parasitoids

In general, genetic diversity has been found to be comparatively low in the few hymenopteran parasitoid species examined (Roderick and Navajas 2003; Lozier et al. 2009) following the overall trend within this order due to their haplodiploidy, life history, and phylogenetic factors (Graur 1985). Compounded with low genetic diversity, some parasitoids have been shown to exhibit a higher degree of genetic differentiation compared to their hosts (Anton et al. 2007), and thus are thought to be more vulnerable to habitat loss and population fragmentation (Holt 2002).

Studies have found geographic genetic structuring for different specialist parasitoid species at scales varying from less than 1 km (Vaughn and Antolin 1998) to regional within several hundred kilometers (Anton et al. 2007) and continental at the order of thousands of kilometers (Lozier et al. 2009; Karam et al. 2008). The importance of host 'races' versus isolation by distance (geographic or landscape parameters) in the structure of parasitoid populations remains an important question to address and is likely to vary on a case by case basis depending on the life history characteristics of the natural enemy and the host.

Within the family Braconidae, both extremes of the dichotomy between resource and distance-based genetic structuring have been documented. Host use patterns have been found to be more significant than distance in *Diaretiella rapae*, a braconid parasitoid attacking two aphid hosts (Vaughn and Antolin 1998). Meanwhile, geographic structure has been marked compared to an absence of host fidelity in *Aphidius transcaspius*, another braconid parasitoid attacking aphids on plums as well as other related fruit trees (Lozier et al. 2009). Due to the spectacular diversity in natural history amongst parasitoids, host use patterns and habitat fidelity (Godfray 1994), it is difficult to make a general prediction about the relevant scales of differentiation and parasitoid population structure. However, a predictive theory based on species characteristics, landscape features, host population connectivity and population sizes is a feasible and desirable goal as more studies are completed (Lozier et al. 2008; Roderick and Navajas 2003; Roderick 1996).

Landscape composition can influence gene flow by way of continuous available habitat and food resources (Holderegger and Wagner 2006; van Klinken and Edwards 2002). Since gene flow is one of the ultimate causes of adaptation (Crespi 2000), the rate and direction of gene flow is an important influence in the coevolutionary process. When gene flow among populations overwhelms local adaptation, parasitoids may be maladapted relative to their host in novel habitats (Crespi 2000). If this occurs in agricultural settings that are sympatric with native habitat of the crop's wild progenitor, gene flow could impede local adaptation of specialist parasitoids in crop fields. This scenario would prove maladaptive in terms of parasitoid efficiency within the agricultural fields. In contrast to studies that have demonstrated 1) that gene flow between wild and agricultural environments is beneficial for crop production in that it prevents rapid evolution of pesticide resistance (Bt resistance) (Tabashnik 2008), and 2) that parasitism within agricultural fields is facilitated by a high proportion of natural habitat surrounding the field (Eliers and Klein 2009; Bianchi et al. 2006; Tscharntke et al. 2005) high rates of gene flow from wild habitats in cases like the one described above would be detrimental to long term CBC efforts.

Sunflower moth and its parasitoid

In this study, we examine the population genetic structure of *Dolichogenidea homoeosoma* Muesenbeck (Hymenoptera: Braconidae), a native specialist parasitoid of *Homoeosoma electellum* Hulst (Lepidoptera: Pyralidae), within the Central Valley of California, U.S.A. The larval host, *H. electellum* is an important native pest of agronomic sunflower, *Helianthus annuus* var. macrocarpa L., consuming the flower and developing seeds of the plant. This herbivore also feeds upon the sympatric wild progenitor of crop sunflower, *H. annuus* L (Chen and Welter 2003). The parasitoid, *D. homoeosoma*, is found in both wild and domesticated sunflower habitats throughout Central and Western North America, but is more successful in parasitizing its host in the wild setting due to architectural changes in the plant resulting from the domestication process that have created a refuge from the parasitoid for the herbivore host (Chen and Welter 2003).

This parasitoid-herbivore complex was selected in part due to the apparently maladaptive traits found in the native parasitoid in agricultural settings including: inefficient search behaviors on agricultural sunflower compared to wild sunflower (Chen and Welter 2003) and ovipositors that are too short to reach host within the enlarged domesticated sunflower seed (Chen and Welter 2005). Both of these factors lead to lower parasitism rates in agricultural compared to wild settings (Chen and Welter 2007).

In the Great Central Valley of California, agricultural sunflower fields have been a common landscape feature in the last 100 years. Small annual self seeding wild populations of sunflower occur in places where the soil has been recently disturbed and seasonally flooded, such as in temporary wetlands or along major roadways throughout the valley. The herbivorous pyralid larvae feed readily on the achenes and florets of both plant types and are present in relatively high densities wherever the plants exist (Chen and Welter 2003).

For the parasitoid, the wild and agricultural habitats might be hypothesized to be isolated host-race patches, where frequency and density dependent selection vary, posing a structured coevolutionary landscape (Thompson et al. 2002). If this were the case, we would expect that genetic structure would be detectable between the habitat types at some scale. Eventually, local phenotypic adaptation to flower morphology (larger seeds and thus more concealed hosts leading to selection towards longer ovipositors or behavioral changes in oviposition for example) could follow resulting in increased effectiveness of the parasitoid in agricultural sunflower habitats over time (Holt and Gaines 1992; Sheck and Gould 1996; Singer et al. 1993). On the other hand, if the parasitoid population exhibits high levels of gene flow at long range scales and thus little or no genetic structure at scales compatible with the agricultural sunflower fields, habitat type specialization is less likely and the genetic architecture necessary to maintain a coevolving metapopulation structure would not be supported (Crespi 2000; Thompson et al. 2002).

With this investigation we seek to describe the genetic population structure within *D. homoeosomae* in order to understand the relative importance of host “race” (in this case wild and agricultural plant habitat types) versus isolation by distance in promoting genetic structure for this species. To this end, we examined genetic differentiation among and within subpopulations of this specialist parasitoid in wild and agricultural habitats in California’s Great Central Valley. To further explore the scale at which this species exhibits genetic structure we included individuals collected in the states of Kansas and Texas, Central United States to compare genotypic parameters at a continental scale.

Methods

Sampling locations

D. homoeosoma females were obtained from seven sites within the Sacramento and San Joaquin Valleys (collectively known as the Great Central Valley) of California (Figure 1). These sites were chosen to be representative of the geographic region where agricultural and wild sunflower co-exist in California. Four of these sites were agricultural sunflower fields and the other three were self seeding "wild" *Helianthus annuus* patches of 1 to 4km^2. The distance between these sites ranged from 20.7km to 474.6km (mean 195.8km), calculated using Google Earth^{TM} 4.2 (Google Inc., Mountain View, CA). The number of individuals obtained per site ranged from 16 to 32 (mean 19.5) with a total of 195 individuals analyzed. We also obtained a total of 29 individual *D. homoeosoma* from host larvae collected in the central United States at two agricultural and one wild site located in Kansas and Texas. This parasitoid species has been reported from the central states of the U.S. (Texas, Kansas, the Dakotas) as well as the west (Colorado and California) (Krombein et al. 1979) thus this set of samples represents a broad sample within the state of California and a small but representative sample from the eastern portion of its continental range.

Sample collection

We collected the pyralid larval host of *D. homoeosoma*, *Homoeosoma electellum* Hulst by dissecting sunflowers collected randomly at each of the above mentioned sites. The host larvae were brought back to the laboratory and reared in individual 28ml plastic cups with artificial diet (Wilson 1990) at 23 ºC. We checked the samples daily and placed any *D. homoeosoma* parasitoids emerging from these larvae in 2ml tubes filled with 95% EtOH. Parasitoids were sexed based on the presence or absence of an ovipositor.

Genotyping

DNA was extracted using a Qiagen (Valencia, CA) DNEasy DNA extraction kit and stored at -20ºC. We genotyped a total of 384 individuals for nine fluorescently labeled microsatellite loci (Dh-27a, Dh-2a, Dh-11a, Dh-8a, Dh-3a, Dh-20a, Dh-19a, Dh-14a, Dh-26a; Douhovnikoff et al. 2006). We performed PCR and +A overhang removal with T4 polymerase as in Douhovnikoff et al. (2006) and suspended 0.5µL of T4-treated PCR products in 9.5µL of a 39:1 Hi-Di formamide (Applied Biosystems, ABI, Foster City, CA): LIZ500 size standard (ABI) solution. Fragments were separated on an ABI 3730 sequencer and data were visualized using GeneMapper version 3.0 (Applied Biosystems, Life Technologies). All steps were performed in 96-well plates with multiple positive and negative (purified water) controls, and the quality of allele size calls were checked manually.

Microsatellite data analysis

We calculated locus specific diversity (Nei and Kumar 2000), F statistics (Weir and Cockerham 1984), and tests for deviations from Hardy Weinberg Equilibrium (HWE) using the software package GENETIX 4.05 (Belkhir et al. 2004) with 3000 permutations of alleles among individuals and F_{IS} as a test statistic. In this program, the distribution of the parameter values under the null hypothesis (of HWE for *F* statistics) is generated by re-sampling of the relevant objects (e.g. alleles between individuals in the case of F_{IS}) using permutations.

We used the web software package GENEPOP (Raymond and Rousset 1995) to test for linkage disequilibrium (LD) with Fisher's method (correcting for the large number of tests with the Bonferroni method (Rice 1989), calculate observed allelic diversity, observed heterozygosity, expected heterozygosity at HWE and the inbreeding coefficient (F_{IS}) according to Weir and Cockerham, 1984. To assess population structure, we calculated pairwise estimates of F_{ST} among the sampling populations, testing for significance by randomizing multiple locus genotypes among populations 2000 times using FSTAT (Goudet, 2001). We tested for isolation by distance (IBD), by determining the correlation between Slatkin's linearized F_{ST} (Slatkin 1995) and geographic distance using Mantel tests (3000 permutations) and reduced major axis regression (RMA) implemented in Isolation by Distance Web Service (Jensen et al. 2005). The same analysis was applied to a reduced data set, including only populations sampled from California to inspect IBD at this closer scale. To test how variance in the molecular data is partitioned among geographic areas and wild host plant locations compared to agricultural sunflower fields, we used analysis of molecular variance (AMOVA) implemented in the program ARLEQUIN 3.1 (Excoffier et al., 2005).

Results

Allele frequency and genetic variability

We detected a total of 84 alleles across the nine microsatellite loci for *Dolichogenidea homoeosoma* (Table 1). The number of alleles per locus averaged 8.4 and ranged from 2 to 16. Several unique alleles were found in the Central States populations (Kansas, Kansas-wild, and Texas); these are unlikely to be useful as population specific markers because of their low frequency (three alleles at locus SSR2, and two respectively at SSR27, SSR11.1 and SSR14.2).

We found no instances of linkage disequilibrium among the loci. However, all loci except SSR3.3 exhibited significant deviations from HWE globally. Across all of the parasitoid populations, all three *F*-statistics were significantly different from zero for seven of the nine loci. The overall F_{ST} (0.02), F_{IS} (0.13) and F_{IT} (0.14) were significant as well. F_{IS} estimates across all populations ranged from -0.006 (SSR3) to 0.325 (SSR2), averaging 0.126. Overall departure from HWE was in the direction of heterozygote deficiency, as indicated by the positive F_{IS} values (Table 1). Homozygote excess is expected for a haplodiploid species (Wright 1933).

Genetic differentiation

In general, within-subpopulation allelic diversity was high (Table 2). Multilocus expected heterozygosity (H_e) ranged from 0.81 to 0.73, with an average of 0.78 across subpopulations; observed heterozygosity (H_O) followed a similar trend from 0.61 to 0.82 averaging 0.71 (Table 2). Observed allelic diversity ranged from 6.7 to 8.3 alleles per locus per subpopulation, with a mean of 7.4. The three related genetic diversity variables (allelic diversity, H_E and H_O) showed similar tendencies amongst wild habitat subpopulations compared to agricultural habitat and California versus the Central States subpopulations. Again, at the subpopulation level F_{IS} estimates indicate that overall departure from HWE was in the direction of heterozygote deficiency, ranging from 0.002 to 0.250. On average, the subpopulations exhibited a 7.2% deficit of heterozygotes.

Genetic differentiation among populations

Levels of population subdivision were quite low overall. Pairwise F_{ST} estimates within the California subpopulations were particularly low, ranging from 0.000 to 0.037 (median F_{ST} = 0.008), while the pairwise estimates for population pairs from California and the Central States were significantly higher at 0.013 to 0.065 (median F_{ST} = 0.031), t= -6.13 Bonferroni adjusted p=0.00. All but one of the total of 21 pairwise F_{ST} values for population pairs between California and the Central States were significant ($p < 0.05$), whereas only 4 of the 24 pairwise F_{ST} values for population pairs within California were found to be significant (Table 3).

<u>Genetic Distance and isolation by distance</u>

Pairwise estimates of genetic differentiation measured by F_{ST} / (1- F_{ST}) are consistently higher for population pairs that are further apart geographically, as demonstrated by Mantel tests (3,000 randomizations); r = 0.63, p = 0.002 based on a one tailed test of the null hypothesis that there is no relationship between genetic and geographic distance, and a reduced major axis (RMA) regression slope of 0.000187 (Figure 2a).

Analysis of Molecular Variance

A test of how genetic variation was partitioned among the subpopulations sampled, the habitat plant type and the geographic areas revealed that 98.25% of the variation occurs within the subpopulations sampled, while only 1.75% of the variation is among the populations. The overall fixation index (F_{ST}) of 0.02 ($p<0.0001$) tested by 1,023 permutations among populations and within populations supports this result, showing very little structure among the subpopulations. Partitioning the AMOVA further into the California subpopulations and the central states subpopulations (among regions, section b, Table 4) shows that, while 96.65% of the variation is still found within subpopulations, 2.71% of the variation is due to regional separation (F_{ST} = 0.033). In contrast, the AMOVA design partitioning among agricultural and wild sunflower habitats (section c in Table 4) results in 99.32% of the variation within the groups (F_{ST} = 0.007). Together, these analyses show that there is extremely low genetic differentiation among the subpopulations sampled; the majority of the population structure identified exists at the continental scale.

Discussion

We are only beginning to understand the importance of gene flow between wild and managed environments from an ecosystem service, conservation science and agronomic production standpoint. Using the tools provided by population genetics and knowledge of parasitoid-host interactions, the land management community stands to make better informed decisions about Conservation Biological Control, a pest management paradigm that, if successful, could help to solve the current crisis of pesticide dependency in agriculture. The scale at which a parasitoid population is structured in space relative to its host's population range may determine the degree to which this natural enemy is able to efficiently exploit the host (Price 1991). For this reason, it is imperative to understand how native parasitoid populations are structured in dynamic landscapes that encompass a range of host habitat types (here wild and agricultural sunflower plants) and potential barriers to gene flow. Our study is among the first to attempt to capture the relevant scale of geographic population structure for a native parasitoid of agronomic significance (Lozier et al 2008; Karam et al 2008; Vaughn and Antolin 1998).

While genetic diversity was relatively high in *D. homoeosomae*, overall population structure was low at regional scales and totally absent at the scale of habitat patches (10's of kilometers). Allelic diversity, expected and observed heterozygosities did not vary amongst regions or habitat types, showing that for the set of neutral genomic makers used here, the parasitoid retains a relatively panmictic geographic structure within its range of endemism across wild and agricultural habitats in our sample (Table 2). Sampling error can not be ruled out as a cause for the observed departure from Hardy Weinberg equilibrium in the direction of heterozygote deficiency at the marker (8 of 9) and subpopulation (6 of the 10) levels. However, similar trends have been documented in other parasitoid species (Kitthawee et al. 1999; Huffbauer et al. 2001; Anton et al. 2007), which indicates that it may be due to strong inbreeding effects that result from a haploidiploid mating system, selection against heterozygotes, or perhaps gene flow patterns influenced by human facilitated dispersal of this species (Anton et al. 2007; Lowe et al. 2004). Further extensive sampling at the regional and local scale and a complete parentage analysis of the markers used (sensu Kim and Sappington 2006) would assist in addressing the source of the heterozygote deficiency.

The pairwise F_{ST} estimates for the subpopulations sampled within California indicate that all sites sampled could functionally be considered one inter-breeding population throughout the Great Central Valley (Table 2). Of the four significant pairwise F_{ST} values documented from within California, one was from between the agricultural site "505", in Yolo County, and the most geographically distant wild site still within California (600km), "SL" in Kern County (F_{ST} = 0.015). We might expect this to be the most differentiated comparison based upon geographic distance. However, two other pairwise F_{ST} values for site 505 and one agricultural and one wild site within the same county less than 30km away ("W" and "Y") were higher (0.037 and 0.030 respectively). Interestingly, three of the four significant pairwise F_{ST} values within California involve site 505. The agricultural area where this sunflower field is located is one where the crop has been planted annually for at least 12 years in large plots of more than 10 acres, whereas most other agricultural sunflower sites in California are under inter-annual crop rotation.

At the continental level, the highest values for differentiation were found for sample pairs between the 505 site in Yolo County, California, and the three sites located in the central United States (0.065 for Kansas agricultural site, 0.065 for Kansas wild site, 0.055 for Texas agricultural site) (Table 3). These values, in addition to the pairwise comparisons of samples from site 505 and sites W and Y from the same county (Figure 2a), represent the four most outlying points above the line of correlation between genetic distance and geographic distance for all of the population pairs (Fig 3).

Notably, several of the California-Texas sample pairs are well below the line of correlation. Differentiation between the wild and agricultural sites in Kansas and among the Kansas and Texas sites was not significant, even though geographic distances between these three sites were sizable (106, 711 and 781km respectively). An RMA regression of the genetic on geographic distance of the sample pairs within California reveals a similar significant but slight pattern of isolation by distance (Figure 2b).

In contrast to the findings of Vaughn and Antolin (1998) for *Diaretiella rapae* (Hymenoptera: Braconidae), we found no evidence of host use pattern by habitat (wild versus agricultural sunflower plants) (Table 4). The highest fixation index (0.033) results from nesting between California and Central States sites, again supporting a geographic distance based population structure as opposed to one based upon habitat type.

While there is a significant signal of isolation by distance, population structure is moderate even at the continental scale represented by the entire dataset. These results indicate that historic gene flow is high in this species, in particular at the regional scale in California's Great Central Valley.

At the continental scale the magnitude of genetic structure is much less than what has been documented for other braconid species at similar scales (Karam et al. 2008; Lozier et al. 2009). The high allelic diversity observed within the species overall may reflect the species' endemism in North America (Kim and Sappington 2006) and its evolutionary potential (Le Rouzic and Carlborg 2008).

Conclusion

Given the estimation parameters for haplodiploid species (Anton et. al 2007, Zayed 2004), the effective population size of the native parasitoid, *D. homoeosomae,* is likely relatively large compared to other hymenopterans studied (Antolin 2003; Cook and Crozier 1995; Frankham 1995; Hedrick and Parker 1997). The high levels of gene flow detected are likely to limit habitat specialization (wild sunflowers versus agricultural sunflowers) under present conditions. There is no detectable population structure based upon habitat type, although we did find some evidence that individuals collected from the most persistent (inter-annually planted in sunflowers for more than 12 years) agricultural habitat exhibit greater differentiation from other individuals collected in California than those from the other sites within the state where sunflowers are planted in rotation with other row crops every two to four years.

Based upon the previously documented ecological barriers to host population suppression by this parasitoid in agricultural habitats (Chen and Welter 2002), the high levels of gene flow and large effective population size found in this study, we would not expect the specialist native natural enemy, *D. homoeosomae*, to provide economically acceptable levels of control of the sunflower moth in California's Central Valley in the foreseeable future. Equally important, the high rates of gene flow from the wild to agricultural systems would be predicted to prevent the evolution of adaptations that might prove beneficial for the parasitoids in the agricultural systems. Current traits such as shorter ovipositors should reflect the selection due to the smaller seed size of wild sunflowers. Similarly, the tendency of the parasitoid to sting a single larva within a sunflower inflorescence and then leave the flower head reflects the fact that a single larvae is typically found in wild sunflower heads with smaller resources, whereas agricultural sunflower heads and sustain >100 larvae per flowerhead (Chen and Welter 2003).

Agricultural sunflowers in California are harvested prior to diapause of many sunflower moths as evidenced by the strong third flight that occurs in wild sunflower systems and the continued production of new larvae late in the season (Chen and Welter 2002). Thus, any sunflower moths occurring in agricultural systems will be selected against unless a significant fraction of the population enters diapauses early in the year and overwinters underground.

As a result, effort and resources directed at Conservation Biological Control of sunflower moth should focus on the compounded impacts of specialist and generalist natural enemies. While there are many documented positive effects, from an agronomic perspective, of wild habitats near agricultural fields (Tschranske et al. 2005; Tabashnik 2008; Eliers and Klein 2009), this study provides an example of a potentially detrimental impact of nearby wild habitat: gene flow in a specialist natural enemy that could prove maladaptive in the agricultural habitat in terms of pest population regulation. Our results underscore the importance of taking geographic population structure into account when planning for Conservation Biological Control management and a long term strategy for a more economically and environmentally sustainable agriculture.

Figure 1 : sampling locations for *D. homoeosoma*. Sites marked by the ★ symbol were agricultural sunflower fields. Sites marked by the ● symbol were self seeding "wild" sunflower patches.

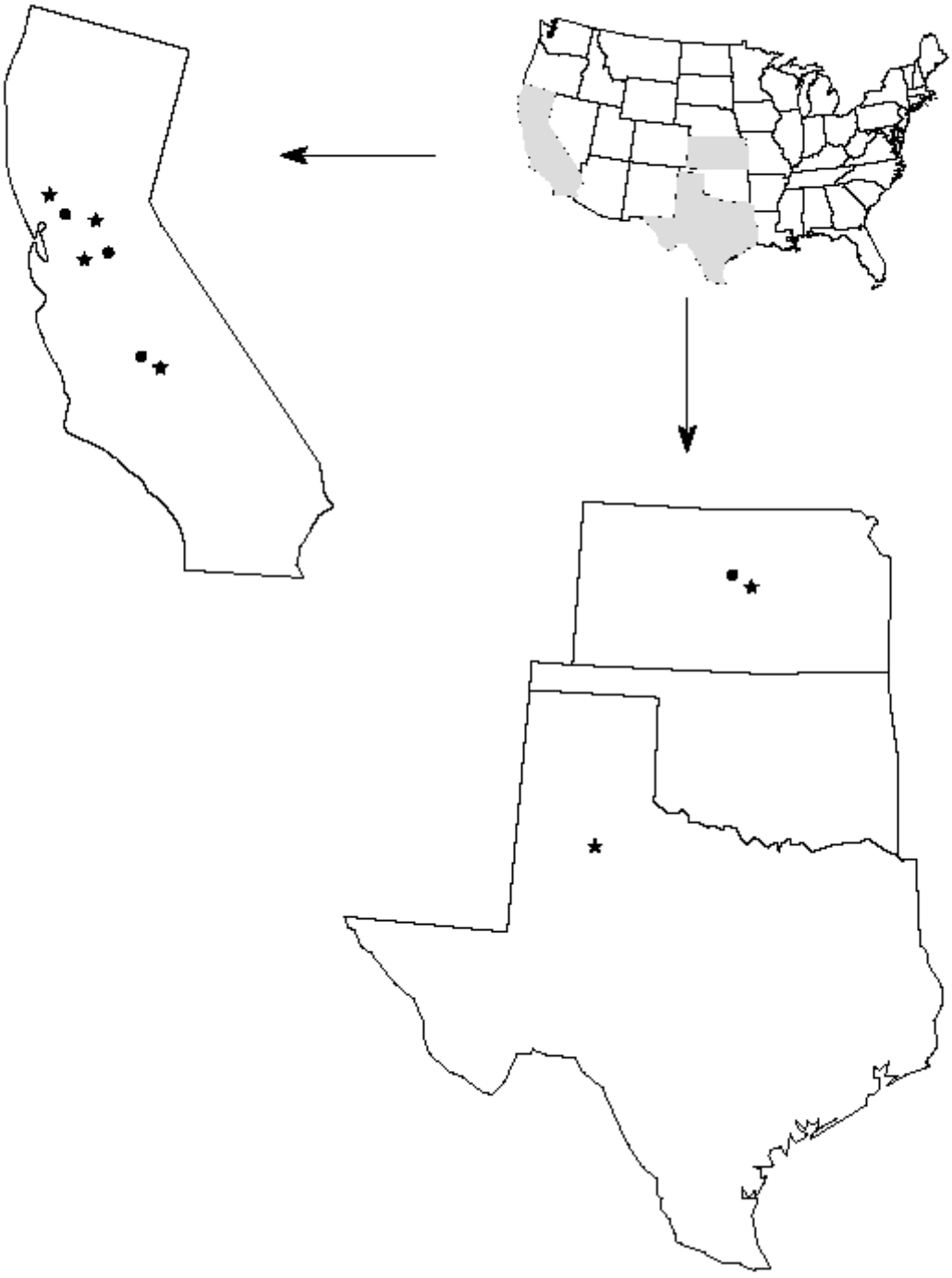

Fig 2a. Correlation between genetic distance, $F_{ST}/(1-F_{ST})$, and geographic distance in kilometers for all population pairs sampled of *D. homoeosomae*. Each point represents a single pairwise comparison of two populations. Symbols represent the possible combinations of within California [CA-CA], between California and Kansas [CA-KS], between California and Texas [CA-TX] so forth. Mantel Test: r = 0.63, z= 1278.43; p = 0.002 (3,000 randmoizations). Linear model (RMA): y = .0014 + 0.000187x ; r^2 = 0.39. Calculated using the program IBDWS Jensen et al. 2005.

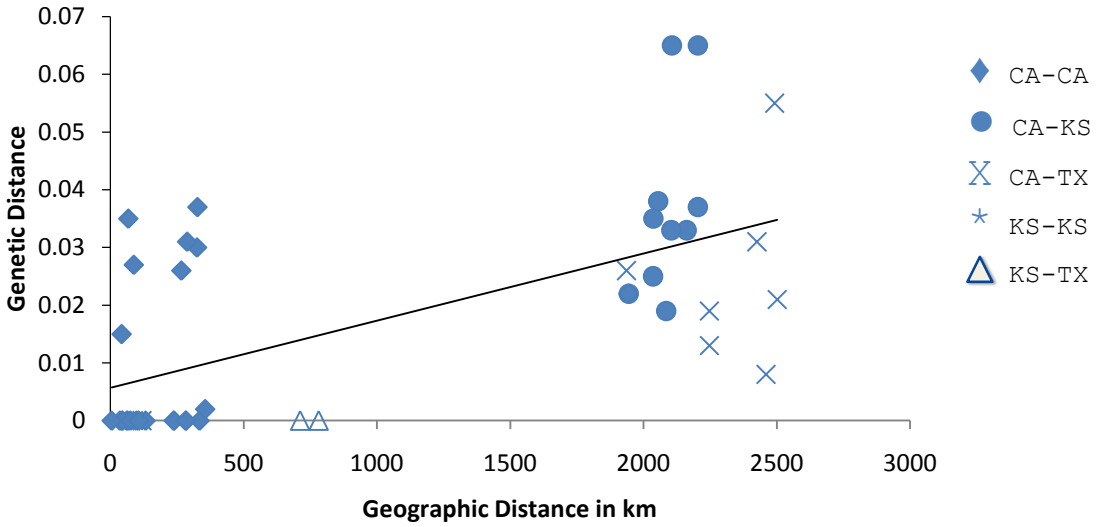

Fig 2b. Correlation between genetic distance, $F_{ST}/(1-F_{ST})$, and geographic distance in kilometers for population pairs sampled within California of *D. homoeosoma*. Mantel Test: r = 0.45, z= 32.12.43; p = 0.029 (3,000 randomizations). Linear model (RMA) y = - 0.01. + .00009 X , r^2 = 0.20. Calculated using the program IBDWS Jensen et al. 2005.

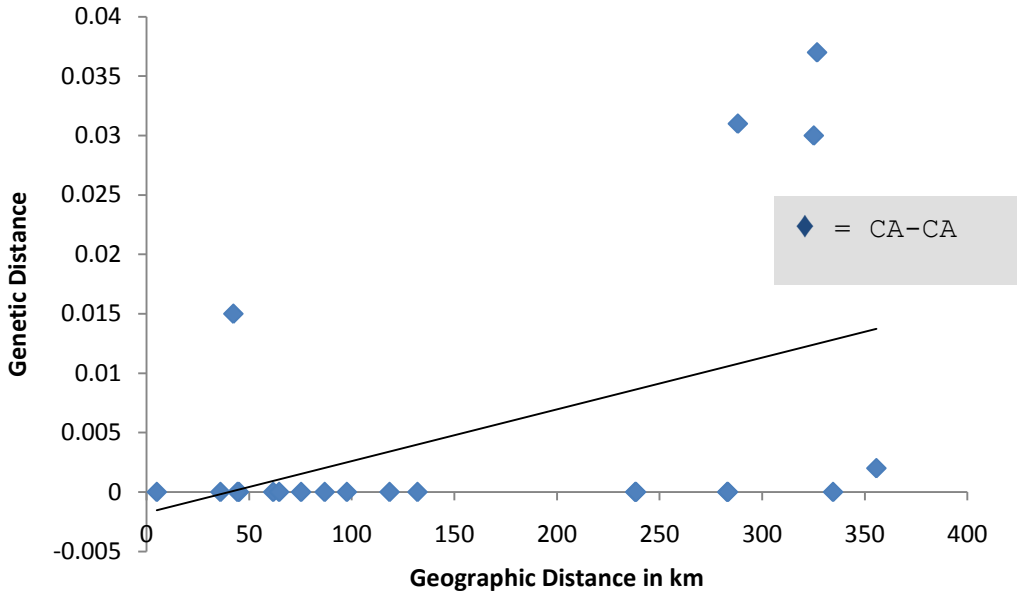

Table 1. Characterization of 9 microsatellite loci analyzed across all *D. homoeosoma* subpopulations sampled.

Locus	*No Alleles*	F_{IS} *(SE)*[1]	F_{IT}*(SE)*[2]	F_{ST}*(SE)*[3]	R_{ST} *(SE)*[4]
SSR27	9	0.116(0.043)*	-0.124(0.041)	0.010(0.008)	0.035(0.020)
SSR2	10	0.327(0.047)*	0.353(0.040)	0.040(0.017)	0.305(0.060)
SSR11.1	11	0.026(0.017)*	0.036(0.018)	0.010(0.001)	0.039(0.020)
SSR8	7	0.098(0.013)*	0.109(0.013)	0.011(0.007)	0.016(0.014)
SSR3.3	2	-0.006(0.116)	0.002(0.106)	0.009(0.006)	0.008(0.008)
SSR20	8	0.111(0.051)*	0.123(0.043)	0.018(0.019)	0.036(0.025)
SSR19	16	0.155(0.039)*	0.156(0.041)	0.002(0.004)	0.003(0.002)
SSR14	9	0.109(0.038)*	0.128(0.034)	0.021(0.011)	0.037(0.019)
SSR26	13	0.160(0.019)*	0.161(0.021)	0.002(0.001)	0.000(0.000)
Overall		0.126(0.029)*	0.139(0.031)	0.014(0.004)	0.051(0.018)

[1]Correlation of alleles within individuals within populations (F_{IS}), [2]correlation of alleles (F_{IT}), [3]correlation of alleles within populations (F_{ST}).

[4]Measure of genetic differentiation analogous to F_{ST}, unbiased with respect to differences in sample size between populations and differences in variance between loci (appropriate for the stepwise mutation model).

*significant deviation from within population Hardy-Weinberg Equilibrium, P<0.001.

All statistics and their standard errors (in parentheses) estimated by jackknifing over populations calculated in GENETIX (Belkhir et al. 2004) following Weir and Cockerham, 1984.

Table 2. Number (**N**) of *D. homoeosoma* sampled from indicated locations and characteristics of subpopulations calculated for the 9 microsatellite loci:

Subpopulation county location *[latitude, longitude in decimal degrees]*	***N***	***Habitat Type***	***O.A.D***[1]	***Ho***[2]	***He***[3]	F_{IS}[4]
505- Yolo- *[38.50, 121.57]*	16	AG	6.67	0.74	0.73	0.025
Cl- Colusa- *[39.13, 122.11]*	20	AG	7.11	0.61	0.77	0.115*
So- Fresno- *[36.36, 119.30]*	25	AG	7.44	0.66	0.78	0.114*
W- Yolo- *[38.41, 121.49]*	27	AG	7.44	0.68	0.79	0.170
Cs- Sacramento- *[38.15, 121.26]*	32	WILD	7.67	0.71	0.79	0.229*
SL- Kern- *[36.45, 119.35]*	19	WILD	7.56	0.71	0.80	0.002*
Y- Yolo- *[38.33, 121.37]*	17	WILD	7.22	0.74	0.78	0.142
Central States Sites						
TX- Lubbock- *[33.35, 101.44]*	16	AG	8.33	0.75	0.81	0.104*
KS- Saline- *[38.46, 97.36]*	14	AG	7.89	0.74	0.80	0.160
KSW- Chase- *[38.09, 96.33]*	9	WILD	6.89	0.82	0.78	0.081*

[1]observed allelic diversity (observed median number of alleles per locus at that site), [2]observed heterozygosity (Ho), [3]expected heterozygosity (He) at HWE calculated in GENEPOP (Raymond and Rousset, 1995). [4]Inbreeding coefficient (F_{IS}) according to Weir and Cockerham, 1984; calculated in FSTAT (Goudet, 2001). [*]Tests for deviation from HWE significant after correction for multiple comparisons (P<0.00001) correction.

Table 3. Bellow the diagonal: Pairwise estimates of genetic differentiation among *D. homoeosomae* populations, measured by F_{ST} (Weir and Cockerham, 1984) calculated in GENEPOP (Raymond and Rousset, 1995). Above the diagonal: Pairwise estimates of genetic differentiation among *D. homoeosomae* populations, measured by F_{ST} / (1- F_{ST}) (Roussett, 1997) calculated in ISOLDE (Raymond and Rousset, 1995)

	505-ag	***Cl-ag***	***So-ag***	***W-ag***	***Cs-wild***	***SL-wild***	***Y-wild***	***TX-ag***	***KS-ag***	***KS-wild***
505- ag (16)	-	0.000	0.000	0.000	0.000	0.000	0.031	0.056	0.070	0.070
Cl-ag (20)	0.000	-	0.000	0.000	0.000	0.000	0.000	0.032	0.040	0.034
So-ag (25)	0.000	0.000	-	0.000	0.000	0.000	0.000	0.026	0.036	0.027
W-ag (27)	0.037	0.000	0.031	-	0.000	0.000	0.000	0.019	0.022	0.036
Cs-wild (32)	0.000	0.000	0.000	0.000	-	0.000	0.000	0.021	-0.008	0.038
SL-wild (19)	0.015	0.000	0.000	0.000	0.000	-	0.000	0.008	0.019	0.027
Y-wild (17)	0.030	0.000	0.000	0.000	0.002	0.000	-	0.013	0.025	0.024
TX-ag (16)	0.055	0.031	0.026	0.019	0.021	0.008	0.013	-	-0.008	-0.008
KS-ag (14)	0.065	0.038	0.035	0.022	0.033	0.019	0.022	0.00	-	
KS-wild (9)	0.065	0.033	0.027	0.035	0.037	0.026	0.025	0.00	0.000	-

Sample sizes are in parentheses next to population names along the left hand column, statistically insignificant values shown as 0.000. Significant F_{ST} p values at significance level 0.05, using Mantel test with 3,024 permutations (implemented in ARLEQUIN 3.1, Excoffier et al. 2005).

Shaded cells represent measures for population pairs from California and the central states.

Table 4. AMOVA results showing molecular variance distribution for the entire data set.

Source of variation	d.f.[1]	SS[2]	Covariance components	Percentage of variation
a.				
among populations	9	39.95	0.05	1.75
within populations	380	1000.34	2.63	98.25
Total	*389*	*1040.28*	*2.68*	
***b.* among regions**	1	15.89	0.10	2.71
among populations within each region	8	34.053	0.02	0.64
within populations	380	1283.77	3.38	96.65
Total	*389*	*133.71*	*3.50*	
c.				
among groups*	1	3.19	-0.01	-0.34
among populations within groups*	5	24.61	0.03	1.03
within groups*	305	1029.55	3.38	99.32
Total	*311*	*1057.36*	*3.40*	

[1] Degrees of freedom, [2]Sum of squares, *P<0.001 tested by 1023 permutations among populations and within subpopulations.

a) Entire data set: Fixation Index $\mathbf{F_{ST} = 0.02}$, (P<0.0000) tested by 1023 permutations among populations and within populations.
b) California versus Central States subpopulations: Fixation indices: $\mathbf{F_{ST} = 0.033}$ (p=0.00000), $\mathbf{F_{SC} = 0.007}$(p=0.00000), $\mathbf{F_{CT} = 0.027}$(p=0.00684)
c) Agricultural versus wild sites: Fixation Indices: $\mathbf{F_{ST} = 0.007}$ (p= 0.017) ; $\mathbf{F_{SC} = 0.010}$ (p= 0.002) ; $\mathbf{F_{CT} = -0.003}$ (p= 0.790)

*agricultural and wild (self seeding) sunflower host plant sites in California

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Chapter 2.

Influence of the surrounding landscape on parasitism: a case study of the parasitoid guild of sunflower moth (*Homoeosoma electellum*) in the agricultural region of the Central Valley of California

Introduction

While landscape modification is a feature of all civilizations, understanding the impacts of these changes upon ecosystem functions is an ongoing endeavor. The ecosystem service of biological pest control, for instance, is dependent upon many habitat variables and is likely to be impacted by modern agricultural methods at various scales (Bianchi et al. 2006). Alpha diversity, species evenness and top down regulation of invertebrate communities are correlated with habitat complexity at various spatial scales in natural and agricultural systems (Bianchi et al. 2006; Langellotto and Denno 2006; Roland and Taylor 1997; Marino and Landis 1996) but the mechanisms underlying these relationships are not clear. In addition, the domestication of plants has changed plant and habitat level factors impacting herbivore density and fitness as well as the effect of natural enemies upon these populations (Macfayden and Bohan 2010; Chen and Welter 2003; Jackson and Koch 1997). Continuity of population level functions is facilitated by dispersal and gene flow between crop fields and land that is not in production in mixed landscapes (Blitzer et al 2011, in review; Nerney Meyers et al in prep).

The factors likely to impact community composition at the landscape and regional level in both managed and unmanaged habitats include plant, habitat and system level characteristics, but trends are difficult to generalize (Blitzer et al 2011, in review; Marino and Landis 1996). One of the challenges in formulating a predictive theory of landscape level community changes is that the biologically relevant scale at which these occur is likely to vary amongst systems and species. Honing in on the relevant spatial scale and assigning suitable experimental replicates have been challenges in deciphering what specific aspects of landscape composition are directly or indirectly responsible for natural enemy effectiveness in agricultural settings (Tschrantke and Roland 2004).

The landscape context in which agricultural crop fields are embedded influences both natural enemy species diversity and the overall impact of the higher trophic levels upon herbivores (Eiliers 2009; Tschrantke and Roland 2004; Marino et al. 2006; Schmidt et al. 2004). A combination of observational and experimental approaches is gaining significant ground towards identifying possible patterns in natural enemy responses to specific landscape factors (Schmidt 2004; With et al. 2002; Kruess and Tscharntke 2000; Steingrover et al. 2010; Haaland et al. 2011). Both researchers and managers need strategies that allow them to easily compare and contrast landscape contexts and their impacts upon biological control.

Much of the research in this area has focused on parasitoids, which comprise an important group of natural enemies for biological control (Menalled et al. 1999; Marino et al. 2006). A parasitoid is an insect whose immature life stages live as parasites that eventually kill their hosts, which are typically other insects.

Parasitoid activity is readily documented in the form of parasitism rates of field-collected, laboratory-reared larvae and therefore these organisms are a useful model for how we might best consider the landscape context in planning for and assessing an important element of biological control.

Landscape level factors that have been correlated with parasitoid activity include landscape complexity, human caused disturbance, and host and non-host resource distribution. These three are likely inter-related in that they can impact parasitoid behavior and numerical response in multiple ways.

Simple vs. complex landscapes

Landscape complexity is thought to increase parasitoid population stability, species diversity, and overall parasitism. Complexity is defined here as increasing temporal and spatial heterogeneity primarily through plant species diversity, which in turn is the structure of phytophagous host populations (Bianchi et al 2006). Mathematical modeling, field and laboratory experiments support the theory that spatial heterogeneity in host distribution stabilizes parasitoid populations. This is due to both modulated searching behavior according to encounter rate by ovipositing females and limits to host density dependence and search time in host-population incidence function dynamics (Ives 1995; Pacala and Hassell 1994; Chesson and Murdoch 1986; Godfray et al. 1994; Ostman el al 2001; Cronin 2003 but see Esch et al. 2005). Landscapes containing features such as ponds, shelterbelts, meadows and small forests have been found to have high parasitoid species richness and overall biomass in large scale surveys (Ryszkowski et al. 1993; Thies and Tscharntke 1999). Comparisons of structurally simple vs. complex landscapes have shown higher parasitism in more complex landscapes (Marino and Landis 1996; Menalled et al. 1999). But in some cases, key elements such as specific host plants were shown to be the primary drivers of these patterns rather than habitat complexity alone (Menalled et al. 2003; Vollhardt et al 2008).

Disturbance

Life history asynchrony with the host, incompatibility with host-plant phenology and agro-management (harvesting, plowing, insecticide applications) lead to a decline in parasitoid populations in landscapes of exclusively annual crops (Vanbergen 2007; Pfiffner et al. 2009; Hollan and Reynold 2003; Thorbek and Bilde 2004). While the spatial scale of response to habitat structure and disturbance varies amongst species (Roland et al. 2000), host-parasitoid dynamics are more likely to become disrupted following habitat disturbance and fragmentation typical of annual crop fields compared to natural habitats (Kruess and Tscharntke 1994).

Provision of non-host resources within the landscape

Habitats that provide non host resources support a greater number and diversity of parasitoids. Availability of external subsidies to parasitoids such as extrafloral nectaries has been shown to enhance overall control of pests in agricultural areas (Tylianakis et al. 2004; Pemberton and Lee 1996).

To the degree that the dispersal of a parasitoid species and the scale at which these resources are available are compatible, this is an important consideration at the landscape scale (Tscharntke and Brandl 2004). One study in an uncultivated system found that disturbed late successional habitats such as forest clearings and edges are characterized by plants that tend to have more extrafloral nectaries (Bentley 1976).

Host abundance and distribution

Parasitoid populations are at least partially host density dependent. The distribution of hosts, searching efficiency, and the number of female parasitoids produced per host attacked, impact parasitism rate and long term parasitoid population stability (Hassell and Waage 1984). However, there is a limit to the direct correlation between host density and parasitoid attack rate, depending on immigration, emigration and landscape structure factors (Cronin 2007).

From a biological control perspective, the natural enemies should keep the host population bellow an economic threshold, but above a critical minimum density to sustain long term stability in parasitoid population numbers.

Two aspects of a parasitoid guild play an important role in providing host population suppression with long term stability: species richness and the relative role of generalist parasitoids in the guild. According to the broadly accepted insurance hypothesis, high species richness should reduce the vulnerability of a community through buffering against the impact of environmental change upon any one species (Yachi and Loreau 1999). This general hypothesis has been investigated in terms of how increased parasitoid species richness relates to the overall host population regulation and stability of the host-parasitoid population dynamics (Tschranstke 2007). Results in the field have been inconsistent. Some studies have found a strong positive relationship between parasitoid species richness and total parasitism (Hawkins and Gagne 1989; Hawkins and Gross 1992; Tylianakis et al. 2006; Mailafiya et al. 2010), but others have found no clear correlation (Rodriguez and Hawkins 2000; Macfayden et al 2009).

While the species richness of a parasitoid guild may confer higher rates of overall parasitism, there is potential for interactions amongst the parasitoids in a guild to negatively impact parasitism. Intraguild predation, and direct or apparent competition mediated by hyper-parasitoids are possible mechanisms by which parasitism rates could be impacted (Cusumano et al 2011). The detailed characterization of parasitoid feeding ranges is complicated by extreme taxonomic and life history diversity within the group, and has only been accomplished rigorously for a few species (May 1988). Temporal and spatial competition for hosts amongst parasitoid species negatively impacts overall parasitism in dynamic modeling studies (Hanski 1995; Wu and Levin 1994; Hassell 2000). In systems with a specialist parasitoid and generalist predators, both additive effects upon parasitism (Snyder and Ives 2003) and disruption of biological control (Snyder and Ives 2001) have been shown. To date, no studies have explicitly examined the relative roles of specialist and generalist parasitoids in controlling population levels of the same host species.

From a biological control perspective, the impact of both generalists and specialists is important and interactions between the two may result in complementary effects (Chang and Kareiva 1999; Sunderland 1997). In the context of invasion biology, it has been shown that introduced herbivores are released from parasitism by a dual mechanism of enemy release and geographic spread, and it is thought that lower rates of parasitism on introduced species even after a long period of time are largely due to the lack of specialist parasitoids (Grabenweger et al. 2010).

The relative impact and importance of enhancing populations of specialists versus generalists in a C.B.C framework are poorly understood. Additional information from a variety of agroecosystems is needed to determine the interplay between specialists and generalists in overall parasitism. Obtaining baseline field data on the comparative role of specialists and generalists in agricultural systems with native herbivore pests and natural enemies is an important step towards understanding these dynamics.

In this study, we focus on the relationship between overall parasitism, parasitoid species richness and the role of generalist parasitoids in sunflower fields near four habitat types: annual crops, orchards, riparian habitat and self seeding sunflower habitat. For this purpose, we are defining the parasitoids in this complex known to attack other hosts, including sunflower herbivores and those of other crop plants, as generalists and the one parasitoid that has not been reared from other herbivores in the system as a specialist.

Study system

Sunflower, *Helianthus annuus* var. macrocarpus L., is a common agricultural crop in California whose wild progenitor, *Helianthus annuus* L., is of North American origin (Heiser, 2008). The herbivore and natural enemy complex of both the crop and the self seeding plant varieties have been well documented (Beregovoy 1985; Charlet 2001; Chen and Welter 2002). The habitat types measured (annual crops, orchards, riparian habitat and self seeding "wild" sunflower habitat) represent typical surroundings of crop sunflower fields in the region of California's Central Valley. Our approach combines a three year observational survey of parasitism parameters with a sentinel larvae experiment examining the same parameters in these landscape contexts to address our central question: is there a relationship between surrounding landscape habitat type and the structure of the parasitoid guild of sunflower moth?

Annual crop habitat

Annual crops, like sunflowers, experience high disturbance in the form of crop and soil management. We predicted that these structurally simple habitats with little provision of alternate host or non-host resources support less species diversity of parasitoids, lower impact by generalists compared to specialists and lower overall parasitism compared to the other landscape context categories.

Riparian habitat

Many of the plants in the riparian context are longer lived, woody perennial species. This could provide a less disturbed source of habitat, host and non-host resources for parasitoid species. The architecture of this habitat type lends itself to a greater "edge" effect in terms of extrafloral nectaries (Bentley 1976). Overall parasitism and parasitoid species richness has been associated with proximity to riparian habitat in other systems (Pfannenstiel et al 2010; Letourneau and Goldstein 2001;Mineau and McLaughlin 1996). We expected a relatively higher level of parasitism, higher impact by generalist parasitoids, and greater parasitoid species richness in areas with more riparian habitat.

Orchard habitat

In terms of parasitoid assemblages of North American pest Lepidoptera, the importance of late succession habitats in providing alternate hosts and other resources has been highlighted as mentioned above (Marino et al. 2006). Though orchards are a managed habitat, these tree crops possess some of the characteristics of forest habitat that are important to parasitoids: alternate hosts and comparatively low disturbance. The provision of alternate hosts for parasitoids has mostly been explored from the perspective of improving control of herbivores within orchards via groundcover and edge-row plantings (Irvin and Hoddle 2010; Sarvary et al 2007). The potential for "spillover" of parasitoids and other natural enemies from orchards to annual crops certainly exists, and the synergism between the two types of crops in terms of conservation biological control strategies is worth exploring.

In California, fruit and nut orchards are managed by pruning, insecticide applications and understory weed suppression via mowing and or herbicide application. Unless there is a pest outbreak, most orchards are not often disturbed during the fruiting season. We expected that the relatively complex structure, mid level disturbance regime and potential for provision of host and non host resources characterizing the orchards would result in overall parasitism, impact by generalists and species richness of parasitoids in fields near orchards somewhere between that of fields near annual crops and riparian habitats.

Self-seeding "wild" sunflower habitat

The wild progenitor of crop sunflower (*Helianthus annuus* L.) is found in dense patches in uncultivated areas throughout the Central Valley and in some instances can be found very close to crop sunflower fields. Cultivation guidelines for parent seed varietals require that they be isolated and thus growers try to eradicate self-seeding sunflowers from within 2km of their fields, though they do occasionally occur as weeds and are not usually removed when the sunflower crop is meant for oil production (Nerney Meyers personnal observations). Previous work has shown that several indigenous herbivore and natural enemy species are found in both the agricultural and self seeding habitats (Chen and Welter 2002), that parasitism and parasitoid species richness are greater in the uncultivated habitat (Chen and Welter 2002) and that, due to the creation of structural refuges in the seed layer of cultivated sunflowers, individual herbivores are more likely to be parasitized in the wild habitat (Chen and Welter 2007). Further, the specialist parasitoid *Dolichogenidea homoeosomae* Muesebeck exhibits a high degree of gene flow between cultivated and uncultivated habitats and thus is unlikely to specialize in one host plant type (Nerney Meyers et al. in preparation). For this reason, we included wild sunflower habitat in our measures of habitat types surrounding field sites, with the expectation that the presence of wild sunflower habitat in the landscape context of a field would increase parasitism rate, contribution of generalists, and species diversity of parasitoids.

Methods

I. Parasitism parameters survey

A. Site selection

We performed three annual surveys of *H. ellectellum* (2003, 2004, and 2005) at a total of 60 agricultural sunflower fields in California's Central Valley to compare parasitism the parameters: percent parasitism, parasitoid species richness, and impact of generalist versus specialist parasitoids. "Impact" here is defined as the proportion of total parasitism due to attack by each species of parasitoid. We conducted the surveys across all the landscape contexts: annual crops, orchards, riparian habitat and self seeding sunflower habitat. The sites were selected from a pool of available agricultural sunflower sites to represent the existing range of habitat context in California's Central Valley.

B. Landscape categorization

We identified and assigned habitat categories within a 1 km radius area from the center of each of the 60 crop sunflower field sites in the survey. Habitat/ vegetation type was determined using aerial photography data available on Google Earth (from the year of the survey or prior, US Geological Survey and USDA Farm Service Agency data, Google Earth (Version 5.2.1.1588) [Software], Mountain View, CA: Google Inc. 2009). We also performed ground inspections of the area surrounding the field to verify vegetation cover type during the year of the respective survey. The area within a 1km radius of the center of the field (determined using Google Earth^{TM} scaled ruler tool) was plotted and a true to scale circle was superimposed on the image using AutoCAD (Computer Aided Design software application, Autodesk, Inc. 1982). Polygons were drawn over each identifiable habitat patch (those that we were not able to identify or were urbanized, gravel etc. areas were marked as "other"). The true to scale area of each polygon in each of the categories was then determined and summed to obtain estimated scale measurements of the proportion of the total area covered by each habitat type surrounding each of the sites. We then divided the total area measurements in each habitat category by the area of the circle (3.14km^2) to obtain the proportion of surrounding landscape (see appendix 1 for map overlays, and data legend for each site). All habitats within the circle were weighted equally in the proportion calculations. Landscape categorization calculations are summarized in Appendix ii and the autoCAD image overlays for each site are contained in Appendix iii.

The habitat categories are defined as follows:

Annual: annual crops such as tomatoes (*Lycopersicon esculentum*), sunflowers (*Helianthus annuus)*, wheat (*Triticum spp*.), various hay crops that are harvested bi-annually (*Dactylis glomerata,* etc.), broccoli (*Brassica oleracea*), rapeseed (*Brassica napus*), peppers (Capsicum annuum), lettuce (*Lactuca sativa*) and various cut flower species.

Riparian: Seasonal wetland, creek, or other waterway habitat with at least one of the following perennial plant species common: Big leaf maple (*Acer macrophyllum)*, alder (*Acer negundo),*willow *(Salix* spp.), blackberry (*Rubus* spp).

Orchard: Tree crop plantings including almond (*Prunus dulcis*), walnut (*Juglans regia*), pear (*Pyrus spp*.), and a variety of stone-fruits (*Prunus spp*.).

C. Site locations

Due to some inter-year variation in the location of crop sunflower plantings, the sites are never in the same exact locations for more than one of the annual surveys, though they are within the same regions and counties. All of the sites included in the survey are agricultural oilseed sunflower (female hybrid plants) fields (*Helianthus annuus* var. macrocarpus L.) between 3,000 and 20,000 meters^2 in size. Only female hybrid plants were used in the survey. To check for possible effects of location (latitude), we divided the sites into 3 regions: region 1 is the southernmost including Fresno and Tulare counties, region 2 is the middle latitude section of the Central Valley including Madera, Merced, Stanislaus and San Joaquin counties and region 3 is the northernmost area where the sites were located including Solano, Yolo, Colusa, Sacramento, Glenn and Butte counties (Figure 1).

D. Survey methods

Adult female sunflower moths lay eggs singly or in groups of 4-5 on the florets of sunflowers between 3 and 6 days after bloom begins. Larval development and floret development are synchronized such that the larval stage consumes the progressive stage of the flower (Aslam and Wilde 1991). Because the aim of the survey was to measure parasitism parameters of sunflower moth, the developmental stage of the larvae collected (via the flowering stage of the host plant) was held constant across sampling sites. To maximize exposure to the most common larval parasitoids, the moth larvae were collected during the 3rd or 4th (final) instar (Rogers, 1978), when the sunflowers were between the R6 and R7 flowering stage (Schneiter and Miller, 1981). Planting dates vary within the Central Valley of California from mid May to mid July. Depending on temperature and daylight length, the plants take between 60 and 90 days to bloom.

A random sample of 100 flower heads was collected at each site and placed in brown paper bags (8 liter volume). The samples were kept between 20 and 25ºC during transport to the laboratory. Sunflower moth larvae were present at all of the sites. Infestation rates per flower ranged from 0 to 62 larvae (average across sites of 11.1 larvae/ flower head; standard deviation 8.8) and are similar to those found in previous studies (Chen and Welter 2002). Each flower head was dissected and all sunflower moth larvae found within were placed in individual covered 28ml plastic cups with artificial diet at 23 ºC (Wilson 1990; Chen and Welter, 2003). We checked the diet cups daily and placed any parasitoids emerging in 2ml tubes filled with 95% EtOH. Parasitoids were identified with assistance from Yolanda Chen, University of Vermont; Michael Sharkey, University of Kentuky; Norman Woodley, USDA, and Robert Zooparko,

California Academy of Sciences. We then assigned each species as "specialist" or "generalists" based upon published feeding records for sunflower moth and other common sunflower herbivores in agricultural and wild sunflower habitats in California (Chen and Welter 2002, 2003, 2007; Nerney Meyers unpublished data) (Table 1). Total numbers of sunflower moth larvae found in each 100 flower survey, as gross parasitism rates for each site and estimated area of each sunflower field are reported in Appendix i.

Herbivore density in the 100 flower samples ranged from 103 larvae to 156, with a mean of 126.6; S.D. = 12.5 (Appendix i). To obtain equal samples of larvae from all sites we took a random sample from within each survey of 100 larvae using the random sample function in Excel (Microsoft Corporation 2007). For each site, we recorded: 1) parasitism rate calculated as P/(A+P) where A is the number of adult moths and P is the number of larvae parasitized (dead individuals were not included in the calculation because their exact fate could not be determined) 2) total parasitoid species reared 3) total number of generalist and specialist parasitoids obtained in the sample.

We then converted the total number of generalist and specialist parasitoids into a proportion calculated as g/ P and s/P respectively (where s is the number of specialists reared, g is the number of generalists reared and P is the number of larvae parasitized). The region (1, 2, or 3 as described above) for each field was also coded to check for possible effects of latitude upon parasitism parameters. Finally, we noted the presence or absence of wild sunflower habitat within a 5km radius area of the site (this was coded as a separate binary variable from the proportion of wild sunflower habitat within the 3.14km^2 circle).

E. Survey data analysis

To examine trends within this observational dataset, we first used visualization tools (Buja et al. 2003) to plot each of the response variables: parasitism percentage, parasitoid species richness, impact by generalist parasitoids, impact by specialist parasitoids, against the independent variables: habitat type, yes or no within 5km of wild sunflower habitat, year of survey and region. We followed this with descriptive statistics to test for normality in the distribution of the dependent variables. Because many of the variables are in the form of proportions (ie: of landscape in a certain habitat, of parasitism due to generalists), we used both generalized linear models and logistic regression to examine relationships amongst them. In all cases we found that the test statistic significance values were very similar, but we report both for comparison.

We examined combinations of each of the independent variables (habitat context, proximity to wild sunflower habitat, region and year of survey) and the dependent variables (overall parasitism, impact by specialist and generalists and parasitoid species richness) as well as interactions amongst the terms in a multiple generalized linear model framework using the software package R (a language and environment for statistical computing) (R Development Core Team 2010).

II. Sentinel larvae experiment

In order to experimentally test the influence of landscape context and proximity to wild sunflower habitat upon the parasitism parameters (percent parasitism, parasitoid species richness, impact by generalist vs. specialist parasitoids), we used "sentinel" (sensu Walker and Welter 2004; Benson et al. 2003) sunflower moth larvae reared in the lab and then placed in an array of field situations described bellow to expose them to ambient parasitism. We established a laboratory colony of sunflower moth using field collected larvae from throughout the Central Valley of California following the procedure described in Wilson 1990.

A. Site selection

For this experiment, we selected both agricultural sunflower fields and patches of self seeding (wild) sunflowers that were embedded predominantly by each of the surrounding habitat types. Fields categorized as "annual" had a minimum of 70% of the 1km radius circle around the center of the field in annual crops as described above for the survey sites. Fields categorized as "orchard" had a minimum of 70% of the 1km radius circle around the center of the field in orchard crops as described above for the survey sites. Fields categorized as "riparian" had a mix of annual crops and orchard crops and a minimum of 5% of the 1km radius circle around the center of the field covered by riparian habitat as described above for the survey. We choose the 5% value for riparian categorization for two reasons 1) there are very few sites in the central valley that have much more than 5% riparian habitat around them and 2) preliminary surveys suggested even a small amount of riparian habitat can impact the parasitism parameters in question. These categorizations mirror the realistic scenario of habitat context that we quantified for the survey sites as described above.

B. Sentinel larvae exposure

At each of 36 sites (6 agricultural and 6 wild sunflower fields in each of the three landscape context categories of orchard, annual and riparian), we placed 5 wild type (*H. annuus*) sunflower plants (at R6 blooming stage). The plants were grown in a greenhouse and then hardened to exterior conditions for one week before the experiment. Plants were placed at random within the field. We then placed 10 laboratory colony raised larvae in the 3rd instar on the flowers of each of the 5 plants (n=50). For five days, we kept the sentinel plants watered and checked for bird or wind damage (plants with damaged flowers were eliminated from the study). After five days of exposure, we collected all of the flowers from the sentinel plants and dissected them to remove all remaining sunflower moth larvae. Sentinel larvae recovery rates averaged 52.1% (st dev 12.8; n=26.3 +/- 6.4) (Table 3). Larvae that were not of the same age class as the sentinel larvae were removed from the study under the assumption that they were from eggs lair prior to or after placement of the sentinel larvae. The sentinel larvae recovered were placed in individual covered 28ml plastic cups with artificial diet at 23 ºC as above and checked daily for parasitoid or adult moth emergence.

C. Sentinel data analysis

We analyzed the resulting data on parasitism parameters (percent parasitism, parasitoid species richness and impact by generalist parasitoids) and the independent treatment variables/ levels (agricultural field, wild field; landscape context categories: annual/orchard/ riparian; distance from wild sunflower habitat: near, far) using generalized linear models followed by MANOVA and Pillai's tests to compare the model fit (McCullagh and Nelder, 1989). We tested the data for overdispersion by measuring dispersion using Pearson's chi-square, divided by the degrees of freedom (Ihaka and Gentleman, 1996). Since over-dispersion was not present, we used a binomial distribution. We also ran the analysis of variance model using Welch's method for relaxing the variance assumption (since variances were not equal, and could not be transformed to make them equal, Figures 13-17) (Dalgaard 2002) again using the software package R (R Development Core Team 2010).

Results

I. Parasitism parameters field survey

A. Overall parasitism, habitat proportions, geographic region and field area

The mean rate of parasitism found amongst all of the sites was 9.7% (standard deviation = 3.3%) and ranged from 5% to 17%. The mean number of attacks by specialist parasitoids per site was 5.7 (standard deviation= 1.7) and that by generalists was 4.0 (standard deviation= 3.4). The proportion of parasitized larvae per site attacked by generalists ranged from 0.0 to 0.8 (average = 0.36, S.D. = 0.23). The mean number of parasitoid species found per site (species richness) ranged from 1.54 to 3.31 (maximum of 4 species, minimum of 1) (Table 2).

The mean proportions of habitats across sites were: annual crop 0.63 (standard deviation= 0.31), orchard 0.24 (S.D. = 0.31), riparian 0.06 (S.D. = 0.09), "other" (including equipment yards, barns, gravel lots and mayor roads) 0.05 (S.D. =.05), wild sunflower habitat 0.02 (S.D. = 0.03) (Table 2). Proportion of annual crop habitat within a 1km radius circle ranged from 0 to 1.0, orchard from 0 to 0.9, riparian from 0 to 0.4, "other" from 0 to 0.2, and wild sunflower habitat from 0 to 0.2 (Table 2). Proportion of each habitat type for each site is listed in Appendix ii and detailed in Appendix iii.

There were no significant relationships amongst any of the dependent variables (parasitism parameters) and the three geographic areas, therefore we kept the data pooled across geographic areas and survey years (G statistic for parasitism percent, species richness, proportion of specialists, proportion of generalists respectively 1.5, p= 0.56; 1.2, p= 0.67; 1.1, p= 0.35; 1.6, p= 0.45).

We found a slight positive correlation between the area of the sunflower field sampled and the total number of sunflower moth larvae found in the 100 flower survey, adjusted R^2= 0.08, p=0.04 (Figure 2). There was also a similar positive relationship between the gross percent parasitism at each site and the number of larvae found in the 100 flower survey, adjusted R^2 = 0.10, p=0.01 (Figure 3), indicating a slight density dependent response. Correlations between field size and parasitoid species richness and impact by generalists were not significant (p=0.12 and 0.18 respectively).

While we found that parasitoid species richness was higher for sites within 5km of identified wild sunflower habitat (Tukey's Honest Significance test adjusted p = 0.044), parasitism rates and impact by generalist or specialist parasitoids at sites within 5km of identified patches of wild sunflowers were not significantly higher than at those sites not within 5km of wild sunflower habitat (Tukey's Honest Significance test adjusted p = 0.76, p= 0.09, p=0.44 respectively). Notably, the strongest of these relationships was between sites within 5km of wild sunflower habitat and proportion of impact by generalist parasitoids (p=0.09).

B. Parasitism and habitat types

Percent parasitism of sunflower moth at the survey sites was significantly positively correlated with proportion of the area surrounding the field in orchard habitat (adjusted R^2= 0.44, p=0.025 $E^{-}7$) (Figure 4) and negatively correlated with proportion annual habitat (adjusted R^2= 0.45, p=0.002$E^{-}7$) (Figure 5). There was no significant relationship between parasitism and proportion of riparian habitat (p= 0.46) or wild sunflowers (p= 0.44).

C. Parasitism and the other parasitoid guild composition factors

Parasitism rate increased as the number of species (species richness) found per site increased (adjusted R^2= 0.29, p=0.02) (Figure 6). Likewise, parasitism rate and proportion of parasitism caused by generalist parasitoids were positively correlated (adjusted R^2= 0.35, p=0.03 $E^{-}5$) (Figures 7).

D. Species richness and landscape composition

The number of species found per site increased with the proportion of the 1km radius around the center of the field that contained orchard habitat (R^2 = 0.22, p=0.01 $E^{-}7$) (Figure 8), while it decreased with the proportion of the area that contained annual crop habitat (R^2 = 0.28, p=0.01 $E^{-}3$) (Figure 9). There was no significant correlation between number of species and proportion riparian habitat (p = 0.21) or proximity to wild habitat (p=0.07).

E. Relative impact by generalist parasitoids

The proportion of larvae parasitized by generalists and the amount of orchard habitat surrounding the sunflower field were positively correlated (R^2= 0.37, p = 0.01 $E^{-}6$) (Figure 10). It was inversely correlated (though less strongly) with the amount of annual crop habitat (R^2= 0.28, p = 0.01 $E^{-}3$) (Figure 11). The amount of riparian and wild sunflower habitat was not related to the proportion of parasitism due to generalist parasitoid attacks (p= 0.11 and p= 0.09 respectively).

F. Multiple Linear Regression Models

Linear regression model fits are summarized in Table 3. The best fit describing the relationship between percent parasitism and the independent landscape habitat variables (proportion annual crops, orchards, wild sunflower habitat and riparian habitat) is one that includes only the negative impact of proportion annual habitat (F statistic = 48.8, 58 degrees of freedom, p= 0.02 E^{-7}, R^2 = 0.45) (Table 3). To describe species richness, the best model fit includes only the negative effect of proportion of annual crop habitat (F statistic = 23.5, 58 degrees of freedom, p= 0.01 E^{-4}, R^2 = 0.28) (Table 3).

Two of the single variable modes for proportion of parasitism by generalist were tied for the best fit: the model fit including the negative term for annual crops (F statistic = 79.9, 58 degrees of freedom, p= 0.08 E^{-10}, R^2 = 0.55) and the model including only the positive effect of orchard habitat (F statistic = 72.9, 58 degrees of freedom, p= 0.08 E^{-10}, R^2 = 0.55) (Table 3).

II. Sentinel experiment

The results of the sentinel larvae experiment are summarized in Table 4. We recovered 5 of the 10 total parasitoid species found in the field survey from the sum total of the experimental sites (see Table 1, *). The actual numbers of individual parasitoids upon which the parasitism parameters are reported are relatively low (with a mean of 4 individuals of 26 recovered parasitized). This results in low statistical power with regards to the parasitism parameters under an MANOVA model framework. However, there are some appreciable trends in the data. The range of percent parasitism overlaps for the three landscape categories (annual, orchard, riparian), but the highest mean value (19% parasitism) is for the riparian landscape category sites, with a notable outlier site in the annual landscape category with 31.2% parasitism (Figure 12). The variable of species richness also has overlapping ranges for the three landscape categories, but the mean for annual and riparian is at 2, while that for orchards is at 3, and only orchard category sites have more than 3 species (maximum number of species found in one site = 4) (Table 4). Although the variance is overlapping, the proportion of parasitism caused by generalists follows a similar trend to that seen in the field survey, with the mean for annual sites being 0.2 and that for orchards being 0.8 (Figure 14). Finally, the raw data comparing parasitism rates at the agricultural and wild field types shows that the wild sites displayed a wider range of parasitism rates though the mean for both wild and agricultural sites was very close to 17% parasitism (Figure 15). The maximum number of parasitoid species found per site (4 species) was only found in wild sunflower patch sites (Table 4), and while there was not a significant difference between agricultural fields and wild sunflower patches in terms of species richness, the wild sites exhibited a greater range (Table 4). Not surprisingly, an ANOVA model fit to the independent variables we tested did not result in any significant factors, with all p values above 0.3 (Table 5).

Discussion

I. Are parasitoid species richness and parasitism rate correlated in this survey?

In our three year survey of 60 agricultural sunflower fields in the Central Valley of California, we found that percent parasitism and the total number of parasitoid species responsible for that parasitism were positively correlated (Fig 6). Though the total number of parasitoid species found in our study was 10, only 2 of these were relatively common across sites, with the maximum number of species found at any one site being 4 and 68.2% of the total parasitism across sites due to the specialist *Homoeosomae ellectellum* and the generalist *Macrocentrus ancylivorous*. The rest of the 8 species were each responsible for an average of 4.0% of the total parasitism events (Table 1). Given that the life stage feeding niche of this parasitoid guild is the later instar larvae of *H. ellectellum*, it is quite possible that the total number of parasitoids that attack this host throughout its life cycle is much greater. The evidence from our study about the relationship between species diversity and overall parasitism agrees with trends found in studies of several other host taxa (Hawkins and Gagne 1989; Hawkins and Gross 1992; Tylianakis et al. 2006; Mailafiya et al. 2010), and provides strong evidence that in this system parasitoid species richness is correlated with overall parasitism.

II. What is the variation in proportion of generalists? What is their effect on parasitism rates?

Remarkably, the specialist *D. homoeosomae* was present at every one of the 60 sites (though in one case one individual was greatly outnumbered by other generalist species). This speaks to the pervasiveness and importance of this one species in the food web. Contrary to expectation, sites with more specialists (or with a greater proportion of parasitism caused by specialists) were not more likely to exhibit higher rates of parasitism, in fact, the opposite is true; there was a strong positive correlation between the percent parasitism and the proportion of parasitism caused by generalists (logistic regression G statistic = 26.0, adjusted $R^2 = 0.35$, $p=0.03^{E-5}$) (Figure 7). This evidence supports the idea that generalists provide more "efficient" suppression in this system, given that the specialists are 1) more prevalent across the sites and 2) responsible for about half of all parasitism events in our survey (Table 1), yet parasitism is higher in sites where generalists play a more prominent role in parasitism (Figure 7).

III. Is there support for our predictions about the influence of each type of habitat?

A. Annual crop habitat

We predicted that fields in areas with more annual crop habitat would exhibit lower parasitism rates, lower species richness, and lower proportions of overall parasitism caused by generalists. The survey data supports all of these hypotheses. The proportion of annual habitat around a field was associated with lower parasitism (logistic regression G test = 36.9, $p=0.02^{E-7}$) (Figure 5), lower parasitoid species richness (logistic regression G test = 19.9, p=0.00) (Figure 9), and lower proportion of parasitism by generalists (logistic regression G test = 19.3, $p=0.01^{E-3}$) (Figure 11).

The proportion of parasitism caused by specialists was higher in the sites with a greater proportion of annual crop habitat with almost 50% of the variation in parasitism due to specialists explained by the variation in proportion of annual crop habitat surrounding the field (adjusted R^2= 0.49) (Figure 11; Table 3).

B. Orchard habitat

Based upon what we perceived to be the potential habitat qualities of an orchard from a parasitoid's perspective (alternate hosts, possible extrafloral nectaries, less disruptive management such as pesticides), we thought that fields surrounded by more orchard habitat would exhibit somewhat more parasitism, more generalists parasitoids and greater species richness than those with annual fields and somewhat less than those sites with stronger influence from riparian areas or wild sunflower habitat. The amount of orchard habitat surrounding a field was the strongest predictor of parasitism rate (logistic regression G statistic = 35.54, R^2= 0.44, p= 0.03 $^{E-7}$) (Figure 4). This combined with the positive correlation between orchard habitat and number of species (logistic regression G statistic =15.1, R^2=0.22, p=0.01^{E-7}) (Figure 8) and that between orchard habitat and proportion of parasitism caused by generalists (logistic regression G statistic = 28.2, adjusted R^2= 0.37, p= 0.01 $^{E-6}$) (Figure 10) supports that the relationship is stronger than we expected.

C. Riparian habitat

We expected to see the highest levels of parasitism, species richness and proportion of parasitism caused by generalists in the areas with greater riparian habitat quantity based upon our preliminary sampling and studies in other systems (Pfannenstiel et al 2010; Letourneau and Goldstein 2001). However, there were no significant correlations between any of the parasitism parameters and the proportion of surrounding riparian habitat, nor was this variable significant in any of the multiple regression models. This observation could be related to the landscape features of California's Central Valley in relation to other areas where similar studies have been carried out. Dense patches of riparian habitat are relatively rare in this agricultural area and our survey sites reflect this, with on average less than 6% riparian habitat present in the 1km radius area around a field. While this limits the power of our ability to seek patterns about the impact of this habitat type, we are still somewhat surprised to find that even the sites with the greatest proportion of riparian habitat (SFAG05-20, with 40% riparian habitat and SFAG04-14 with 38%), did not have higher than average parasitism rates (10% and 6% respectively, average parasitism rate = 9.8%), species richness (both had 2, average was 2.31) or impact by generalists (both had 30%, average was 36%) (Table 2). One possible explanation for the incongruence between our results and the theory of parasitoid habitat influence (Bentley 1976, Price 2001), is that the surface areas of the water bodies themselves were included in the area estimates, so this may not have reflected the actual habitat availability

IV. The influence of orchard habitat upon parasitism parameters

Increasing orchard habitat in the immediate surroundings of a sunflower field in this observational data set was associated with percent parasitism, impact by generalist parasitoids and species richness of parasitoids. In addition, the proportion of impact by generalist parasitoids was positively correlated with the total parasitism. While a great deal of attention in the C.B.C. field has been focused on the role of generalist parasitoids of lepidopteran pests in orchard systems and potential habitat sources for these natural enemies (Bugg and Picket 1998; Coll 1998; Guys 1982), the literature does not yet speak to the influence of orchard habitats upon parasitism within annual crop fields that are adjacent to orchards. This study provides motivation to further explore this relationship and its potential for management implications.

V. Sentinel experiment

We found no significant effects of any of the treatment factors- landscape category: annual, orchard, riparian; distance from wild sunflower habitat: within 5km, more than 5km; type of field: agricultural field, wild sunflower patch. Nonetheless, non-significant trends in the results of the sentinel experiment do complement our findings in the field survey in several important ways and warrant further work in this area.

The overall percent parasitism found in the survey (mean 9.7%, standard deviation= 3.3%) is within the range found in the experiment (mean 17.4%, standard deviation 12.8%). The greater variability in the experiment is interesting. During preliminary studies, we found that the timing of parasitism varies temporally during the summer season, and the experiment allows for a smaller window of opportunity than what is included in the survey. During the experiment, we found 5 of the 10 species of parasitoids reared in the survey (Table 1), but in both the experiment and the survey one specialist*, D. homoeosoma*, was responsible for more parasitism than any other species (total in survey 52.1%, total in experiment 56.0%) (Table 4). The proportion of specialists vs. generalists responsible for the parasitism followed a similar pattern in the survey and the experiment. The proportion of specialists across sites in the survey was mean= 0. 61 standard deviation = 0.22 and in the experiment the mean = 0.56 and standard deviation = 0.28. In both cases this proportion had the highest mean in fields surrounded by more annual habitat (experiment annual treatment proportion of parasitism due to specialists mean = 0.77 standard deviation = 0.24) (Tables 2, 4). The same pattern holds for the relationship between orchard habitat and impact by generalist predators (survey overall mean = 0.40 standard deviation = 3.4; experiment orchard treatment mean = 0.61 standard deviation = 0.37) (Tables 2, 4).

Conclusion

Our approach in this study was to use an existing agricultural landscape and a well known host-parasitoid community to examine possible influences of habitat types on parasitism parameters. Building a realistic model of the influences of different habitat types, including managed and unmanaged as well as a range of plant genotypes (in this case with the wild progenitor of the crop present) is relatively complex, but a good starting point may be to outline the scale at which influences in parasitism parameters can be detected. A recent study successfully examines the influence of rangeland habitat upon another important ecosystem service for agriculture: pollination, at a scale similar to the one we use (in the range of 1 to 3 km) (Chaplin-Kramer et al. 2011). Studies in Europe have also found influences upon parasitism correlated to habitat complexity within the 1 to 3km range (Trsanske et al. 2007). Many more empirical studies combining survey and experimental data are needed to begin to compile a set of recommendations for optimal agroecosystem management at the landscape level (sensu Tchranstke et al. 2007).

Our findings indicate that 1) parasitism and parasitoid guild composition are correlated with surrounding habitat types at the scale of 0-2 km from a field; 2) generalist parasitoids are efficient natural enemies in this system; and 3) increasing parasitoid species richness improves biological control in this system.

A more extensive sentinel experiment with greater sample size and replicates could provide greater insight into the relative effect of each habitat type. It would be beneficial to quantify as a continuous variable the amount of each type of habitat surrounding the sentinel experiment plots and follow with multiple linear regression tests. This would allow for a better comparison with the field survey results. While experimental support of the trends found in our field survey was not statistically significant, the broad trends seen in the sentinel experiment are complementary to the observational data. Further controlled experiments are desirable to determine the strength of the relationship between, in particular, orchard habitats and parasitism by generalists.

Our results support the theory that parasitoid species diversity translates into higher rates of parasitism. A higher proportion of generalists was related to higher parasitism, although the specialist parasitoid in this guild was responsible for the highest proportion of overall parasitism. Generalists may partially compensate for loss of overall species diversity in simplified agricultural habitats, since specialists in particular may be vulnerable to disturbance in the system due to having "all their eggs in one basket" (Tschrasnke 2007). Evidence from this and other studies in the sunflower system indicates that although the specialist parasitoid *D. homoeosmae* is pervasive throughout the Central Valley of California (Chen and Welter 2003; Nerney Meyers et al. in preparation), the role of generalists in this guild remains critical in providing suppression of the herbivore pest.

In light of the population genetic structure of this specialist parasitoid and resulting constraints on adaptive potential, the generalist species found in this survey may become increasingly important in the agricultural sunflower fields over time (Nerney Meyers in prep.)

"Farmscaping" and long term landscape level planning will benefit from further consideration of the potential C.B.C. synergism between sunflower fields and orchard crops. In order to understand the influence of surrounding habitats upon parasitism within agricultural fields, continued research on the factors impacting parasitoid guild composition and the relative importance of host and non host resource distribution is needed.

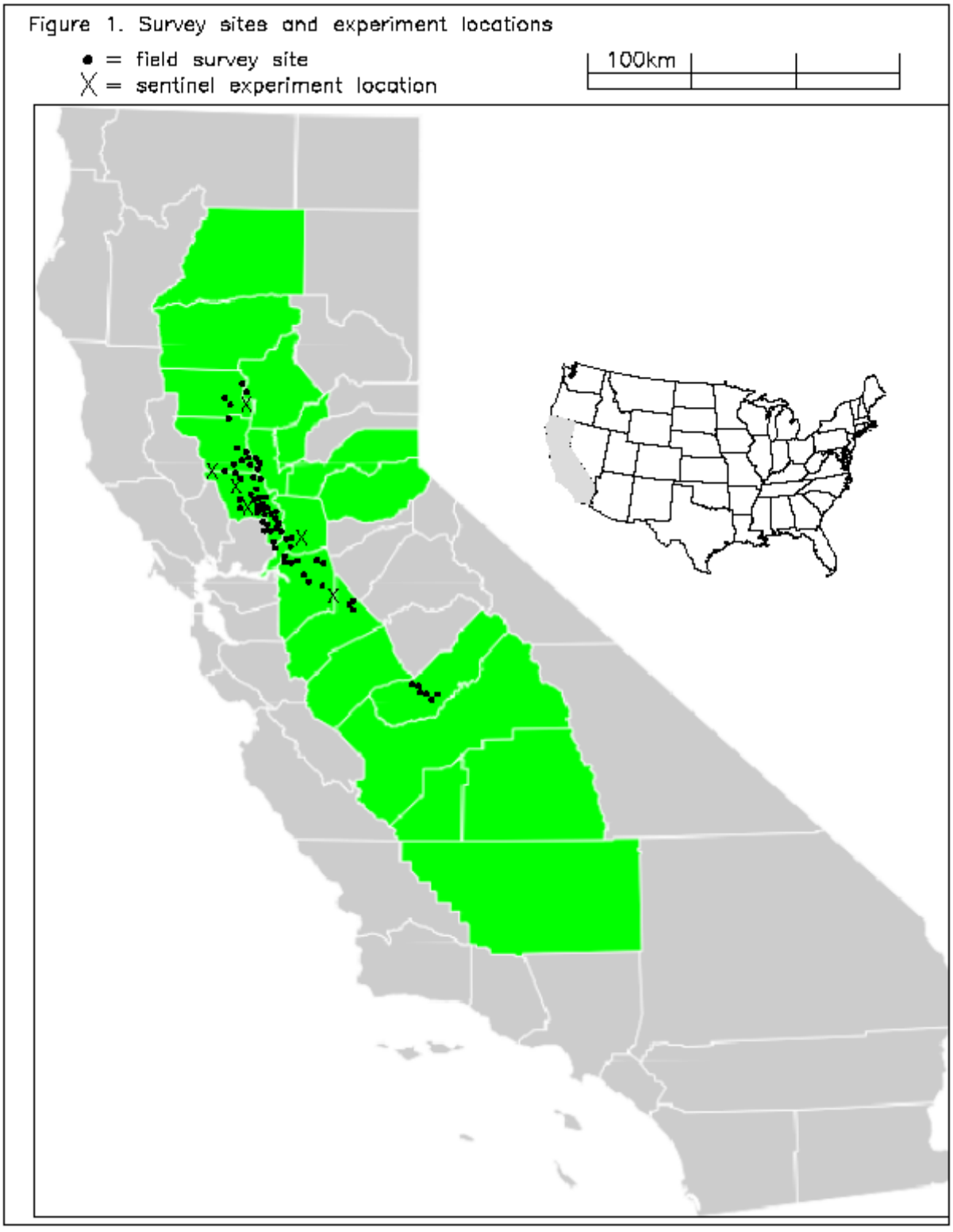
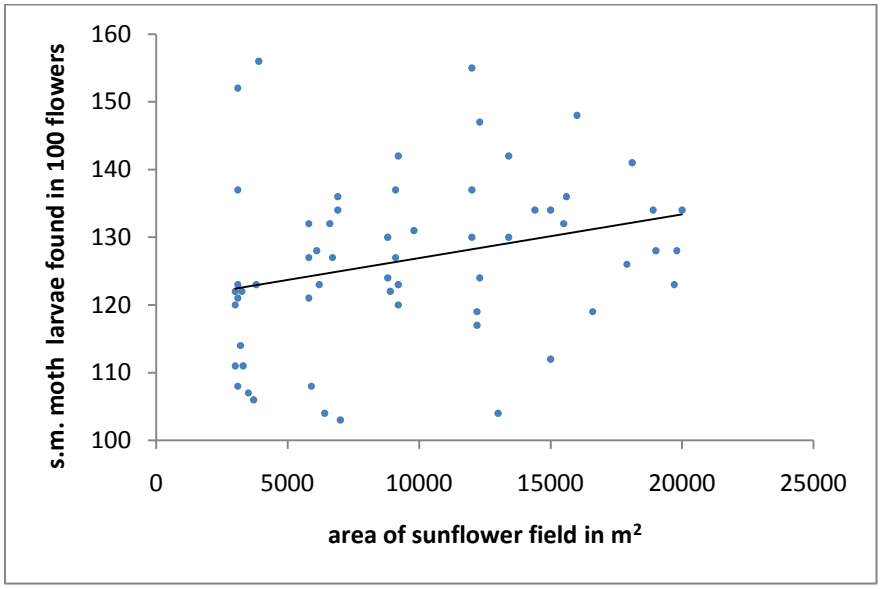


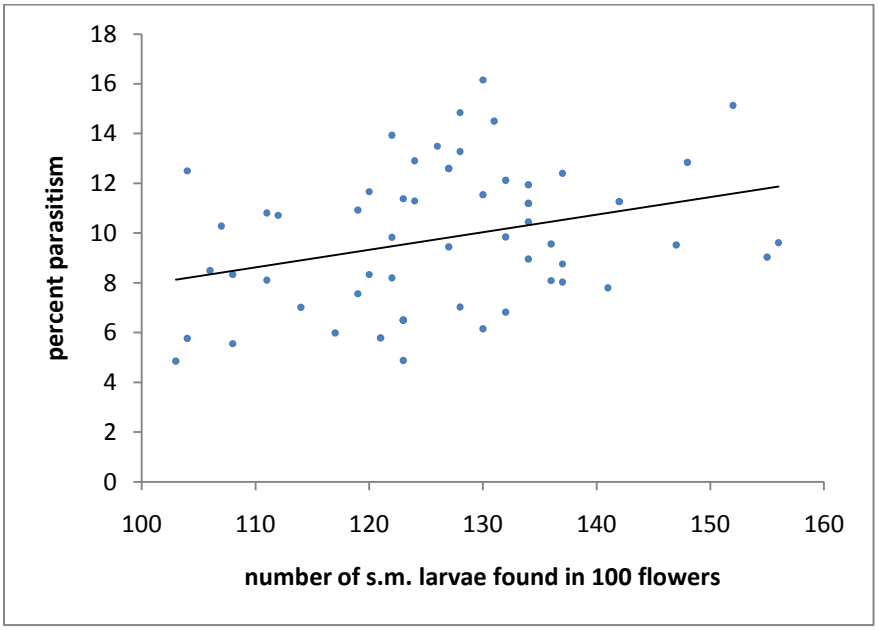
Figure 1. Survey sites and experiment locations
• = field survey site
X = sentinel experiment location
100km

Figure 2. Correlation of density of sunflower moth larvae in 100 sunflower heads and area of the sunflower field



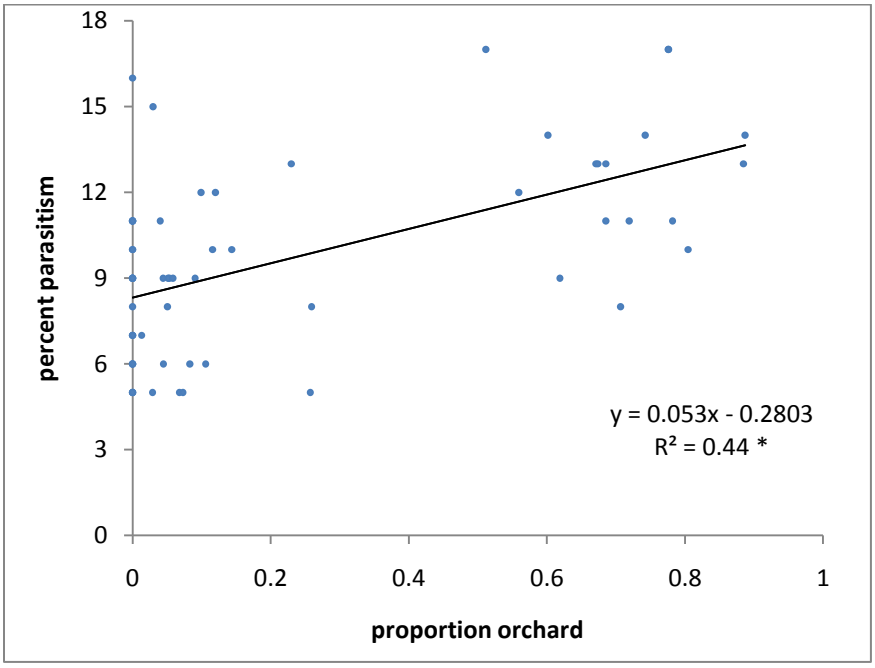
Linear model fit $y = 0.0006x + 120.46$; $R^2 = 0.08$, $p=0.04$

Figure 3. Correlation of overall percent parasitism and the number of larvae found in each survey



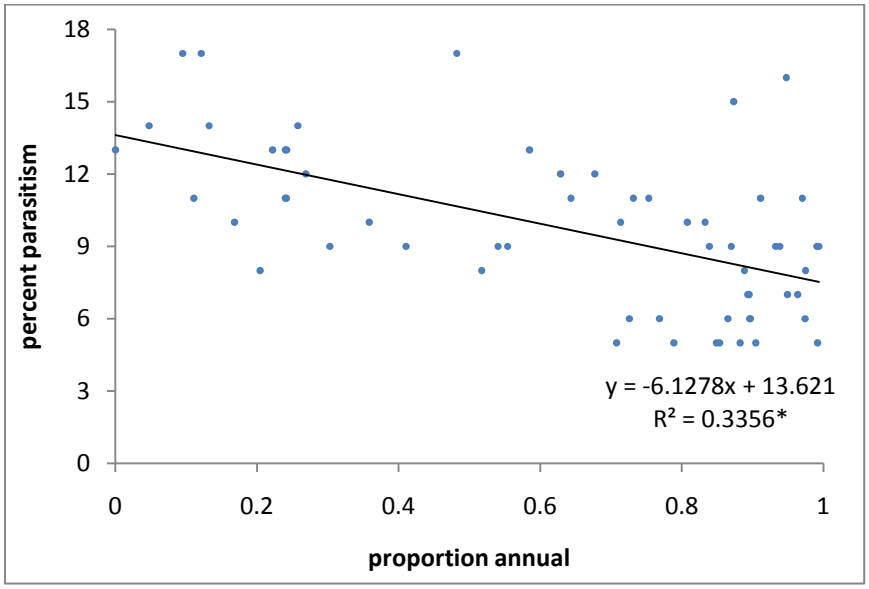
Linear model fit $y = 0.0708x + 0.8384$; $R^2 = 0.10$, $p=0.01$

Figure 4. Correlation of parasitism and proportion of surrounding habitat within a 1km radius of each site in orchards



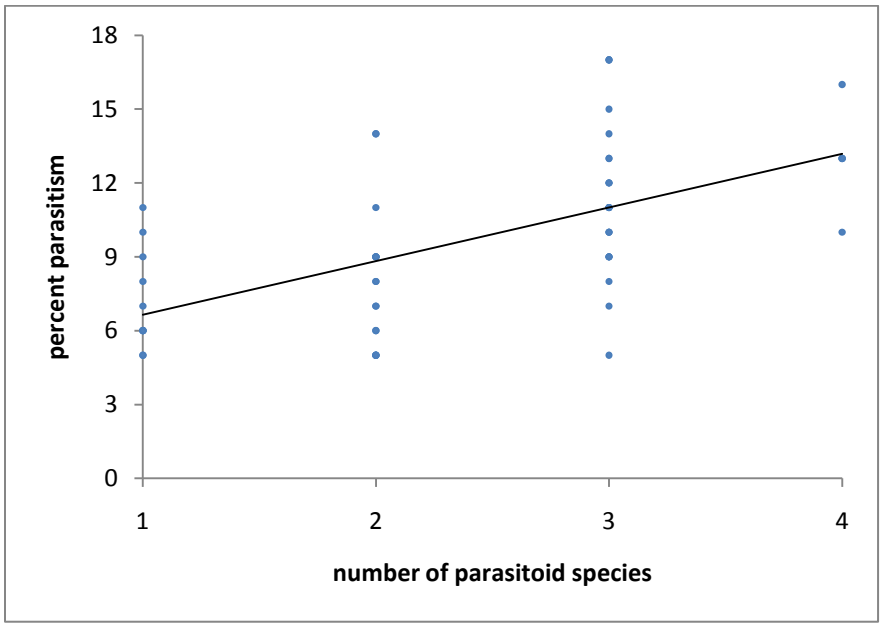
*logistic regression R^2 = 0.44, Model L. R. 35.5 (G statistic), p = 0.025E-7

Figure 5. Correlation of parasitism and proportion of surrounding habitat within 1km radius of each site in annual crops



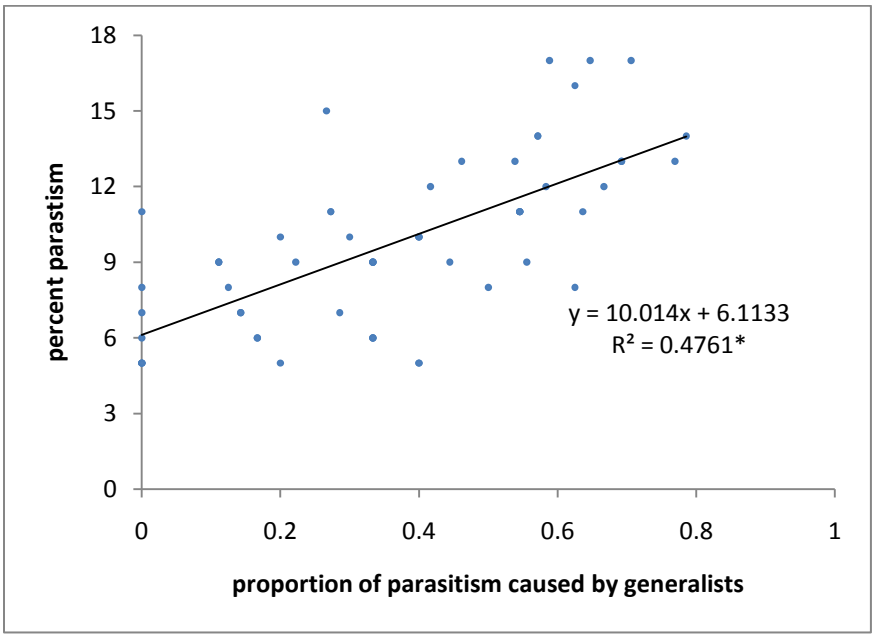
*logistic regression R^2 = 0.46, Model L. R. 36.9 (G statistic), p = 0.015E-7

Figure 6. Correlation of parasitism and species richness per site



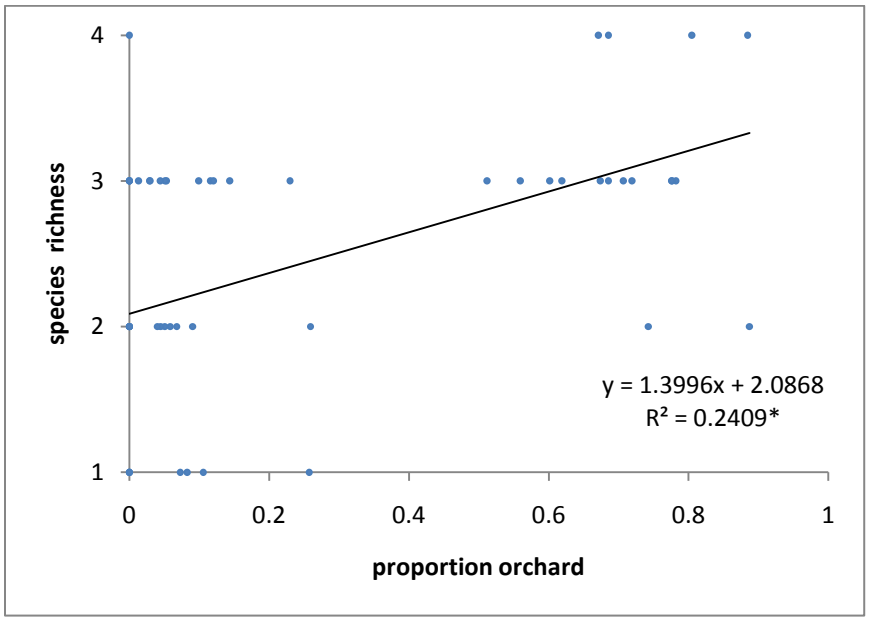
Linear model fit $y = 0.1565x + 0.8936$, $R^2 = 0.29$, $p=0.02$

Figure 7. Correlation of Percent parasitism and proportion of parasitism caused by generalist parasitoids



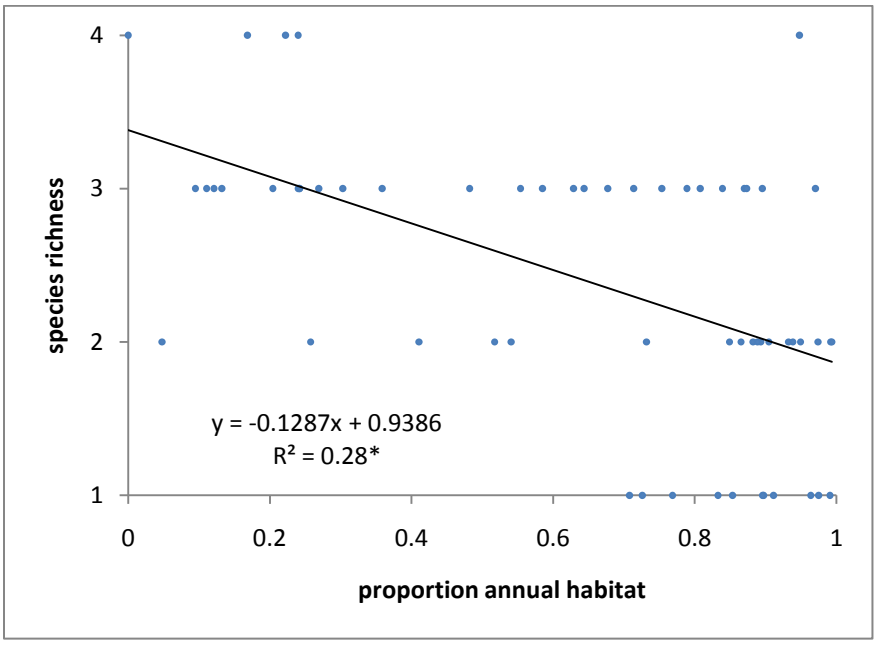
*logistic regression R^2 = 0.35, Model L. R. 26.0 (G statistic), p = 0.034E-5

Figure 8. Correlation of the number of parasitoid species found at each site and proportion of surrounding landscape in orchard habitat



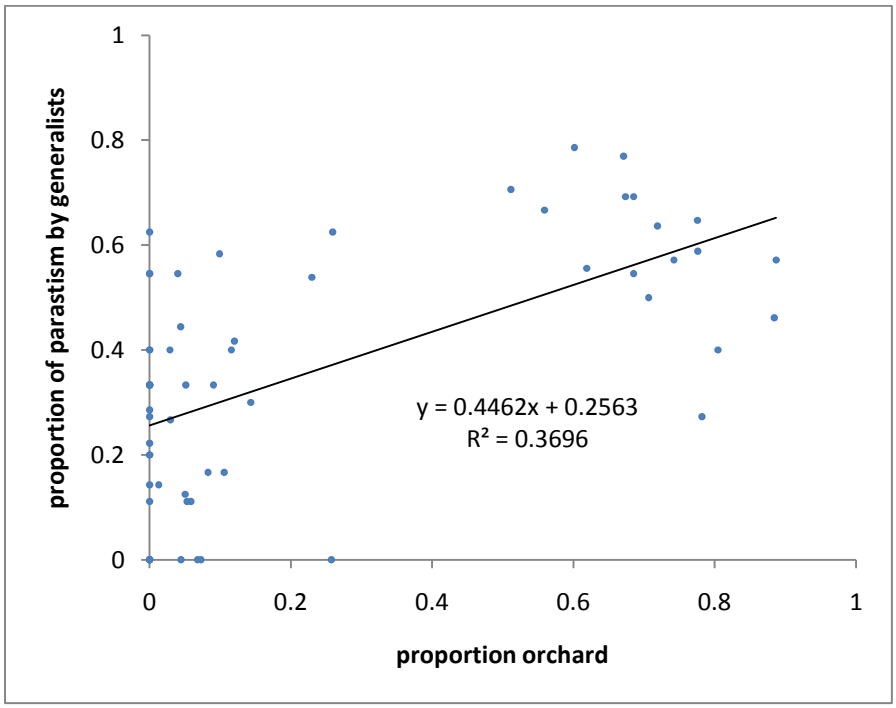
*logistic regression R^2 = 0.22, Model L. R. 15.1 (G statistic), p = 0.01E-7

Figure 9. Species richness and proportion of surrounding landscape in annual crops



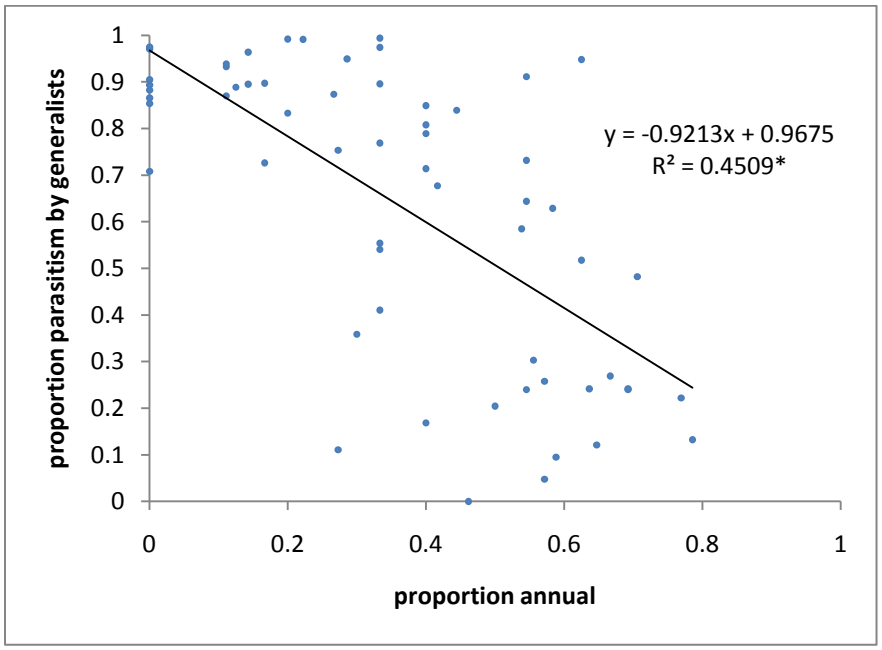
*logistic regression R^2 = 0.28, Model L. R. 19.29 (G statistic), p = 0.01E-3

Figure 10. Proportion of parasitism by generalists and proportion orchard habitat



*logistic regression R^2 = 0.37, Model L. R. 28.2 (G statistic), p = 0.01E-6

Figure 11. Proportion of parasitism by generalists and proportion annual habitat



*logistic regression R^2 = 0.28, Model L. R. 19.3 (G statistic), p = 0.01E-3

Figure 12. Sentinel experiment results of parasitism rates for each of the three landscape categories (agricultural and wild site data is pooled)

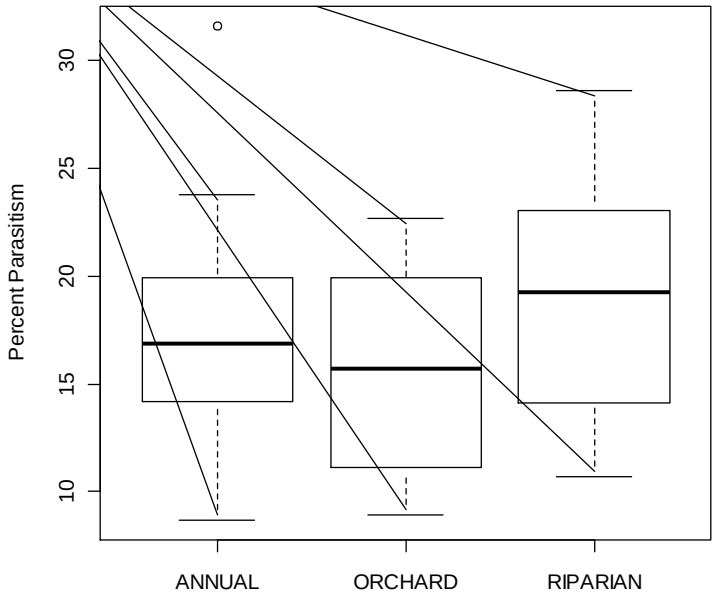


Figure 13. Sentinel experiment results of proportion of parasitism due to generalists for each of the three landscape categories

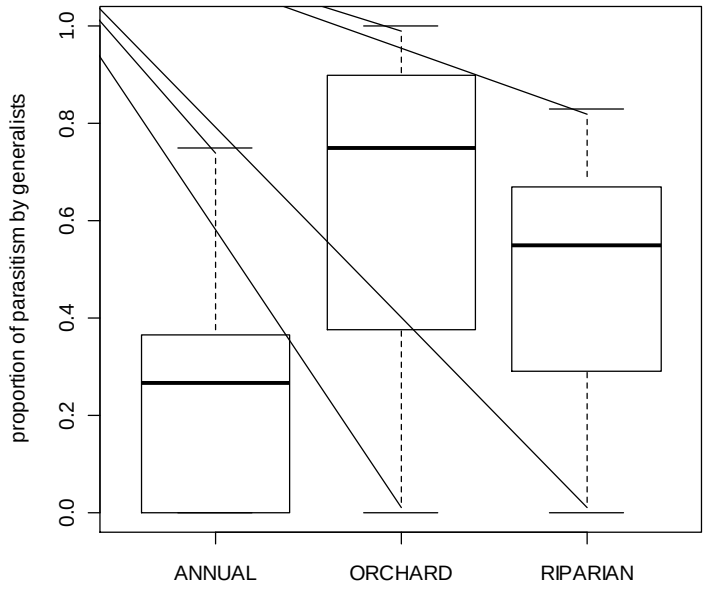


Figure 14. Sentinel experiment results of parasitism rates at experimental sites that were in agricultural fields compared to sites in wild sunflower patches

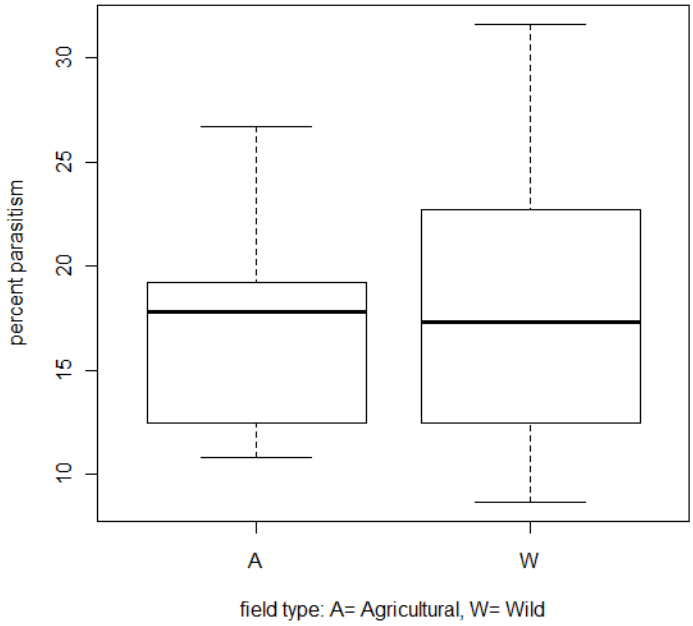


Table 1. Sunflower moth parasitoid species found, host specificity classification, the habitats that they were found in during our study and sources of identification and feeding record information

Family	Species	% of total parasitism[^]	Host range[+] (number of sites/60 present in)	Habitats	Identification and host records
Braconidae	*Dolichogenidea homoeosomae**	52.1%	Specialist (60)	Wild Agricultural	*Sharkey 1987; Krombein et al. 1979; Whal 2002*
	Bracon nuperus	3.2%	Generalist (6)	Wild	*Sharkey 1987; Zooparko 2002; Krombein et al. 1979*
	*Macrocentrus ancylivorous**	16.1%	Generalist (17)	Wild Agricultural	*Ahlstrom 2005; Charlet 1999; Brunner 1993; Beregovoy 1985; Teetes and Randolph 1969; Rowher 1962*
Ichneumonidae	*Pristomerus spinator**	4.6%	Generalist (13)	Wild Agricultural	*Sharkey 1987; Smith 1986; Fabricius 1941*
	*Trichomma maceratum**	6.1%	Generalist (17)	Wild	*Sharkey 1987; Zooparko 2002; Cresson 1927; Thompson 1945; Parker 1951*
	Diadegma openangorum	3.9%	Generalist (9)	Wild Agricultural	*Viereck 1917; Sharkey 1987; Beregovoy 1985*
	*Mastrus sp.**	4.1%	Generalist (19)	Wild Agricultural	*Förster 1869; Krombein et al. 1979*
	Parania geniculata	2.5%	Generalist (13)	Wild	*P Gross 1988; Krombein et al. 1979; Holmgren 1857*
Tachinidae	*Erynnia tortricis*	4.2%	Generalist (19)	Wild Agricultural	*Horn 2004; Krombein et al. 1979*
	Lixophaga variabilis	3.2%	Generalist (14)	Wild	*Coquillett 1895 ; Wood 2006*

[^]this percentage is based upon grand sum total of parasitism events recorded across all 96 sites, except for those parasitoids only found in wild habitats, for which this percentage is based upon total parasitism in the 18 wild type sites used in the sentinel experiment

[+]specialists = one host in this system; generalists = more than one host in this system

*species also reared during sentinel experiment

Table 2. Summary of parasitism field survey results

all field survey sites n=60	**Mean**	**Standard deviation**	**Range**
Parasitism	9.7%	3.3%	5-17%
Specialists reared	5.7	1.7	3-11
Generalists reared	4.0	3.4	0-12
Proportion of parasitism caused by specialists	0.6	0.2	0.2-1.0
Proportion of parasitism caused by generalists	0.4	0.2	0-0.8
Species richness	2.4	0.9	1-4
Landscape categories			
(in proportion of total area of circle of a 1km radius around the center of survey field)			
Annual	0.63	0.31	0-1
Orchard	0.24	0.31	0-0.9
Riparian	0.06	0.09	0-0.4
Wild sunflower habitat	0.02	0.03	0-0.2

Table 3. Summary of multiple regression model fit tests for parasitism parameters field survey

MLR model	***orchard + annual + wild sunflower + riparian***	***orchard + annual***	***orchard***	***annual***
Degrees of freedom= 58	**F statistic/ p value** ***Adjusted R^2 value***			
Percent parasitism	12.7/ 0.02 E^{-05} *0.44*	26.3/ 0.08 E^{-07} *0.46*	49.2/ 0.03 E^{-07} *0.44*	48.8/ 0.02 E^{-07} *0.45**
Parasitoid species richness	6.5/ 0.02 E^{-02} *0.27*	11.6/ 0.06 E^{-03} *0.26*	18.4/ 0.07 E^{-03} *0.23*	23.5/ 0.01 E^{-04} *0.28**
Proportion of parasitism caused by generalists	18.94/ 0.08 E^{-08} *0.55*	39.1/ 0.02 E^{-09} *0.56*	72.9/ 0.08 E^{-10} *0.55**	72.9/0 .08 E^{-10} *0.55**

**represent the best model fits for the dependent variable*

Table 4. Summary of results of sentinel experiment

	Number of sentinels recovered Mean (% of total placed); *average deviation*	**Total number of larvae parasitized Mean (% parasitism); *average deviation***	**Number of parasitoid species represented Mean; *average deviation***	**Number of attacks by generalists Mean; *average deviation***
All sites	26.3 (52.1%); *6.4(12.8%)*	4.1(17.4%); *1.2 (12.8%)*	2.22; *0.67*	0.44; *0.28*
All Agricultural fields (n=18)	26.1 (52.2%); *8.5 (17.0%)*	4.4 (16.9%); *1.6 (4.4%)*	2.00; *0.71*	0.42; *0.36*
All wild sunflower patches (n=18)	26.7 (53.2%); *7.4 (14.7%)*	4.4 (16.9%); *1.6 (4.37%)*	2.40; *0.92*	0.42; *0.27*
Sites within orchard settings (n=12)	26.5 (53.0%); *8.5 (17.0%)*	4.1 (16.1%); *1.2 (5.1%)*	2.52; *1.01*	0.61; *0.37*
Sites within annual crop settings (n =12)	26.3 (52.5%); *8.7 (17.5%)*	4.5 (17.6%); *1.7 (6.1%)*	1.83; *0.72*	0.23; *0.24*
Sites within riparian habitat settings (n=12)	25.8 (51.7%); *6.4 (12.8%)*	4.8 (19.0%); *1.4 (5.7%)*	2.25; *0.62*	0.47; *0.25*
Sites near wild sunflower habitat (n=18)	26.2 (52.4%); *8.2 (16.5%)*	4.5 (18.3%); *1.5 (7.0%)*	2.38; *0.92*	0.44; *0.34*
Sites far from wild sunflower habitat (n=18)	26.3 (52.3%); *7.8 (14.8%)*	4.4 (16.9%); *1.4 (3.7%)*	2.01; *0.75*	0.43; *0.32*

Table 5. ANOVA model fit for the complete set of independent variables examined

	Df	Sum Sq	Mean Sq	Pillai value*	Pr (>Pillai)
Landscape category	2	67.39	33.693	0.9654	0.3928
Agricultural or Wild field	1	3.00	3.004	0.0861	0.7713
Near or far from wild sunflower habitat	1	23.04	23.040	0.6601	0.4231
Number of species	1	4.46	4.462	0.1278	0.7233
Proportion of parasitism due to specialists	1	8.91	8.907	0.2552	0.6173
Residuals	29	1012.17	34.902		

*the Pillai-M. S. Bartlett trace, $\Lambda_{Pillai} = \sum_{1...p} (1 / (1 + \lambda_p))$

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Appendix i. Summary of site information including sunflower moth larval density found in 100 flowers, total percent parasitism based on the entire larval sample, area of the sunflower field in meters^2 and location coordinates

Site name (SFAGyear-#)	Total larvae found in 100 flower sample	Percent parasitism Including all larvae	Estimated area of sunflower field in m^2	Location N	Location W
2003 survey sites 1-20					
SFAG03-01	107	10.28	3500	38°43'5.01"N	121°58'12.28"W
SFAG03-02	122	8.19	3250	38°43'35.02"N	121°56'13.69"W
SFAG03-03	104	5.77	13000	38°50'59.62"N	121°57'14.17"W
SFAG03-04	103	4.85	7000	38°47'44.08"N	121°45'26.74"W
SFAG03-05	106	8.49	3700	38°42'37.68"N	122° 4'38.31"W
SFAG03-06	128	13.28	6100	38°19'8.96"N	121°24'5.25"W
SFAG03-07	123	6.50	9200	37°55'27.33"N	121°24'47.44"W
SFAG03-08	152	15.13	3100	39°37'16.19"N	121°48'58.14"W
SFAG03-09	111	10.81	3000	37°50'38.95"N	121°14'51.13"W
SFAG03-10	134	8.96	15000	37°42'24.79"N	121°12'19.07"W
SFAG03-11	108	5.56	3100	37°42'10.82"N	121° 9'53.52"W
SFAG03-12	114	7.02	3200	38°25'1.77"N	121°50'16.58"W
SFAG03-13	122	9.84	3000	38°33'20.21"N	121°39'18.67"W
SFAG03-14	155	9.03	12000	38°27'17.98"N	121°44'54.93"W
SFAG03-15	132	9.85	6600	38°30'34.72"N	121°38'50.32"W
SFAG03-16	111	8.11	3300	37°48'7.62"N	121° 7'27.70"W
SFAG03-17	141	7.80	18100	38°35'41.52"N	121°56'39.42"W
SFAG03-18	108	8.33	5900	38°23'0.22"N	121°30'24.89"W
SFAG03-19	123	6.50	3800	38°21'24.84"N	121°45'38.08"W
SFAG03-20	156	9.62	3900	38°38'32.89"N	121°49'53.84"W
2004 survey sites 1-20					
SFAG04-01	123	11.38	3100	36°35'47.82"N	119°30'59.48"W
SFAG04-02	132	12.12	5800	36°34'41.96"N	119°31'11.40"W
SFAG04-03	104	12.50	6400	36°35'44.30"N	119°35'31.65"W
SFAG04-04	127	9.45	6700	36°40'41.72"N	119°39'35.91"W
SFAG04-05	127	12.60	5800	37°13'43.50"N	120°15'54.62"W
SFAG04-06	130	16.15	8800	37°32'44.91"N	120°48'18.02"W
SFAG04-07	124	11.29	12300	37°47'58.03"N	120°58'6.05"W
SFAG04-08	124	12.90	8800	38°19'35.74"N	121°28'55.24"W
SFAG04-09	121	5.79	3100	37°16'53.17"N	120°17'47.48"W
SFAG04-10	123	4.88	19700	38°44'2.84"N	121°51'0.83"W
SFAG04-11	136	8.08	15600	38°47'55.04"N	121°54'20.38"W
SFAG04-12	134	10.45	20000	38°46'14.33"N	121°52'16.17"W
SFAG04-13	117	5.98	12200	38°56'1.39"N	121°56'56.75"W
SFAG04-14	126	13.49	17900	39°15'16.13"N	122° 0'38.32"W
SFAG04-15	134	11.19	6900	39°25'17.59"N	121°59'24.70"W
SFAG04-16	119	10.92	12200	38°14'1.60"N	121°19'32.85"W
SFAG04-17	137	12.41	3100	38° 9'13.27"N	121°12'58.73"W
SFAG04-18	148	12.84	16000	38°40'59.23"N	121°54'4.27"W
SFAG04-19	128	7.03	19800	38°38'15.23"N	121°43'0.91"W
SFAG04-20	130	6.15	13400	38°21'43.66"N	121°33'53.46"W

2005 survey sites 1-20					
Site name (SFAGyear-#)	**Total larvae found in 100 flower sample**	**Percent parasitism Including all larvae**	**Estimated area of sunflower field in m^2**	**Location N**	**Location W**
SFAG05-01	120	11.67	3000	38°30'33.01"N	121°57'54.74"W
SFAG05-02	131	14.50	9800	36°40'41.72"N	119°39'35.91"W
SFAG05-03	123	6.50	6200	37°48'3.19"N	120°57'11.94"W
SFAG05-05	134	11.94	18900	38°30'24.89"N	121°40'18.25"W
SFAG05-06	127	12.60	9100	39°34'22.12"N	121°42'55.38"W
SFAG05-07	137	8.76	9100	37°55'21.60"N	121°24'47.49"W
SFAG05-08	121	5.79	5800	38°21'25.55"N	121°45'51.91"W
SFAG05-09	136	9.56	6900	38°27'1.66"N	121°44'17.04"W
SFAG05-10	142	11.27	13400	38°24'11.78"N	121°48'41.59"W
SFAG05-11	132	6.82	15500	38°34'53.00"N	121°55'7.96"W
SFAG05-12	137	8.03	12000	38°44'51.25"N	121°47'33.60"W
SFAG05-13	120	8.33	9200	38°47'5.85"N	121°50'33.69"W
SFAG05-14	119	7.56	16600	38°12'36.79"N	121°23'37.27"W
SFAG05-15	147	9.52	12300	38°15'22.36"N	121°28'30.23"W
SFAG05-16	134	11.19	14400	38°37'39.65"N	121°43'58.89"W
SFAG05-17	112	10.71	15000	38°52'4.67"N	121°48'29.79"W
SFAG05-18	122	13.93	8900	38°14'49.84"N	121°28'37.87"W
SFAG05-19	130	11.54	12000	38°17'58.87"N	121°30'54.04"W
SFAG05-20	142	11.27	9200	39°13'16.44"N	122° 0'7.13"W

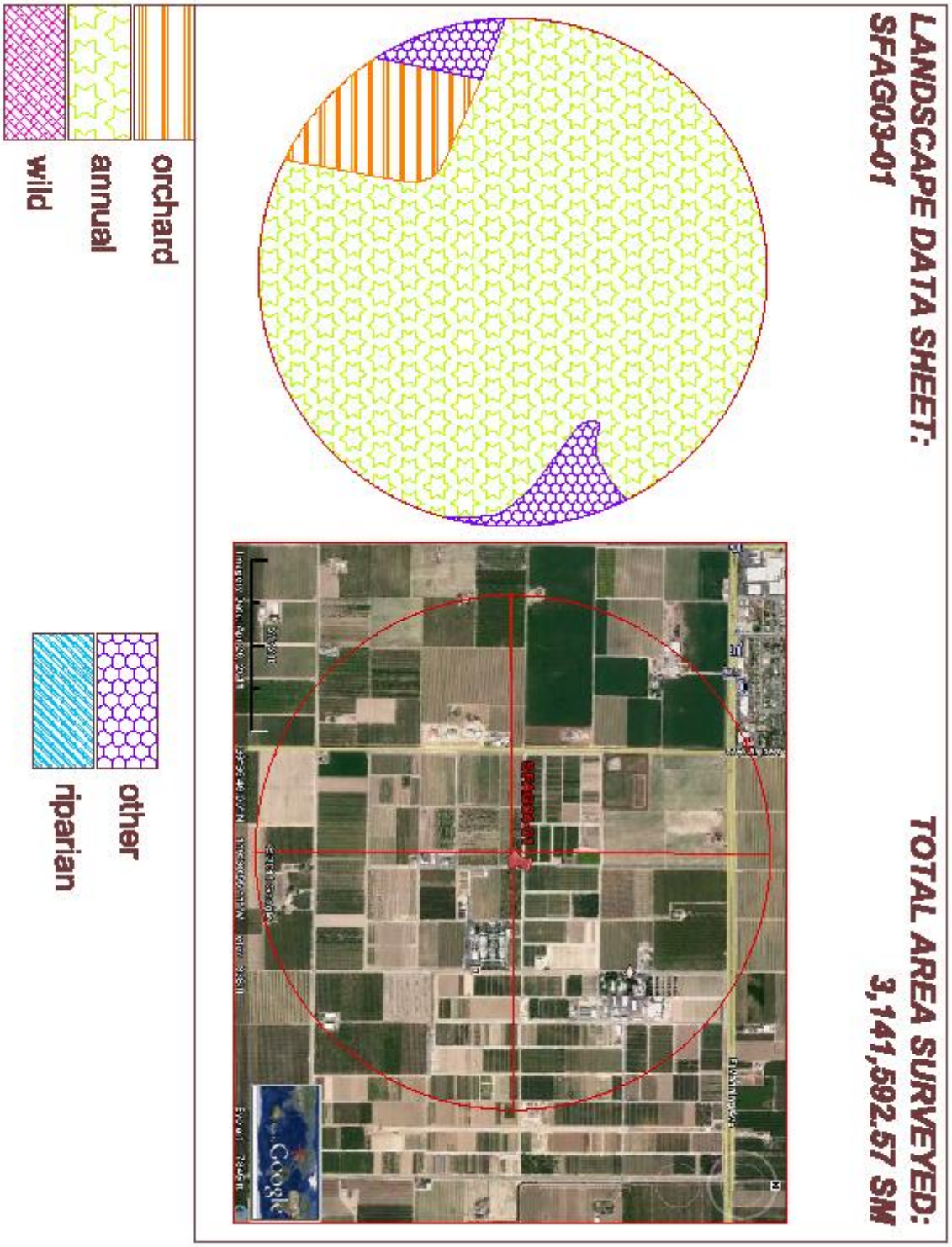
Appendix ii. Summary of landscape categorization for field survey sites. Proportion of total area in the 1km radius circle in each type of habitat is given. Specific figures follow.

Site name (SFAGyear-#)	Annual	Orchard	Riparian	Wild sunflower habitat	"other" category
2003 survey sites 1-20					
SFAG03-01	0.870	0.053	0	0	0.077
SFAG03-02	0.970	0	0.015	0	0
SFAG03-03	0.517	0.259	0.023	0	0.200
SFAG03-04	0.882	0.068	0.050	0	0
SFAG03-05	0.554	0.051	0.150	0	0.245
SFAG03-06	0.222	0.671	0.018	0.017	0.073
SFAG03-07	0.992	0	0	0	0.008
SFAG03-08	0.048	0.887	0.010	0	0.055
SFAG03-09	0.242	0.719	0	0.009	0.030
SFAG03-10	0.897	0.083	0	0.001	0.019
SFAG03-11	0.789	0.029	0.135	0	0.047
SFAG03-12	0.893	0	0	0.074	0.033
SFAG03-13	0.714	0	0.215	0.037	0.035
SFAG03-14	0.932	0.058	0	0	0.009
SFAG03-15	0.808	0.116	0.066	0	0.011
SFAG03-16	0.303	0.619	0	0.054	0.024
SFAG03-17	0.949	0	0	0.019	0.032
SFAG03-18	0.541	0	0.257	0.203	0
SFAG03-19	0.905	0	0.081	0	0.014
SFAG03-20	0.888	0.050	0	0.012	0.041
2004 survey sites 1-20					
SFAG04-01	0.111	0.782	0	0.014	0.094
SFAG04-02	0.168	0.805	0	0	0.027
SFAG04-03	0.062	0.881	0	0.007	0.050
SFAG04-04	0.240	0.685	0	0.012	0.063

Site name (SFAGyear-#)	Annual	Orchard	Riparian	Wild sunflower habitat	"other" category
SFAG04-05	0.132	0.601	0.015	0.016	0.235
SFAG04-06	0.095	0.776	0	0.030	0.099
SFAG04-07	0.242	0.674	0	0.020	0.064
SFAG04-08	0.948	0	0.023	0.029	0
SFAG04-09	0.726	0.106	0.046	0.017	0.105
SFAG04-10	0.865	0.045	0.059	0.015	0.016
SFAG04-11	0.708	0.257	0	0.016	0.019
SFAG04-12	0.939	0	0	0.031	0.031
SFAG04-13	0.974	0	0	0.004	0.022
SFAG04-14	0.359	0.143	0.381	0	0.117
SFAG04-15	0.629	0.099	0.210	0.062	0
SFAG04-16	0.753	0	0.080	0.059	0.108
SFAG04-17	0.585	0.230	0.130	0.006	0.050
SFAG04-18	0.644	0	0.244	0.092	0.020
SFAG04-19	0.769	0	0.113	0.101	0.018
SFAG04-20	0.849	0	0.118	0.020	0.012
2005 survey sites 1-20					
SFAG05-01	0.269	0.559	0.108	0.031	0.032
SFAG05-02	0.121	0.776	0	0.014	0.089
SFAG05-03	0.204	0.707	0	0.024	0.065
SFAG05-04	0.482	0.512	0	0	0.006
SFAG05-05	0.884	0	0.083	0.006	0.026
SFAG05-06	0.258	0.742	0	0	0
SFAG05-07	0.975	0	0	0	0.025
SFAG05-08	0.896	0	0.049	0.032	0.024
SFAG05-09	0.991	0	0	0	0.009
SFAG05-10	0.833	0	0	0	0.167

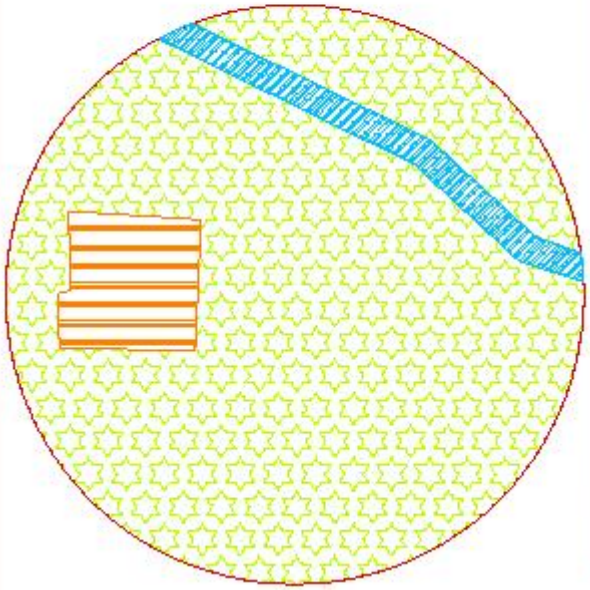
Site name (SFAGyear-#)	Annual	Orchard	Riparian	Wild sunflower habitat	"other" category
SFAG05-11	0.964	0	0	0	0.036
SFAG05-12	0.853	0.073	0.002	0.018817602	0.053
SFAG05-13	0.994	0	0	0	0.006
SFAG05-14	0.839	0.044	0.094	0	0.023
SFAG05-15	0.895	0.013	0.087	0	0.005
SFAG05-16	0.911	0	0.072	0.008	0.009
SFAG05-17	0.677	0.120	0.155	0.044	0.004
SFAG05-18	0.873	0.029	0.086	0.011	0
SFAG05-19	0.732	0.040	0.206	0.012	0.010
SFAG05-20	0.411	0.090	0.402	0.052	0.045

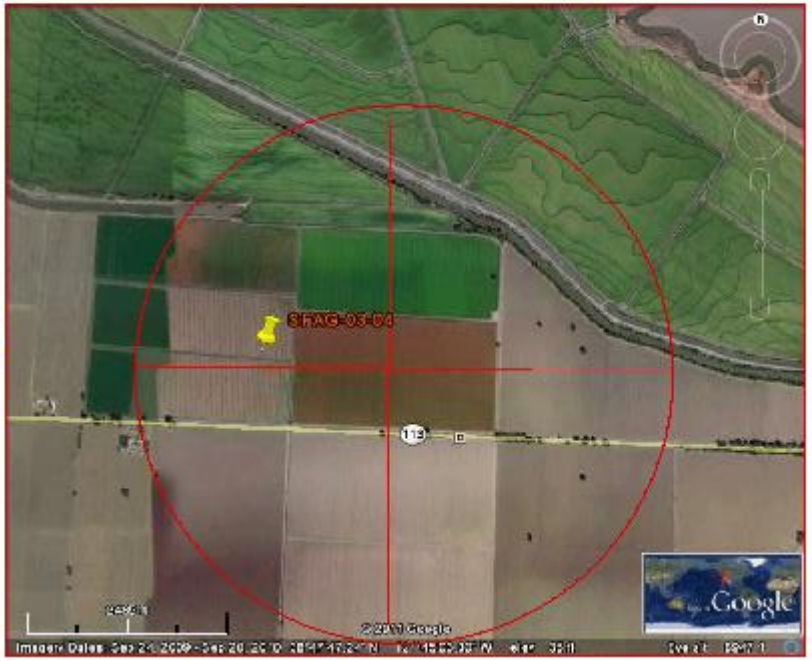
Appendix iii. Landscape composition analysis detail figures.



LANDSCAPE DATA SHEET:
SFAG03-02

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

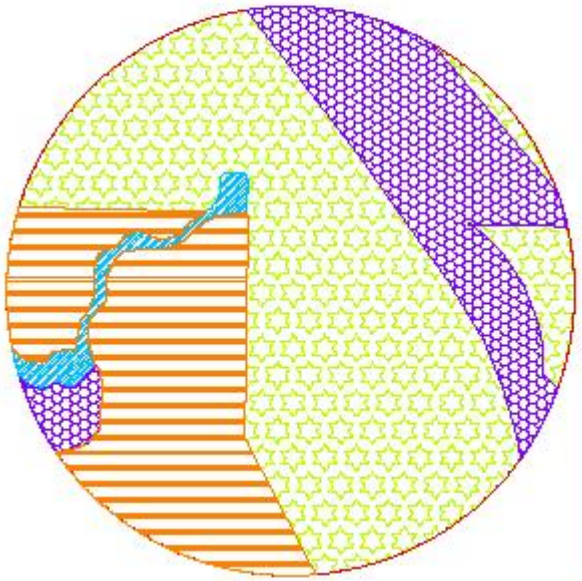
wild

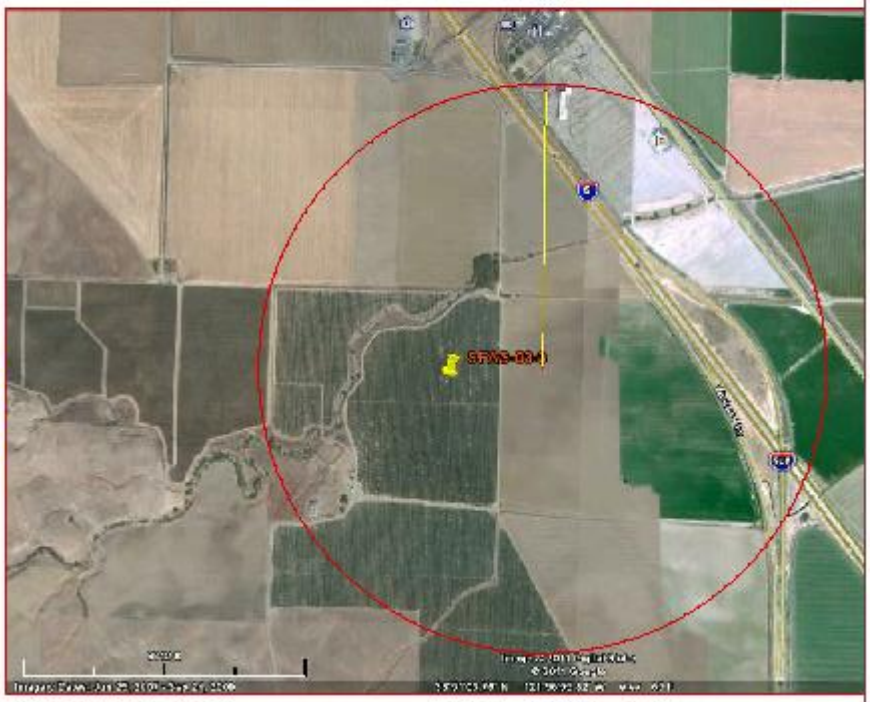
other

riparian

LANDSCAPE DATA SHEET:
SFAG03-03

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

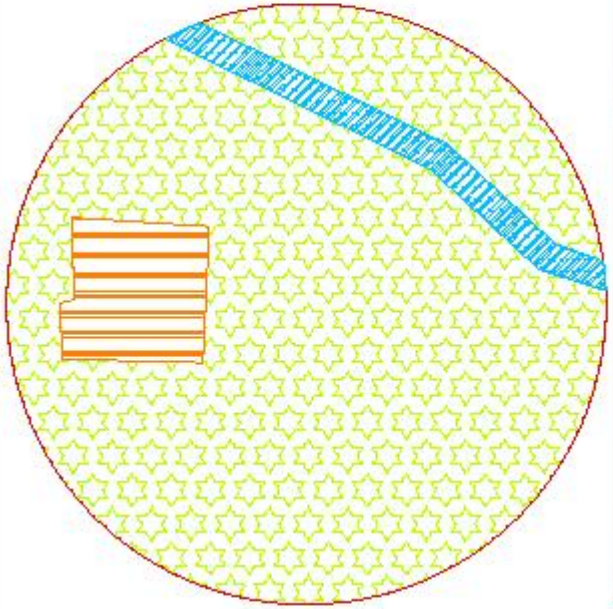
wild

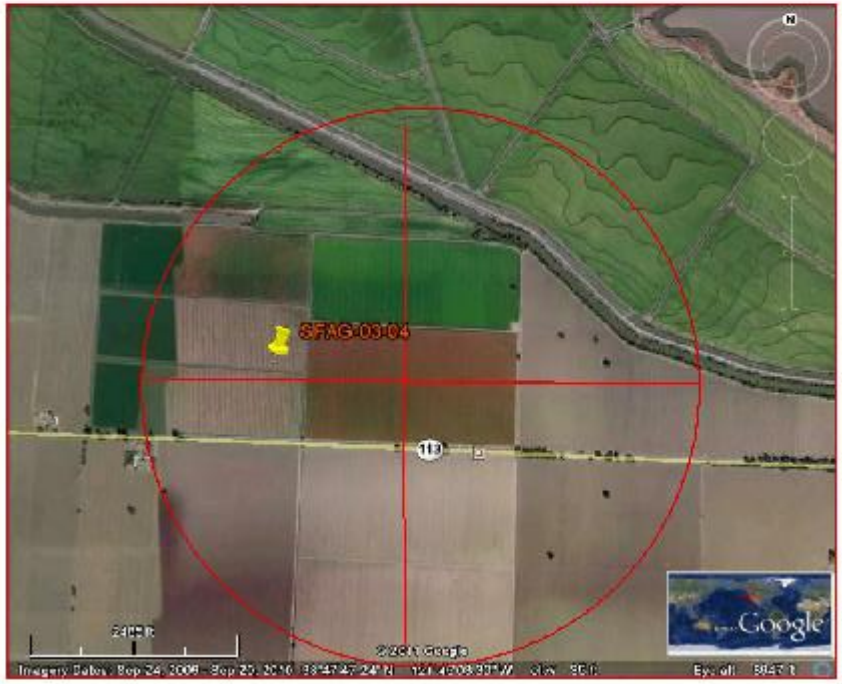
other

riparian

LANDSCAPE DATA SHEET:
SFAG03-04

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-05

TOTAL AREA SURVEYED:
3,141,592.57 SM

orchard

annual

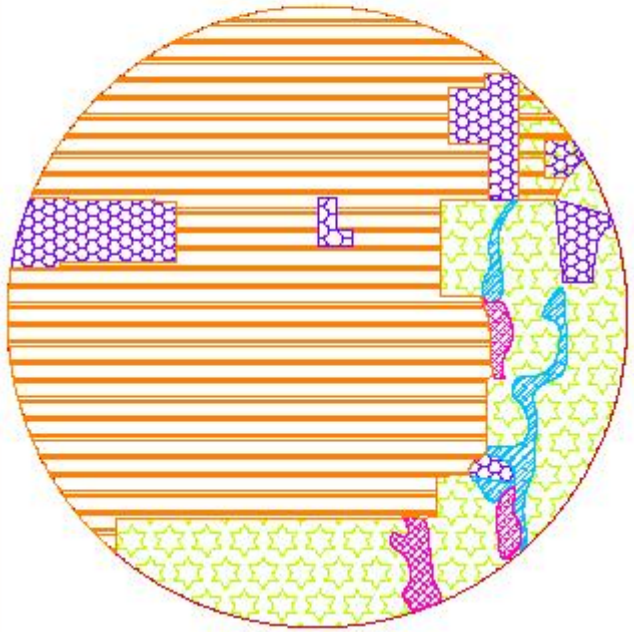
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-06

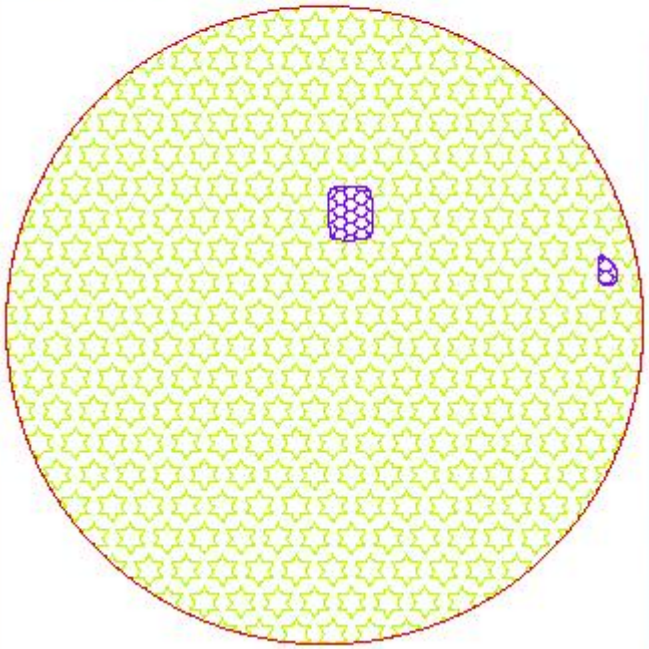
TOTAL AREA SURVEYED:
3,141,592.57 SM

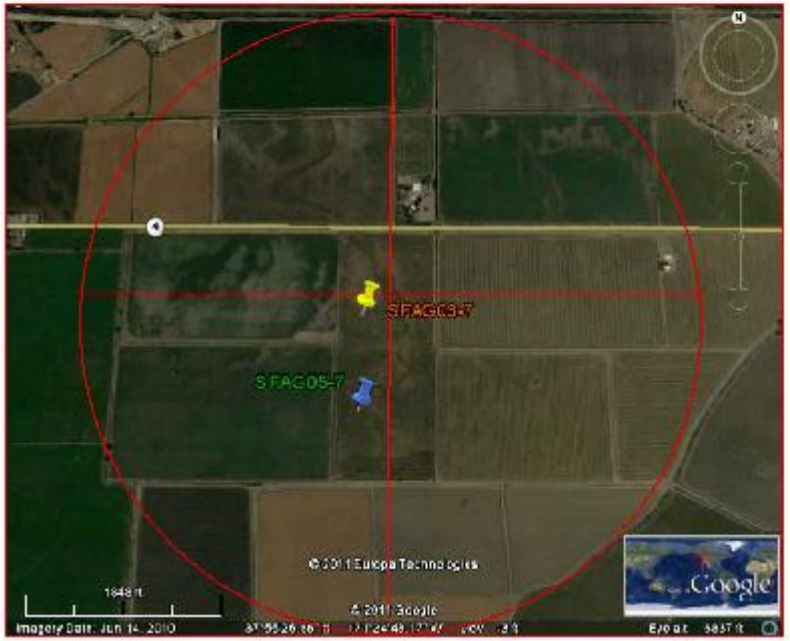


orchard
annual
wild
other
riparian

LANDSCAPE DATA SHEET:
SFAG03-07

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

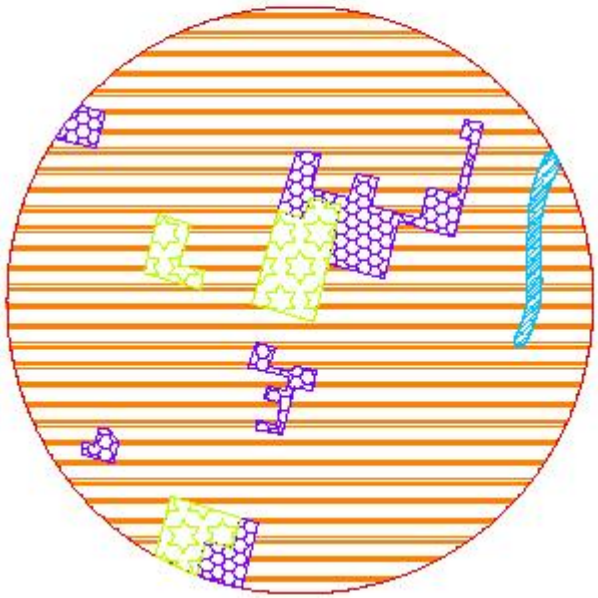
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-08

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

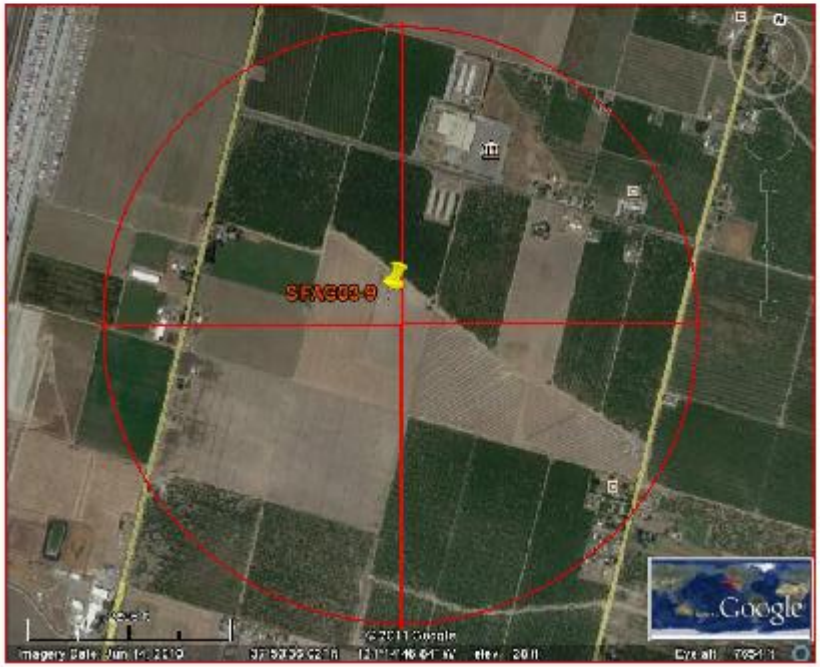
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-09

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

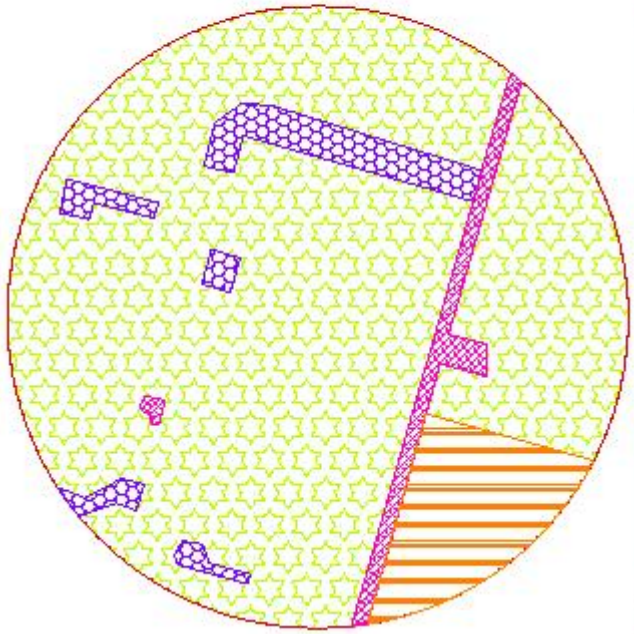
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-10

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

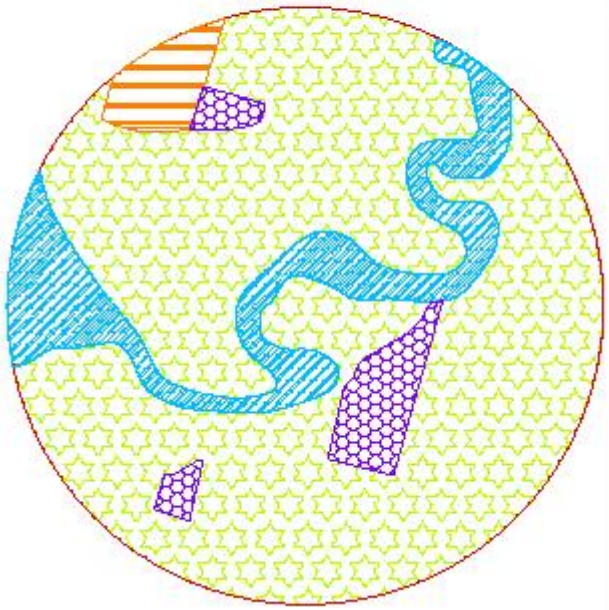
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-11

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

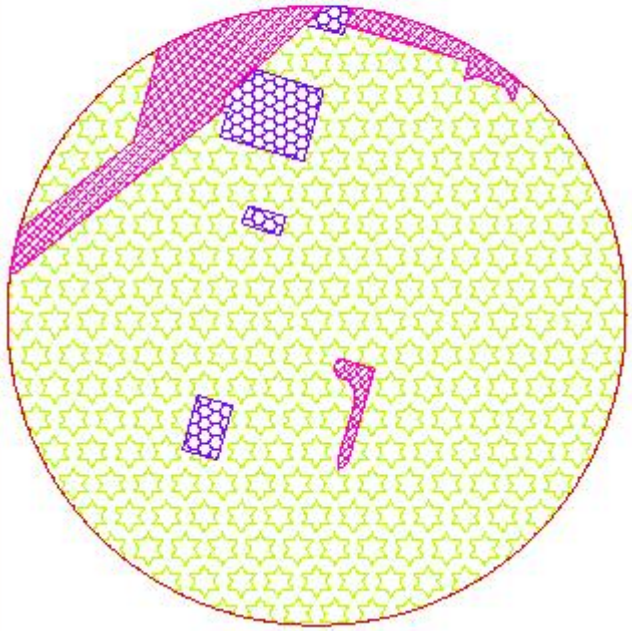
wild

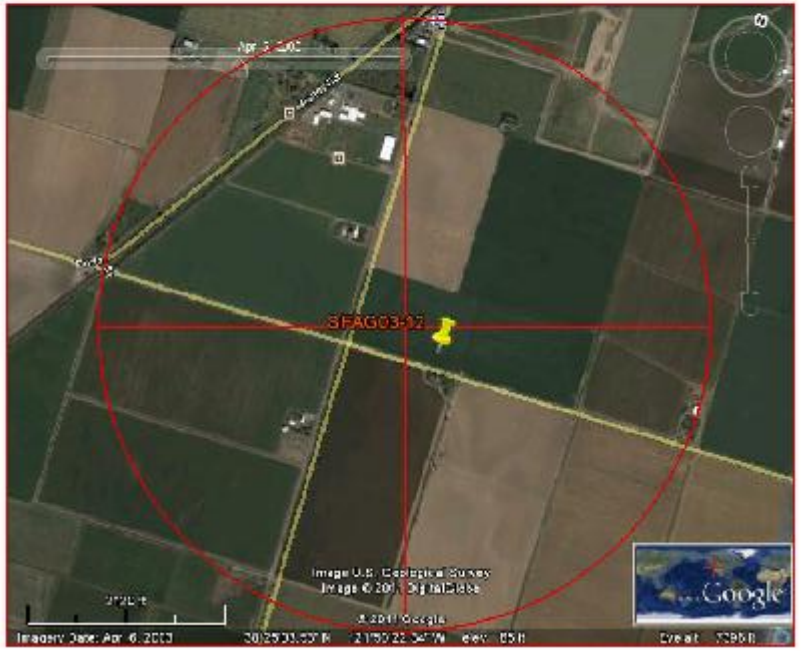
other

riparian

LANDSCAPE DATA SHEET:
SFAG03-12

TOTAL AREA SURVEYED:
3,141,592.57 SM

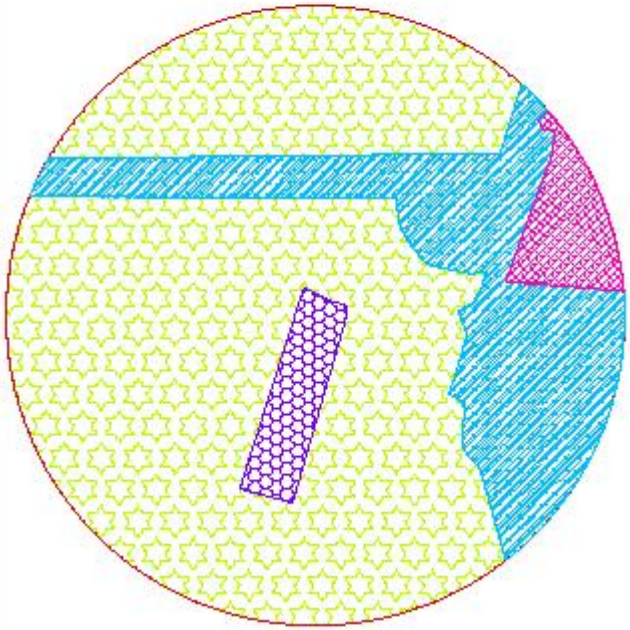




orchard
annual
wild
other
riparian

LANDSCAPE DATA SHEET:
SFAG03-13

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

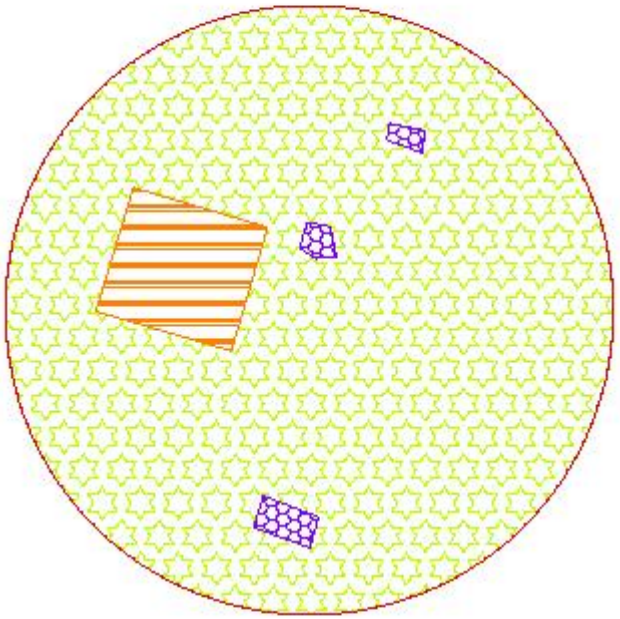
wild

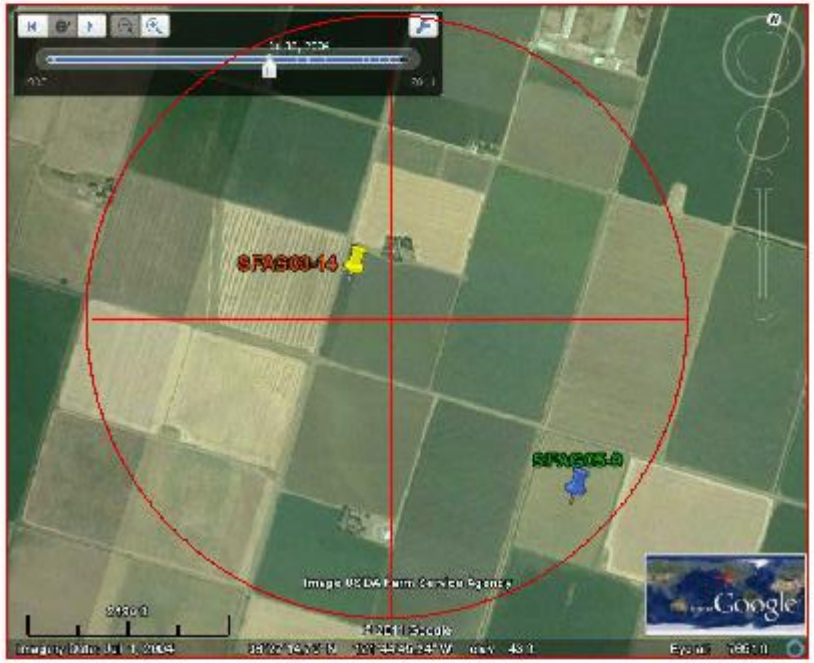
other

riparian

LANDSCAPE DATA SHEET:
SFAG03-14

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

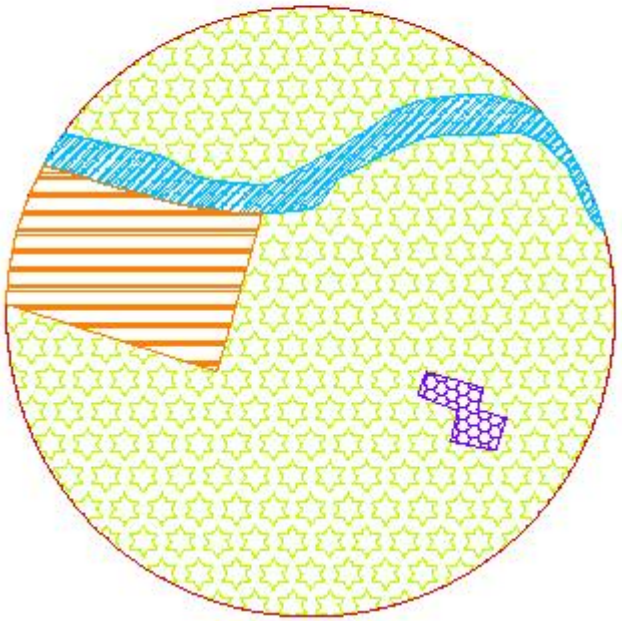
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-15

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

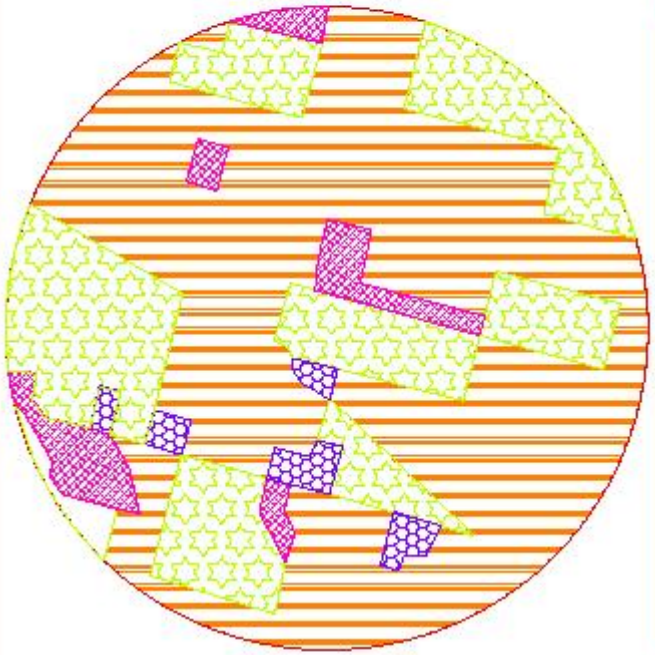
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-16

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

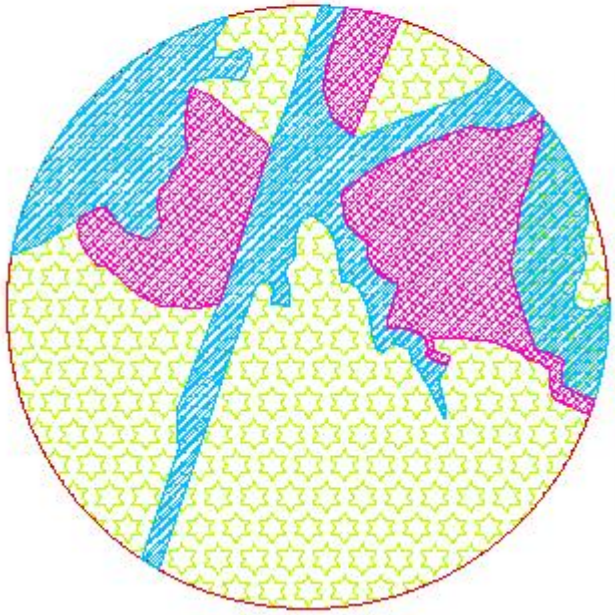
wild

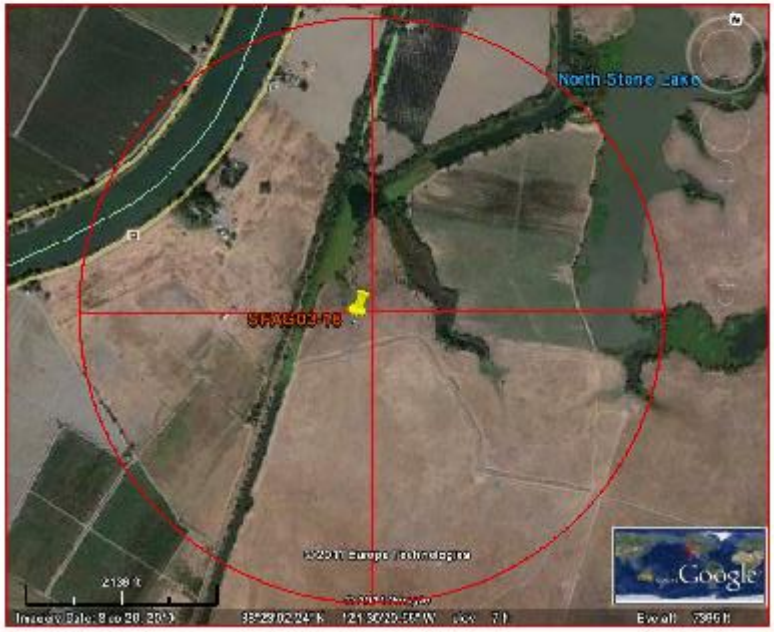
other

riparian

LANDSCAPE DATA SHEET:
SFAG03-18

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

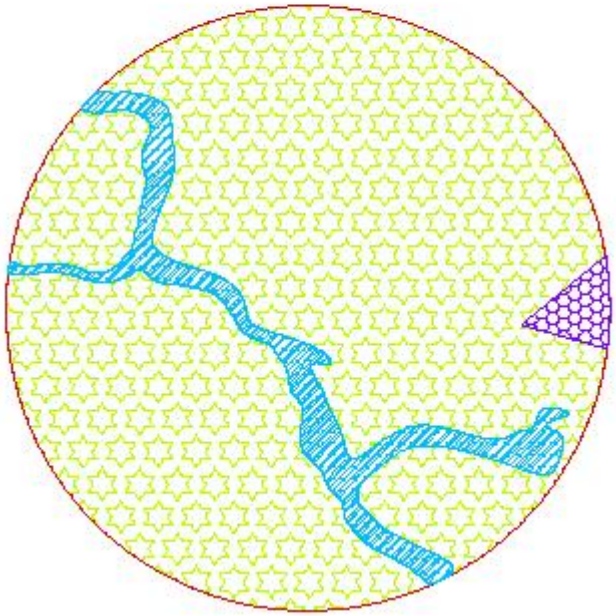
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG03-19

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

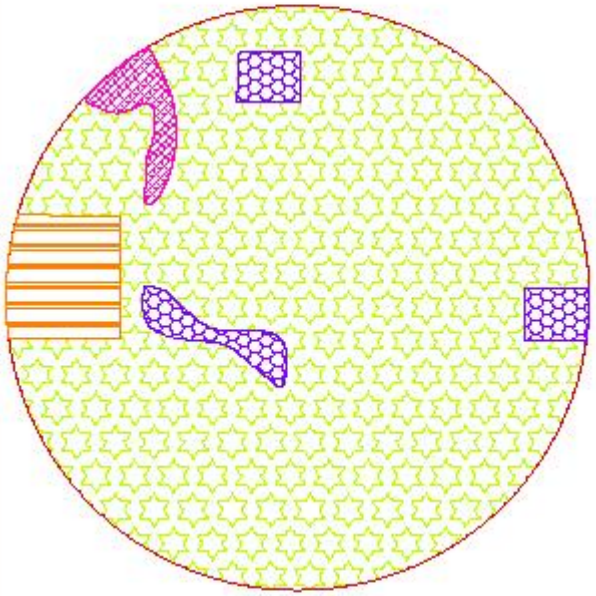
wild

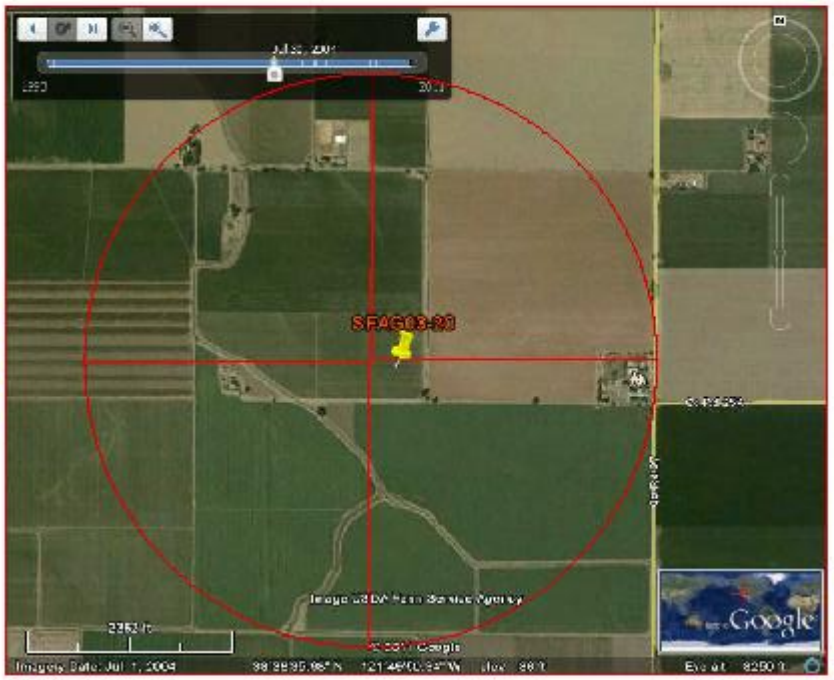
other

riparian

LANDSCAPE DATA SHEET:
SFAG03-20

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

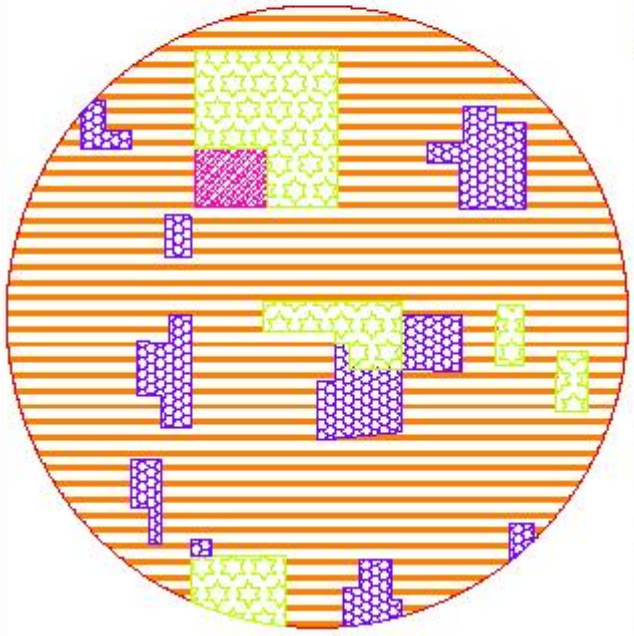
wild

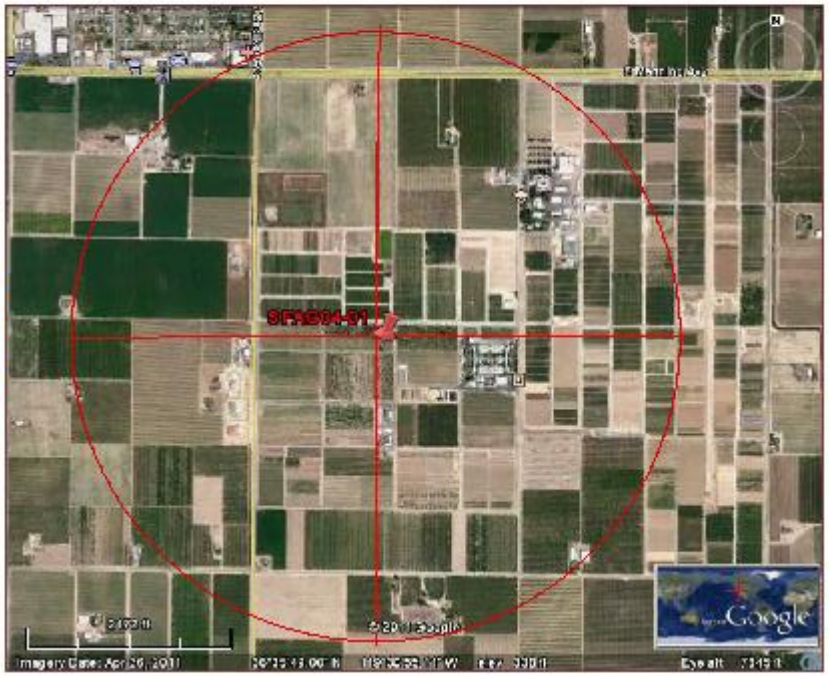
other

riparian

LANDSCAPE DATA SHEET:
SFAG04-01

TOTAL AREA SURVEYED:
3,141,592.57 SM

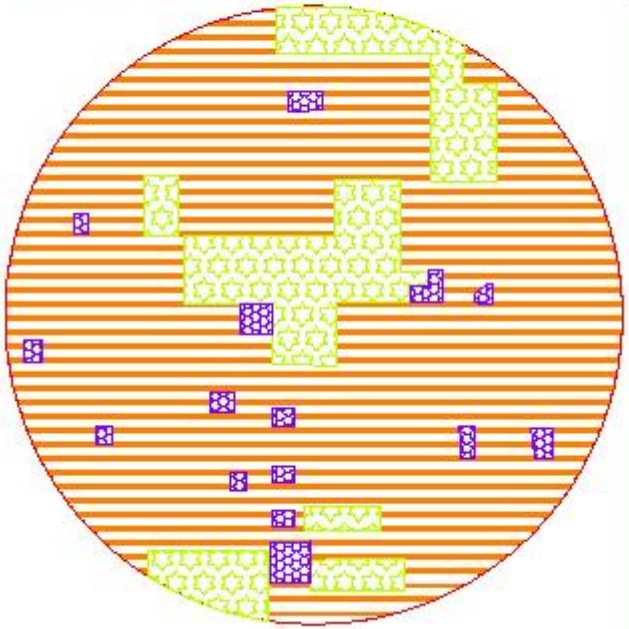




orchard
annual
wild
other
riparian

LANDSCAPE DATA SHEET:
SFAG04-02

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

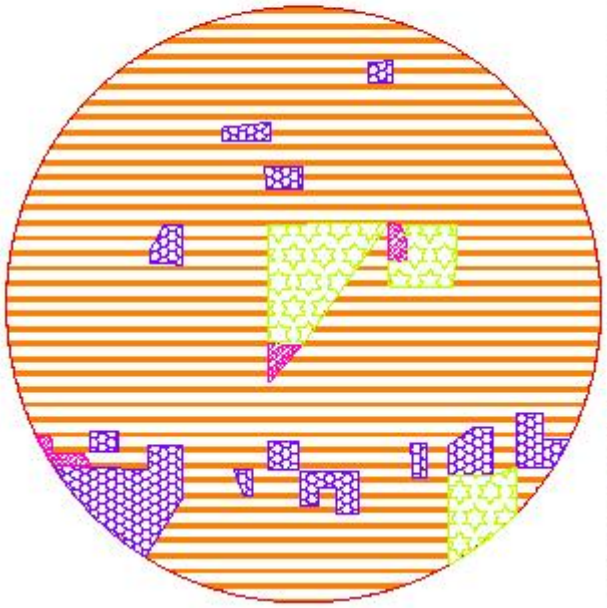
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-03

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

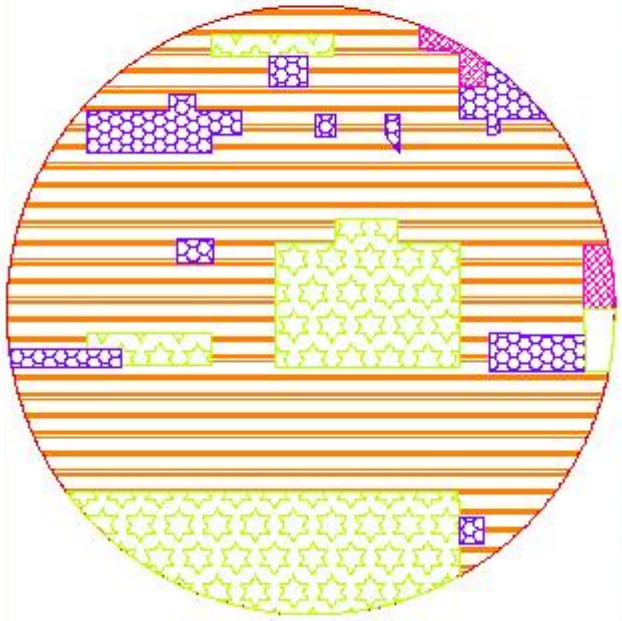
wild

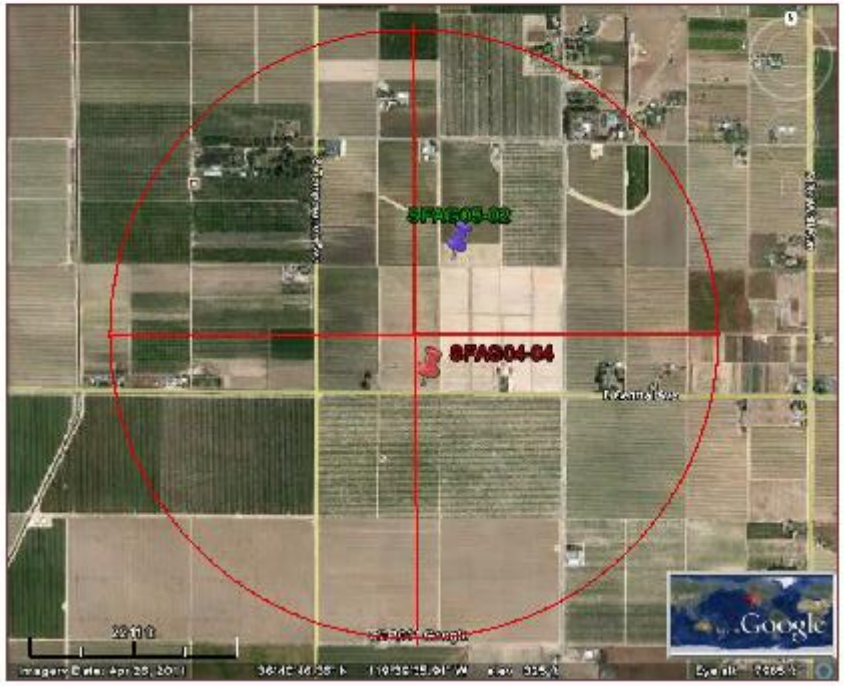
other

riparian

LANDSCAPE DATA SHEET:
SFAG04-04

TOTAL AREA SURVEYED:
3,141,592.57 SM

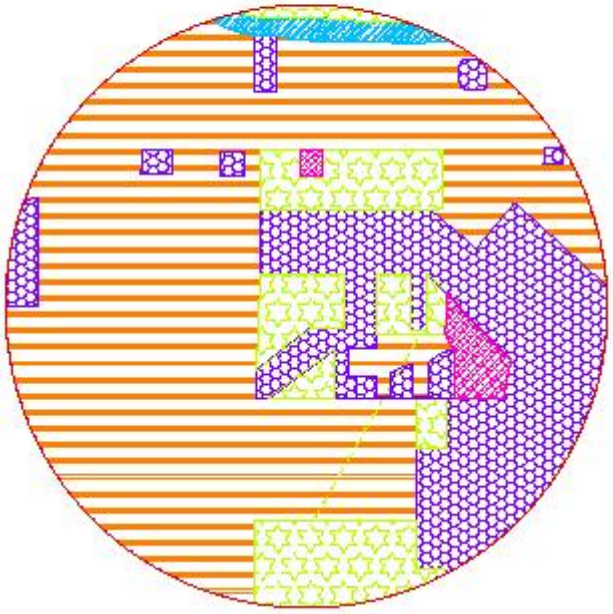




orchard
annual
wild
other
riparian

LANDSCAPE DATA SHEET:
SFAG04-05

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

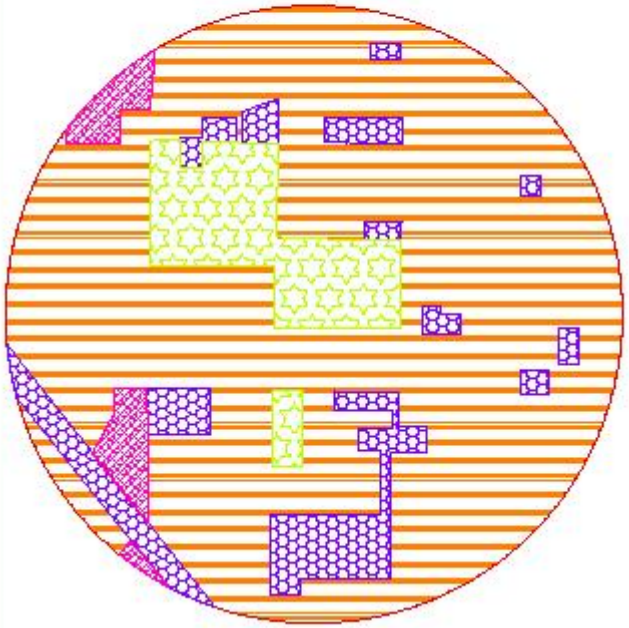
wild

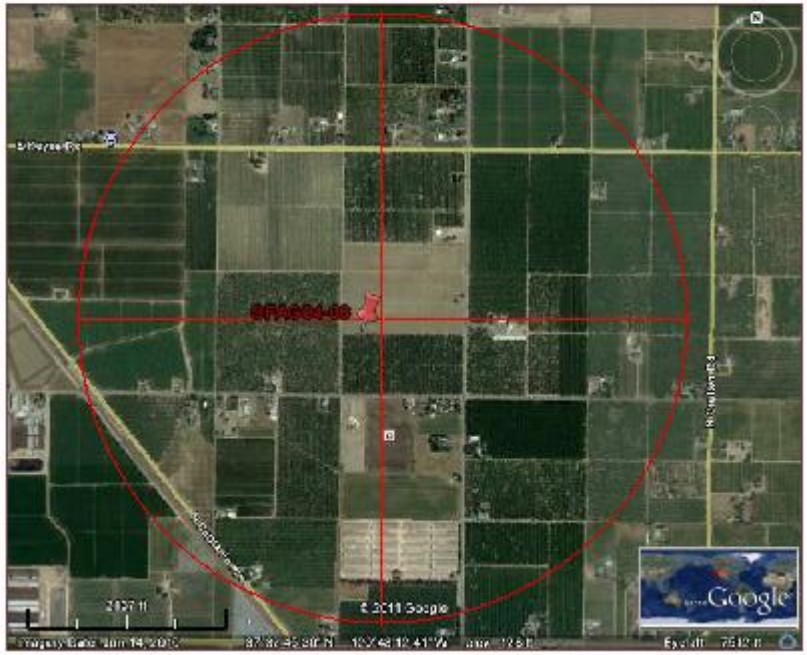
other

riparian

LANDSCAPE DATA SHEET:
SFAG04-06

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

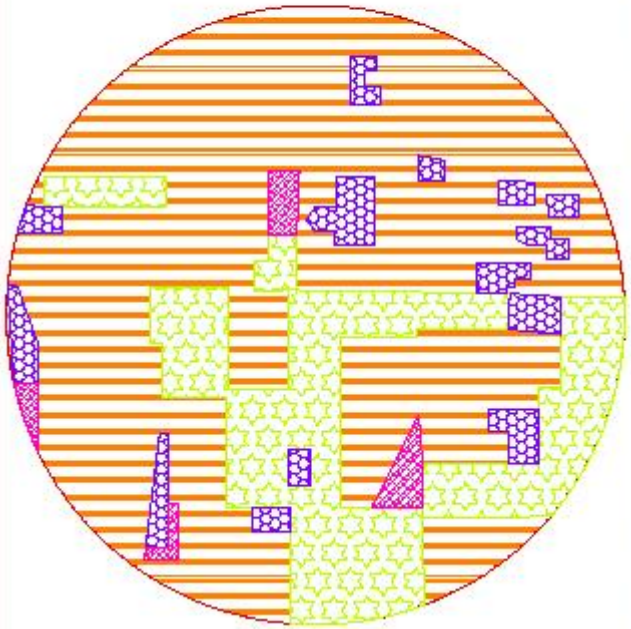
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-07

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

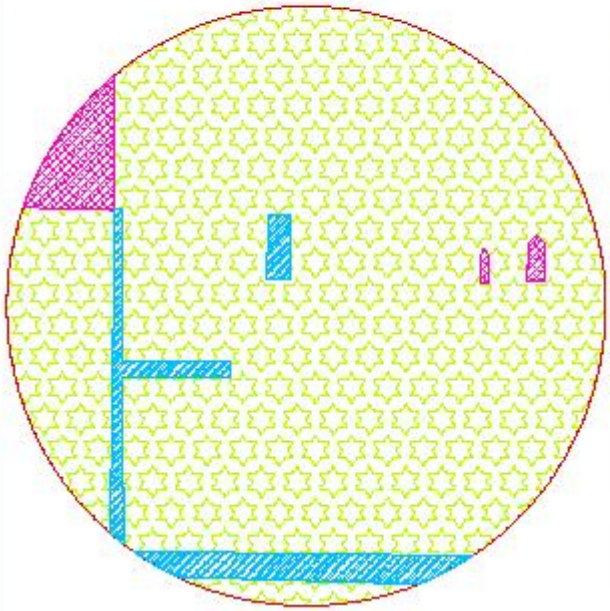
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-08

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

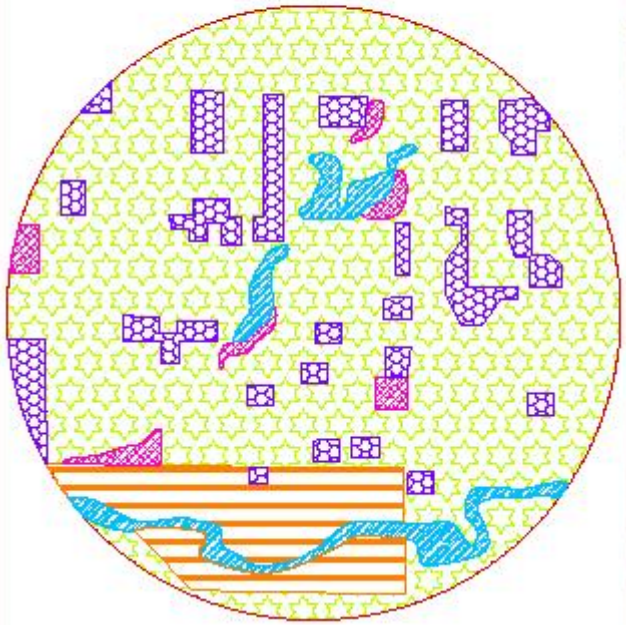
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-09

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

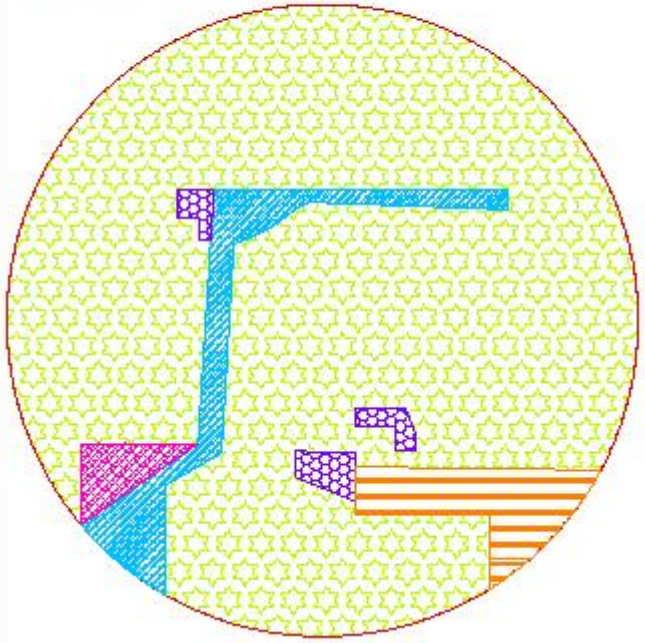
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-10

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

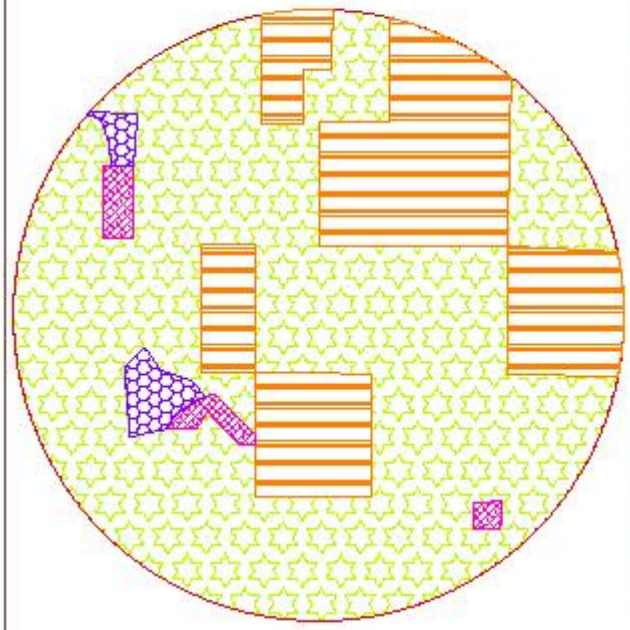
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-11

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

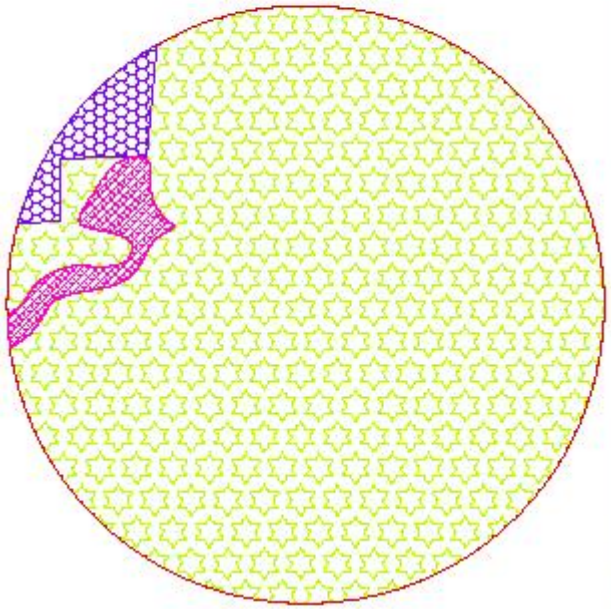
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-12

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

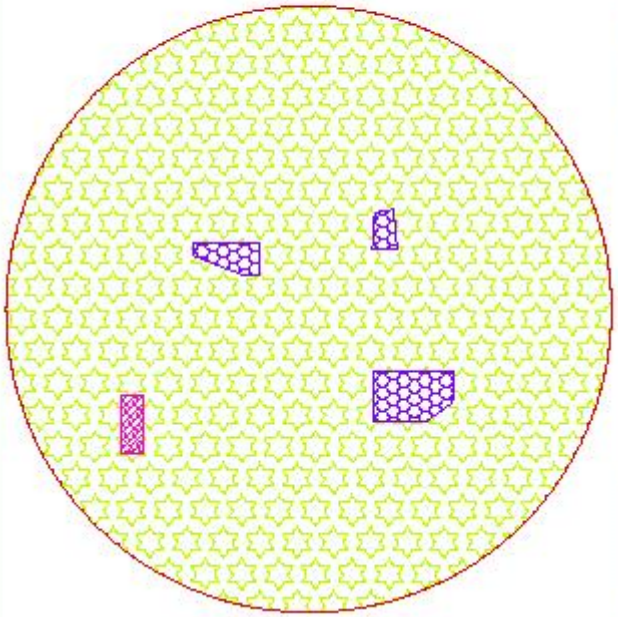
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-13

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

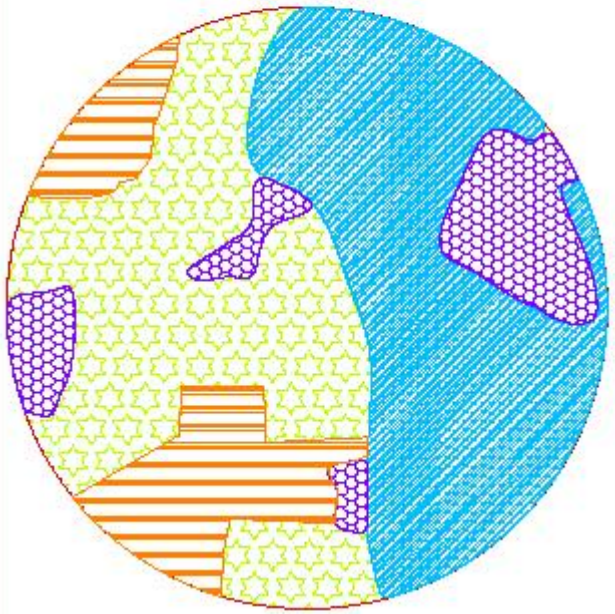
wild

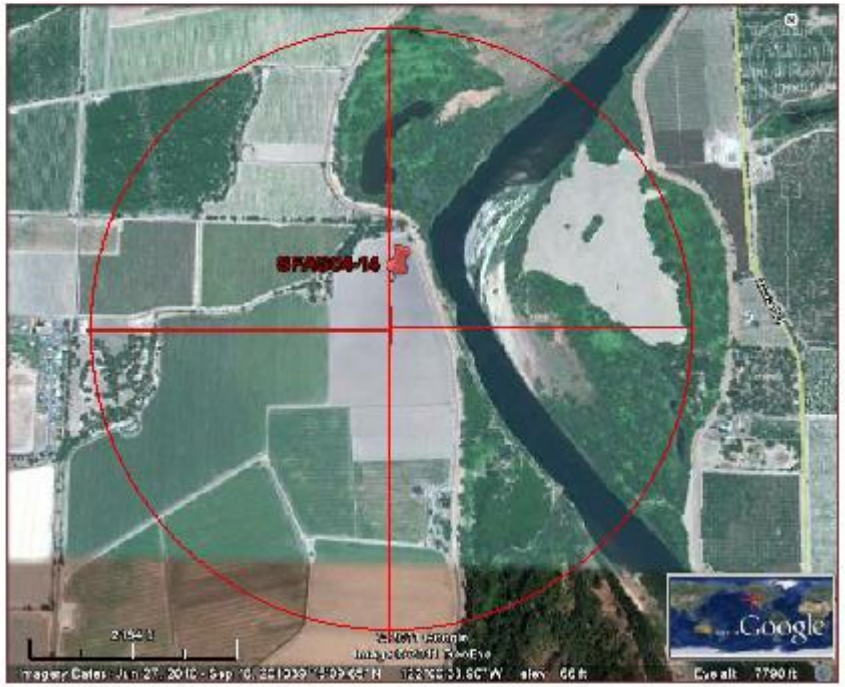
other

riparian

LANDSCAPE DATA SHEET:
SFAG04-14

TOTAL AREA SURVEYED:
3,141,592.57 SM





orchard

annual

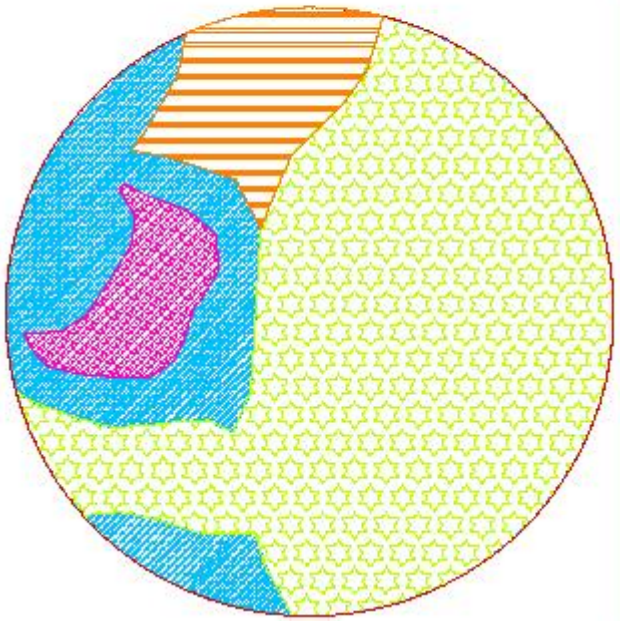
wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-15

TOTAL AREA SURVEYED:
3,141,592.57 SM



orchard

annual

wild

other

riparian

LANDSCAPE DATA SHEET:
SFAG04-16

TOTAL AREA SURVEYED:
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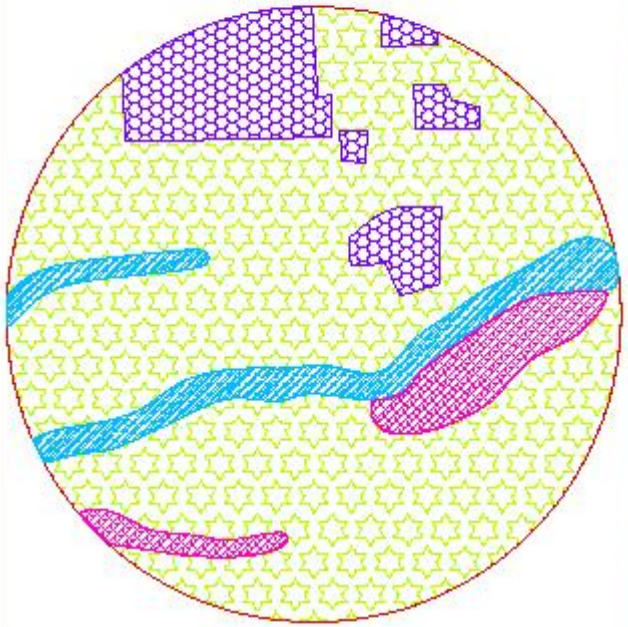

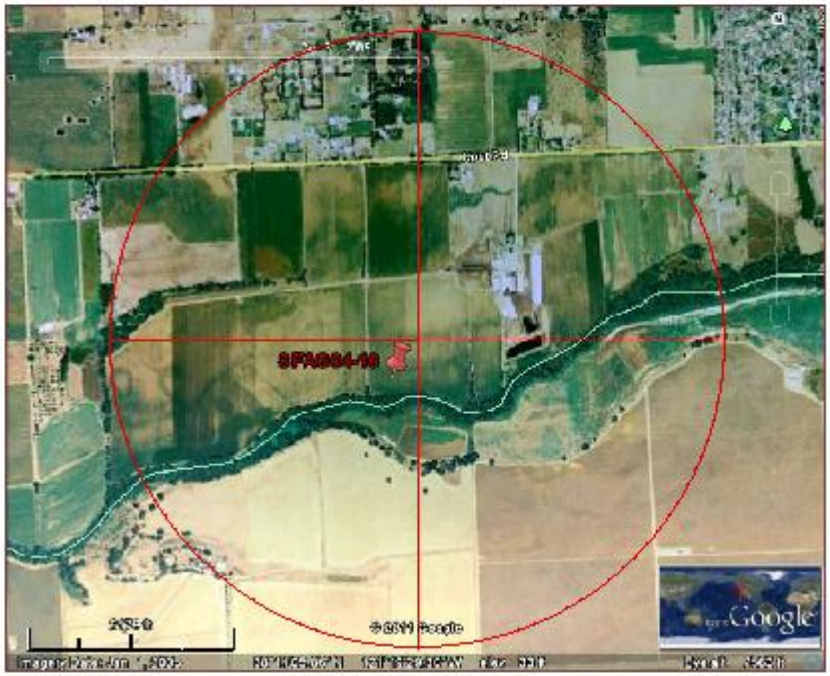

orchard

annual

wild

other

riparian

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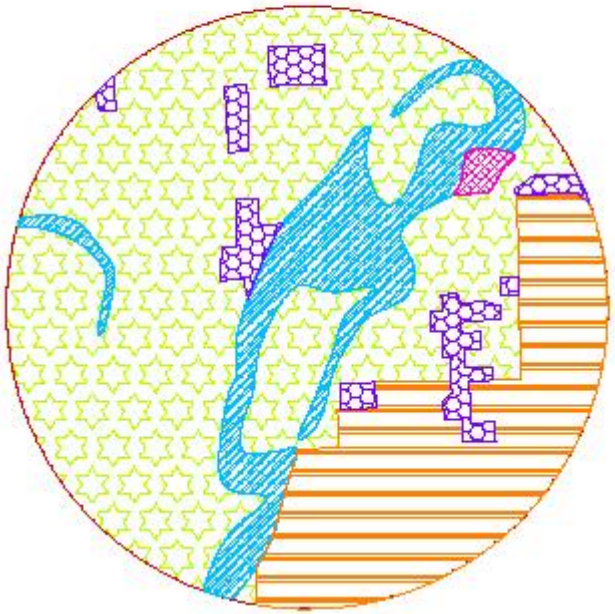

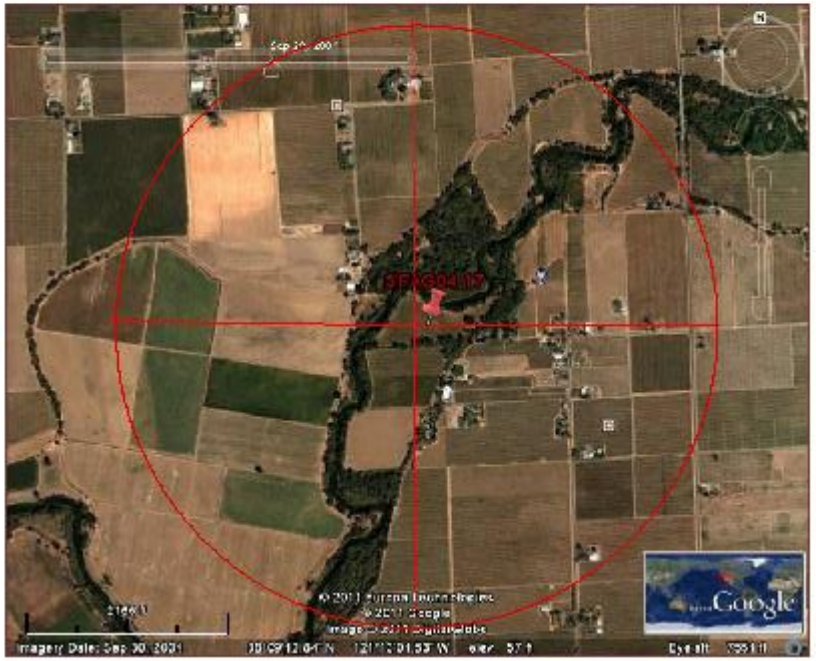

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annual

wild

other

riparian

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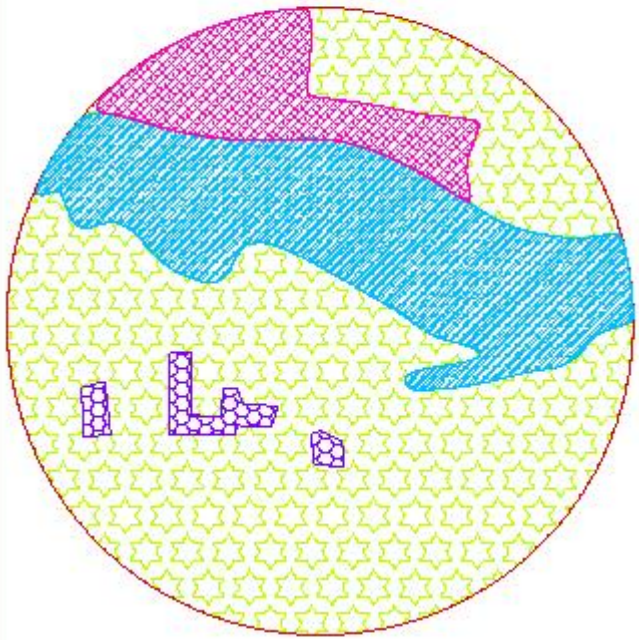

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annual

wild

other

riparian

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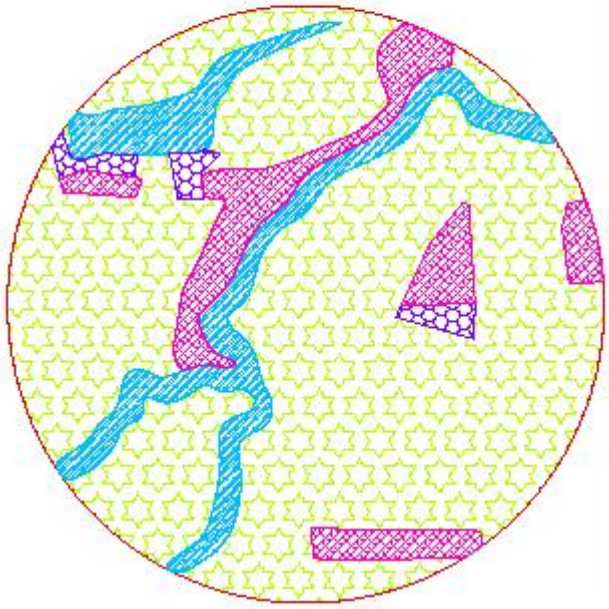

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annual

wild

other

riparian

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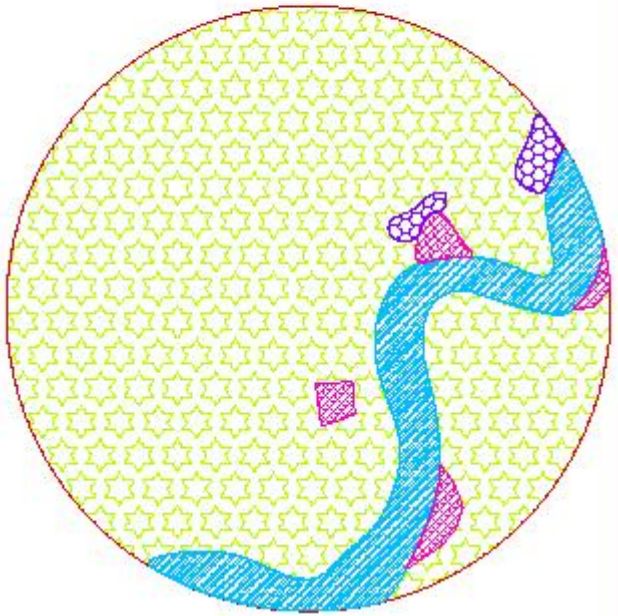

orchard

annual

wild

other

riparian

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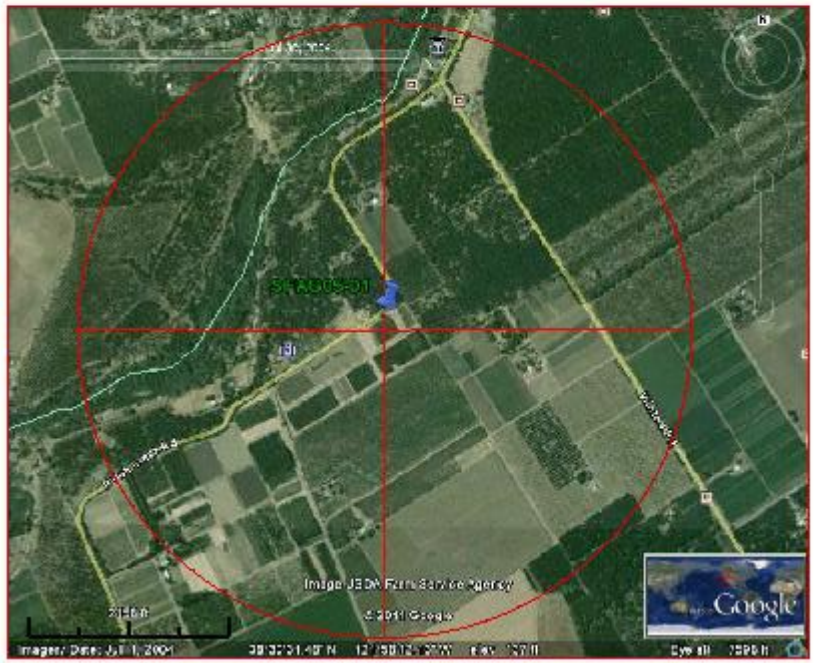

orchard

annual

wild

other

riparian

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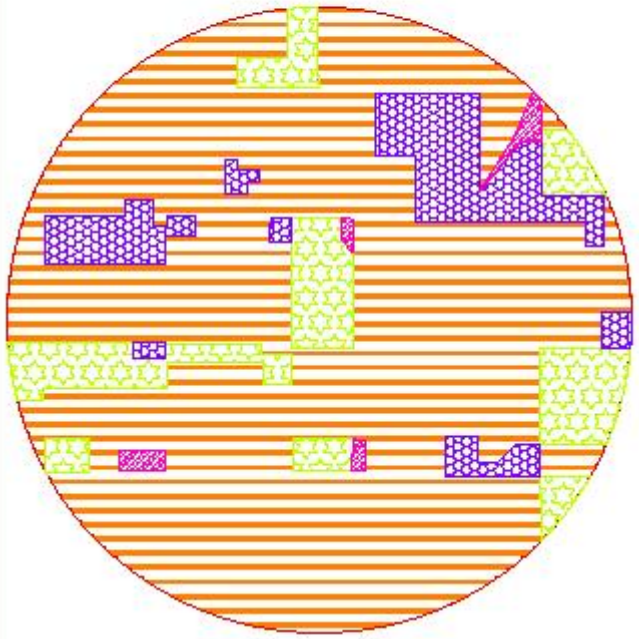

orchard

annual

wild

other

riparian

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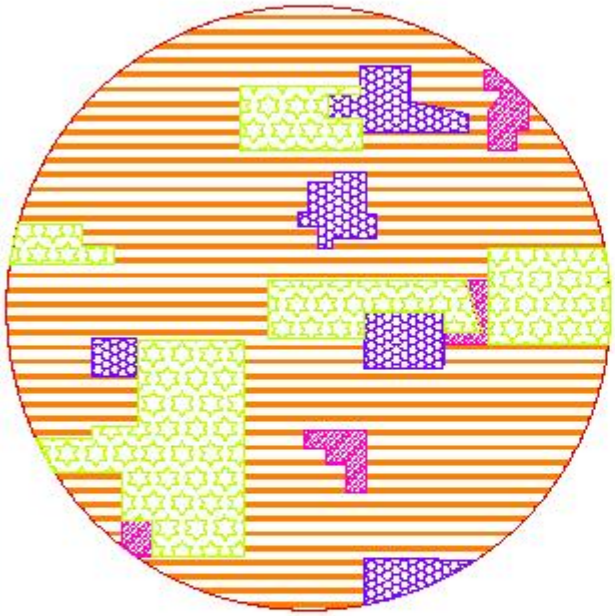

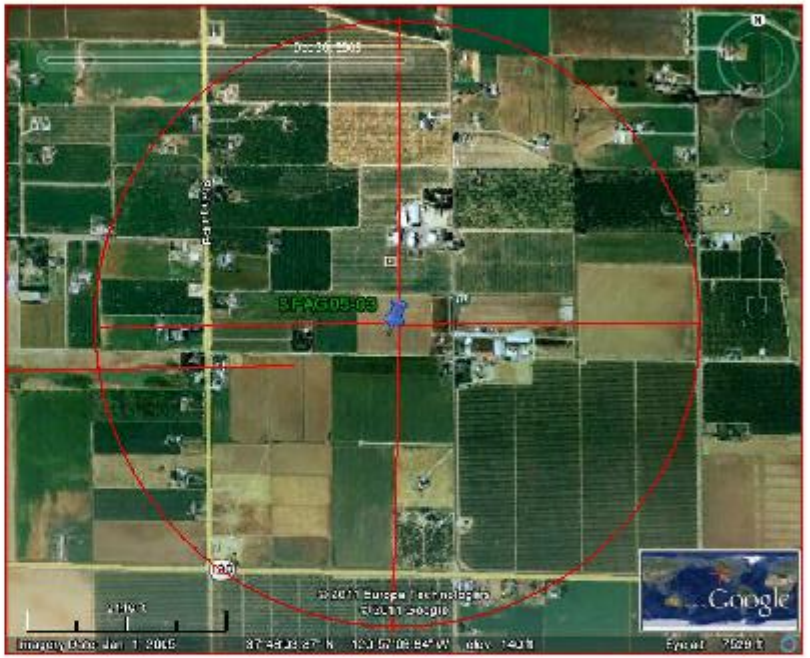

orchard

annual

wild

other

riparian

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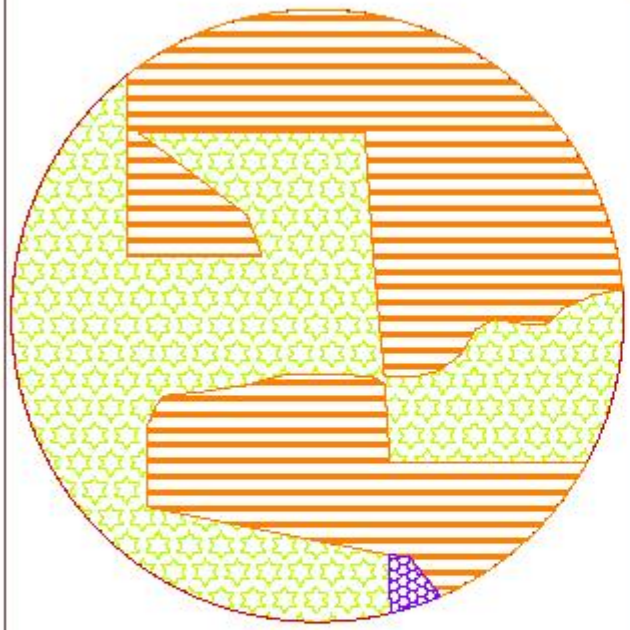

orchard

annual

wild

other

riparian

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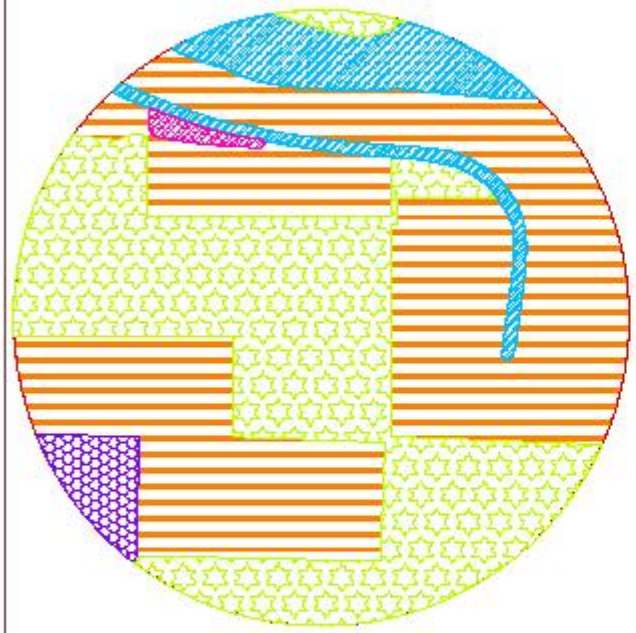

orchard

annual

wild

other

riparian

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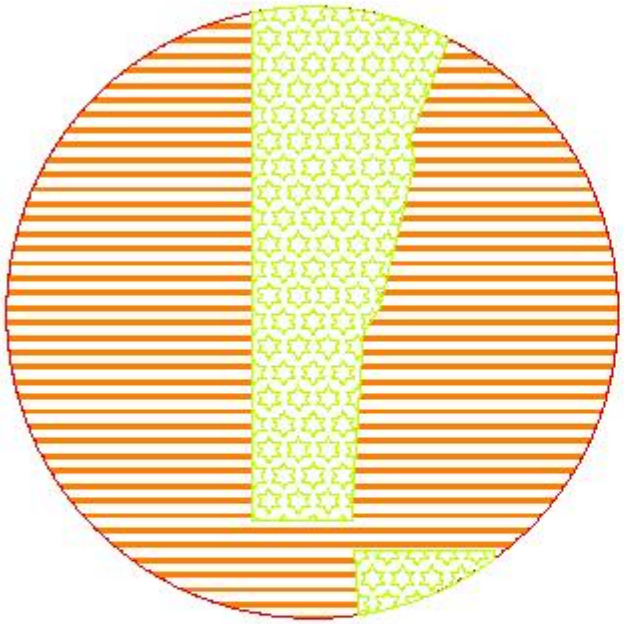

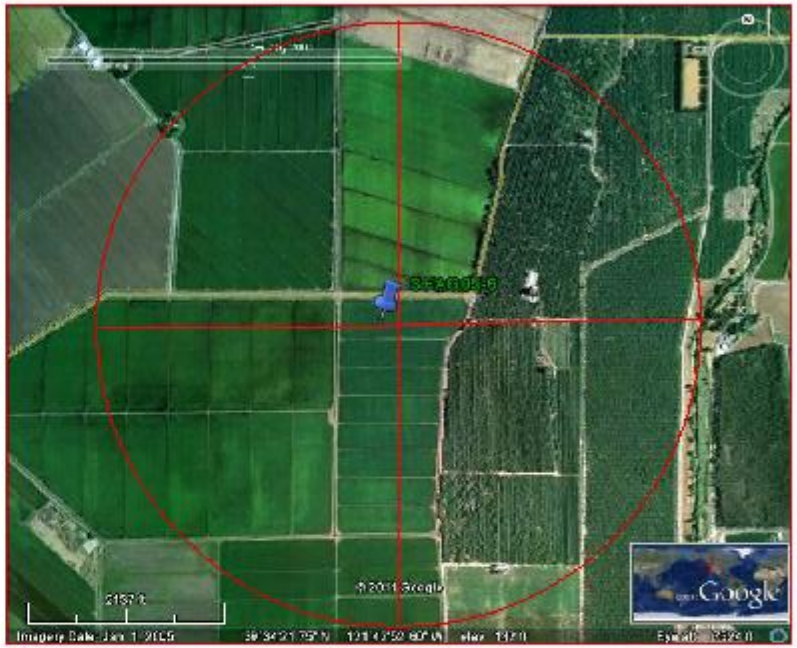

orchard

annual

wild

other

riparian

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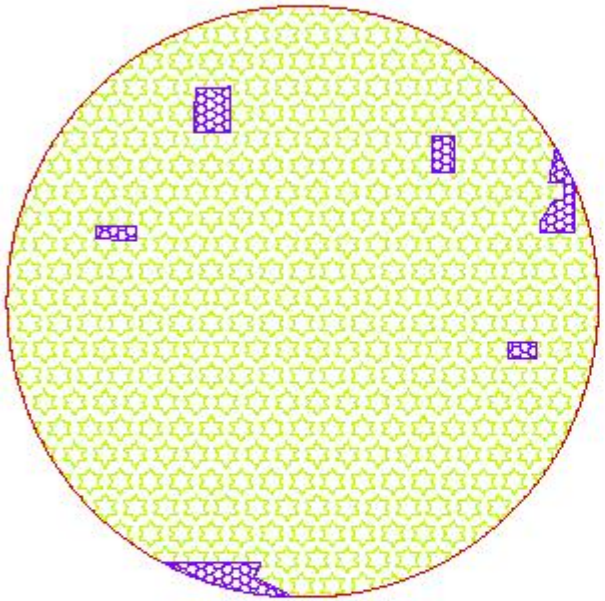

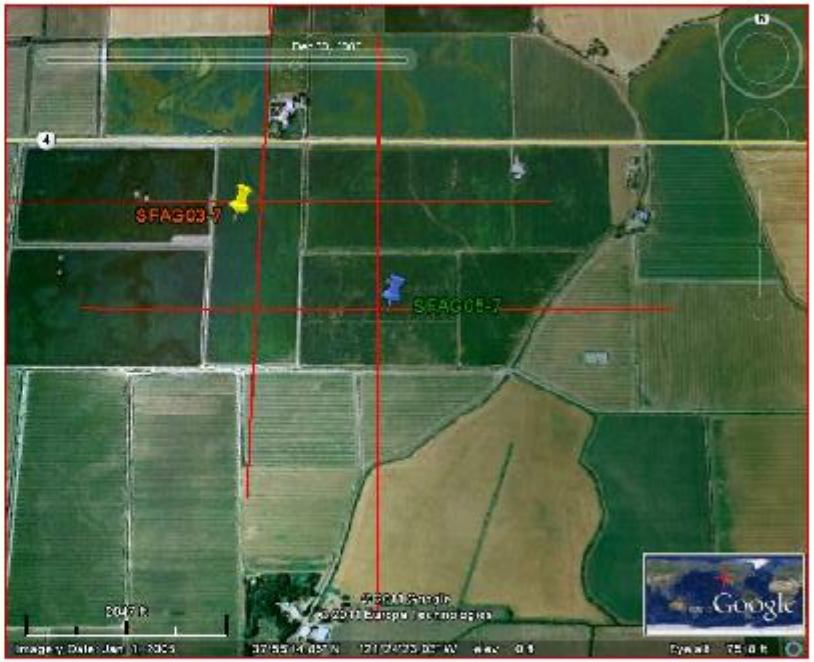

orchard

annual

wild

other

riparian

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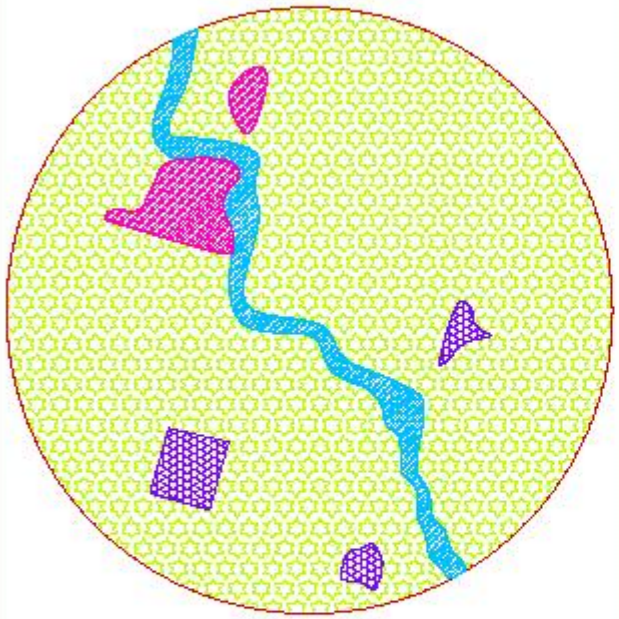

orchard

annual

wild

other

riparian

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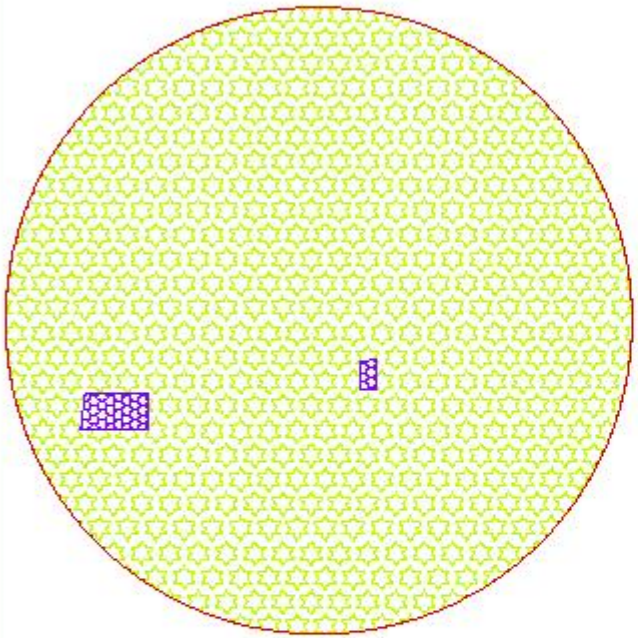

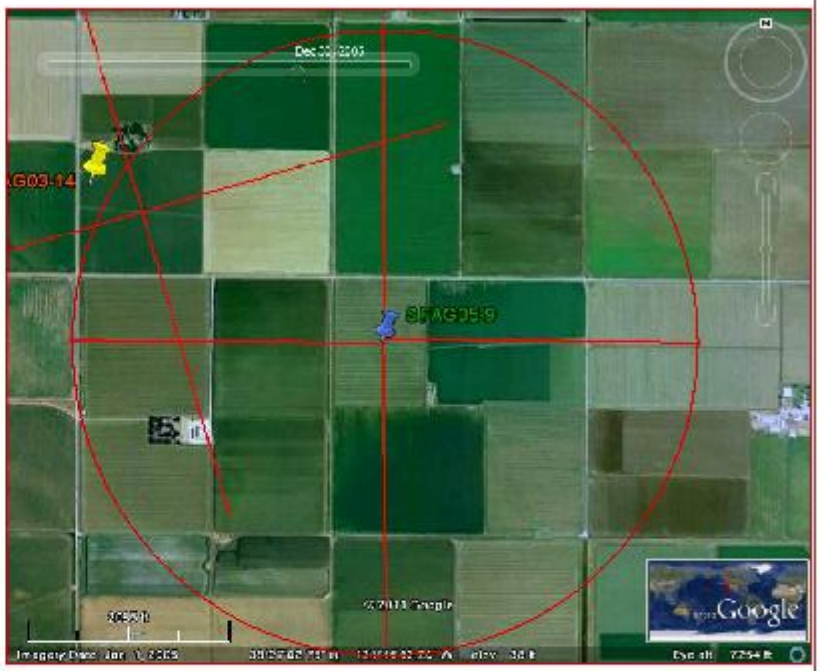

orchard

annual

wild

other

riparian

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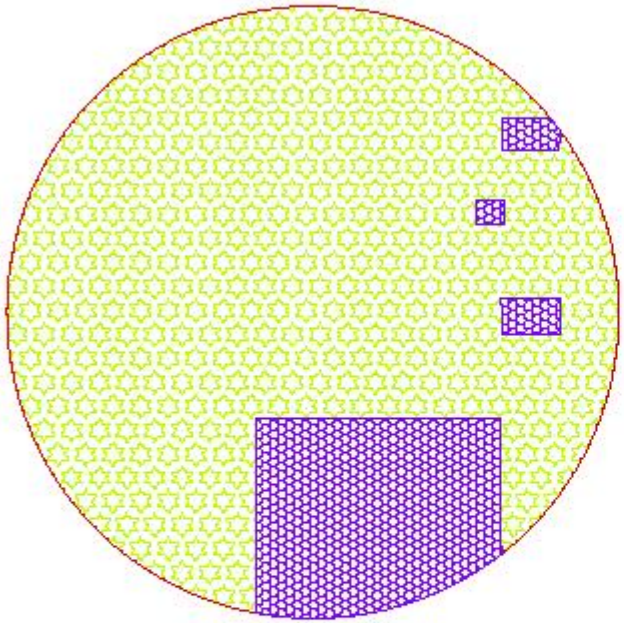

orchard

annual

wild

other

riparian

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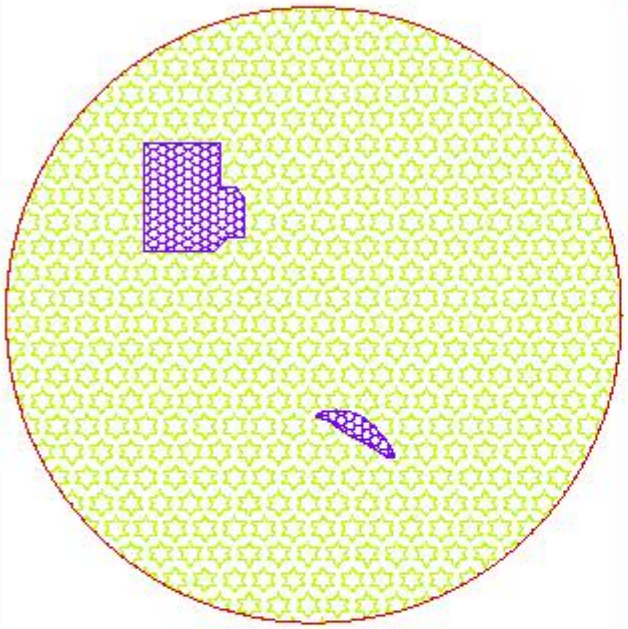

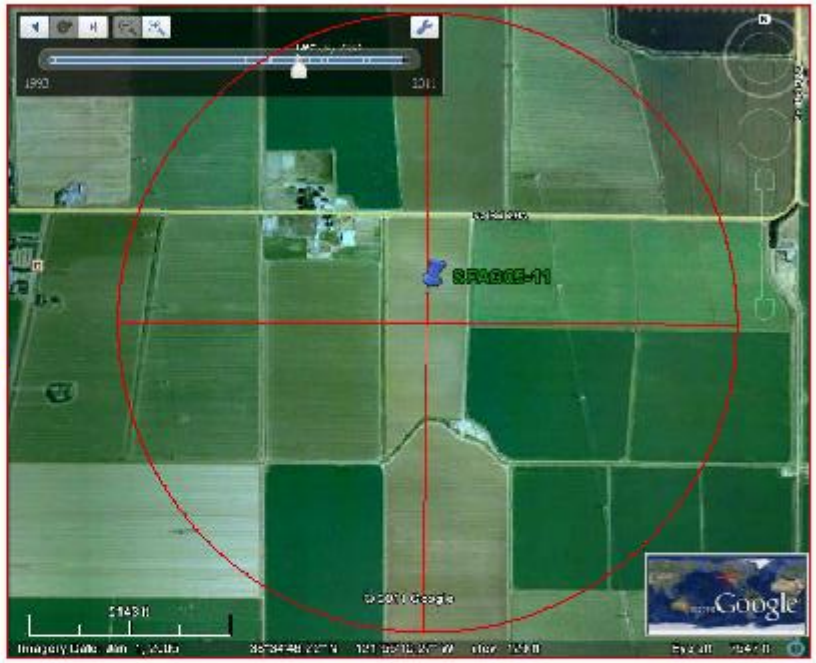

orchard

annual

wild

other

riparian

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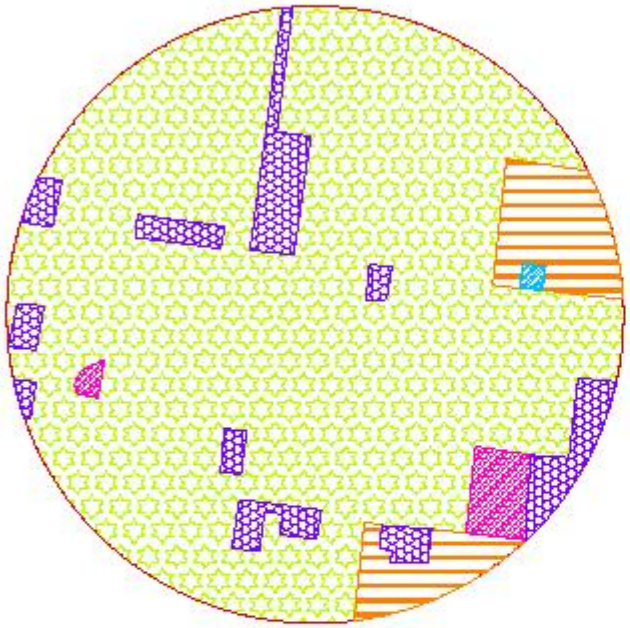

orchard

annual

wild

other

riparian

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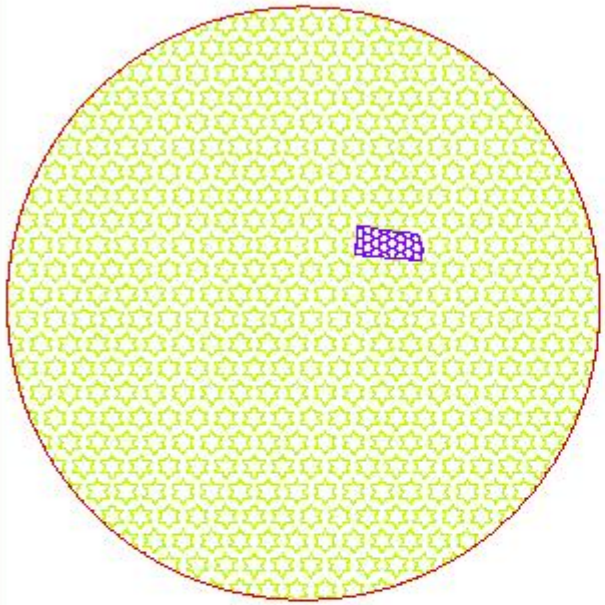

orchard

annual

wild

other

riparian

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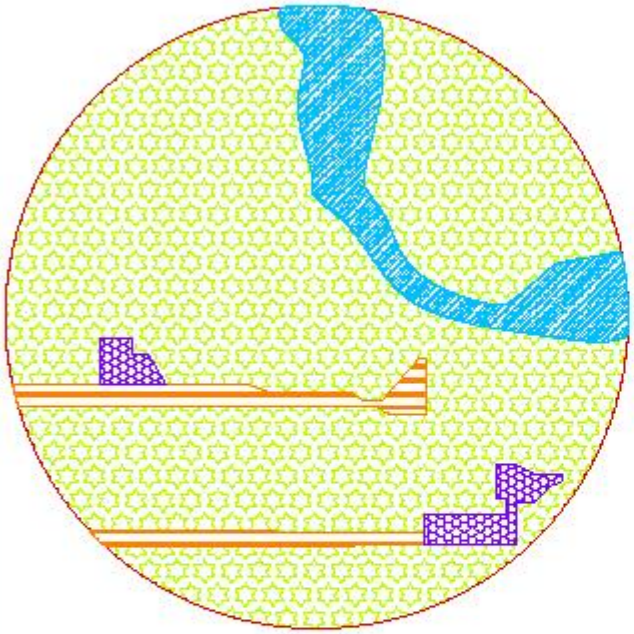

orchard

annual

wild

other

riparian

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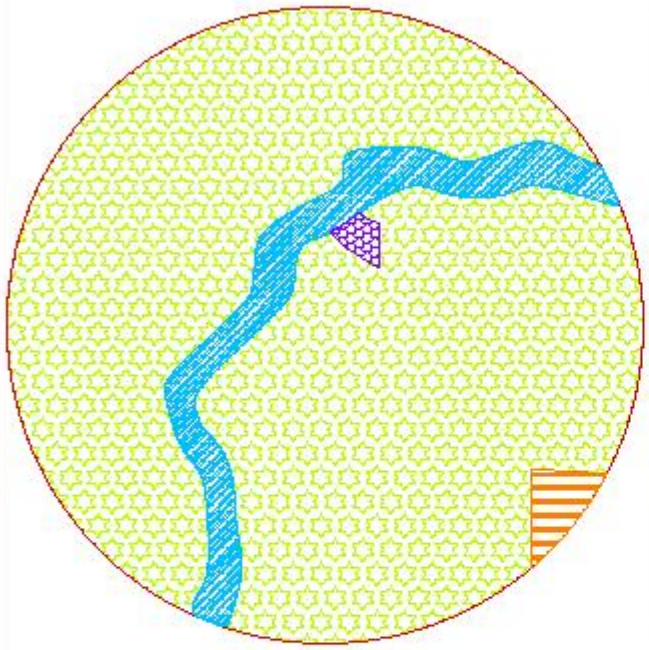

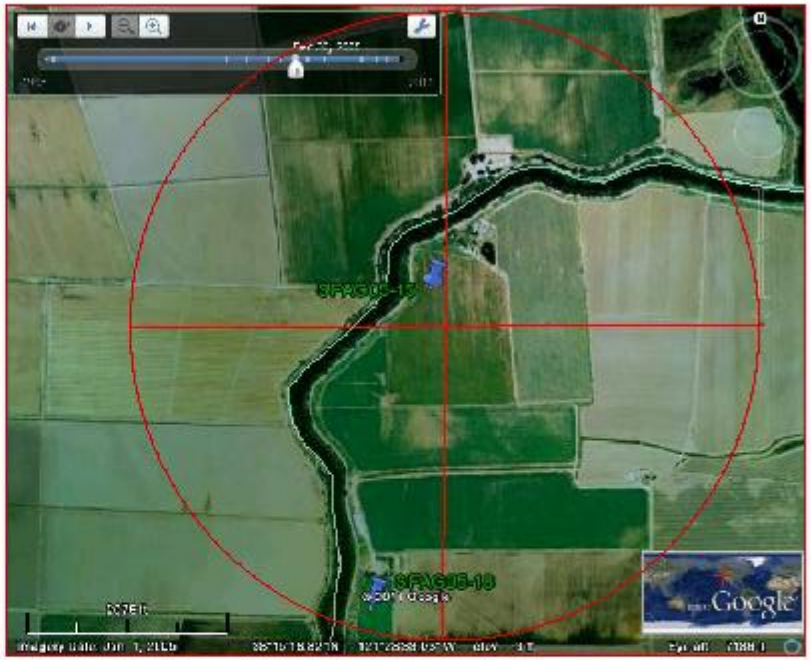

orchard

annual

wild

other

riparian

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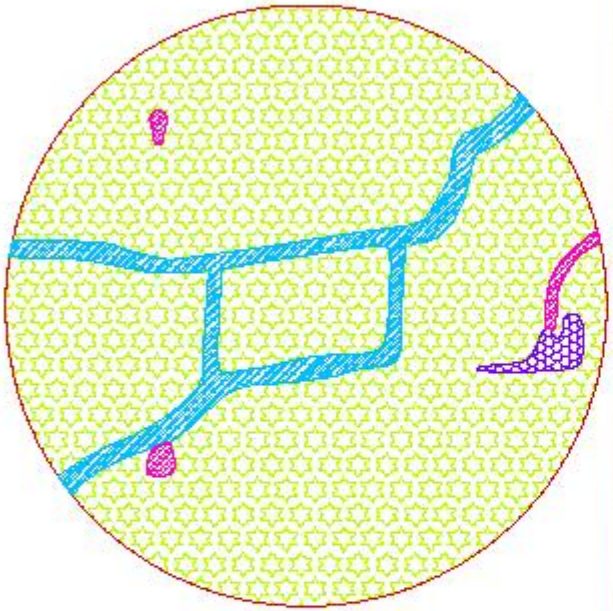

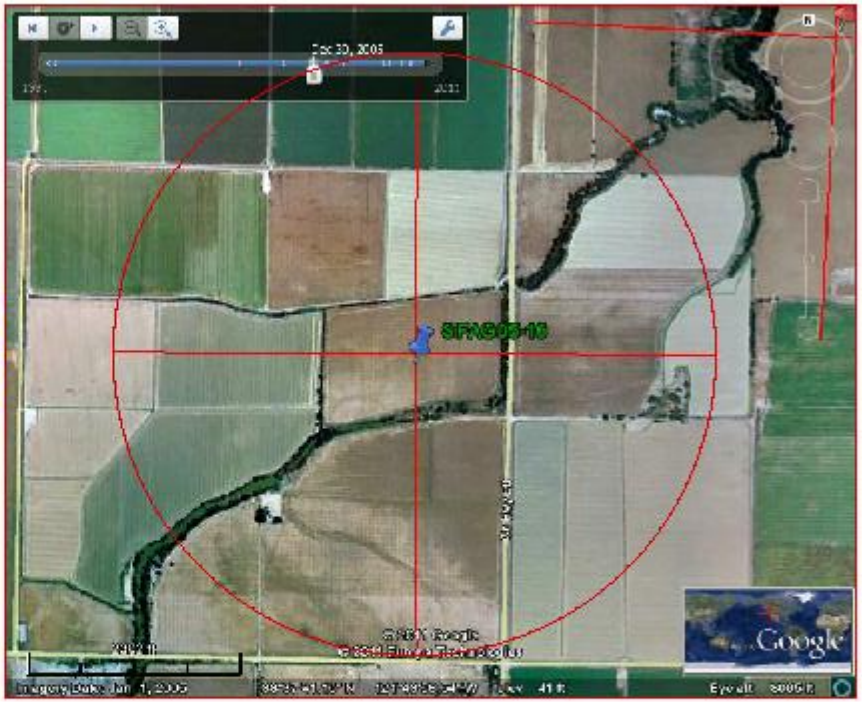

orchard

annual

wild

other

riparian

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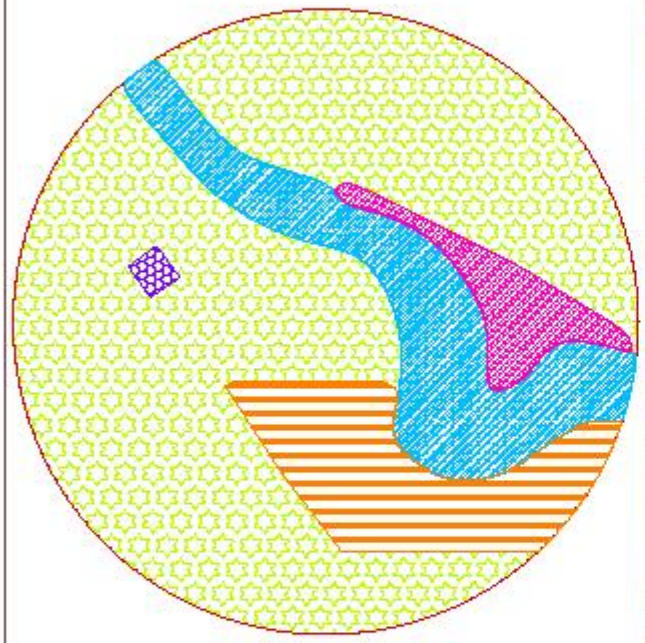

orchard

annual

wild

other

riparian

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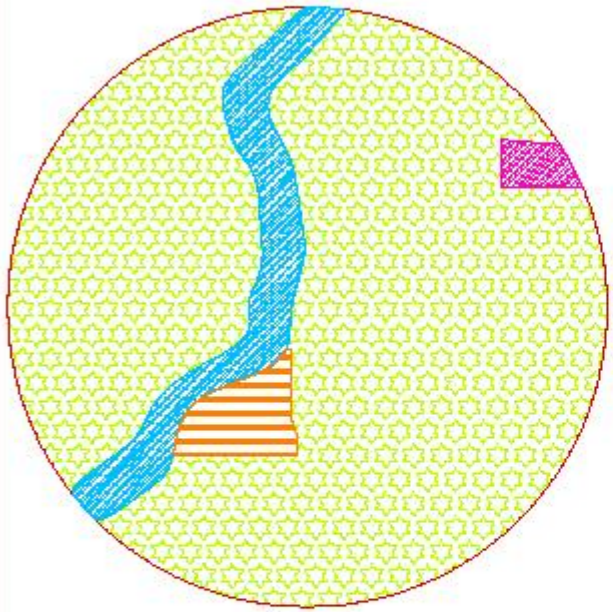

orchard
annual
wild
other
riparian

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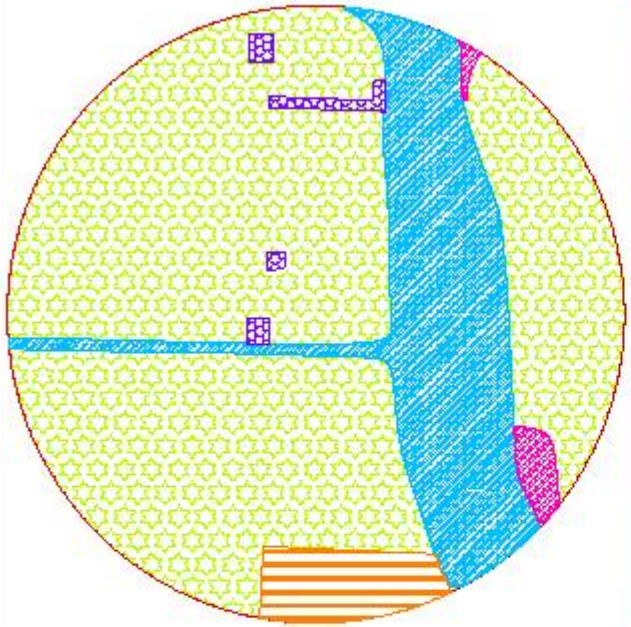

orchard

annual

wild

other

riparian

LANDSCAPE DATA SHEET:
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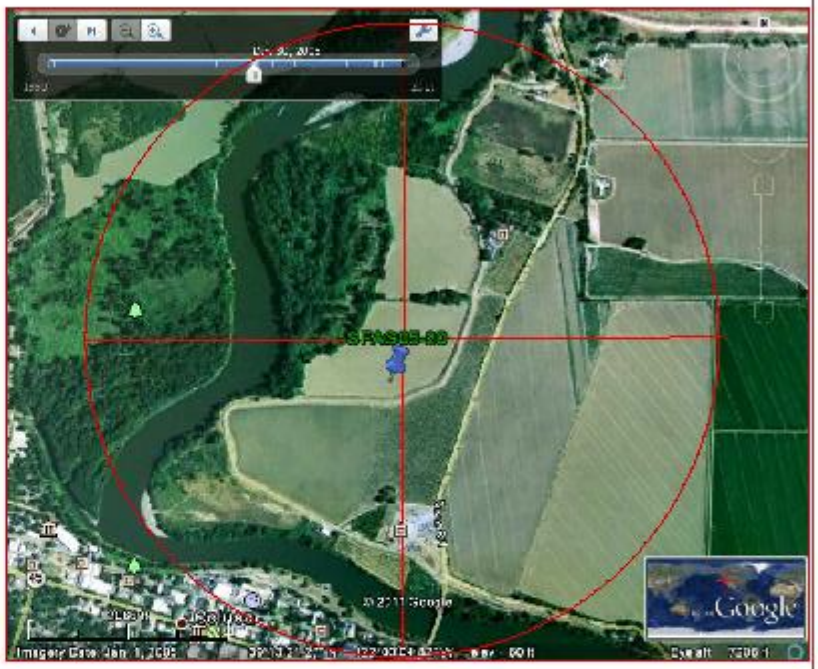


orchard

annual

wild

other

riparian

Chapter 3.

Seasonal abundance and parasitism of flower feeding insects of wild sunflower, *Helianthus annuus*

Introduction

Before the 1980's most ecologists did not consider within-trophic level interactions amongst herbivores to be important in structuring natural communities because they are not usually resource limited (Hairston et al 1960), but increasingly the occurrence and influence of these interactions has been documented (Arim and Marquet 2004). Mechanisms through which herbivores interact range from mutualistic to neutral to antagonistic (Strauss 1988). One herbivore may directly or indirectly interfere with another's use of a host plant through aggression or interference competition (ie: Stahl et al. 2006; Stiling and Strong 1983), explotative competition (Preisser and Elkinton 2008; Karban 1986), changes in plant chemistry and architecture (Gerber et al 2007; Massey et al 2006), associations with other herbivores or natural enemies (Paynter et al 2010; van Veen et al 2009) or apparent competition via a natural enemy (Tack et al 2011; Dyer et al. 2010). The impacts of these interactions upon community structure are interesting from an ecological theory perspective, as well as from an ecosystem management paradigm.

Plant life history, architecture and allocation towards resistance against herbivory have all been modified in crop plants through the domestication process (Welter 2001). Sympatric populations of crop plants and their wild progenitors allow for a unique opportunity to study the relative importance of plant and community level changes for tritrophic interactions (Chen and Welter 2003). Conservation biological control (CBC), which emphasizes the optimal use of existing natural enemies in managing agricultural landscapes (Barbosa 1998), has much to gain from a deeper understanding of these impacts. The ultimate goal of CBC is to adjust plant and system level characteristics to optimize natural biological control, as opposed to the management approach of using pesticides or introduced natural enemies for pest regulation.

In the model system of North American agricultural and wild sunflowers (*Helianthus annuus var. macrocarpus* L. and *Helianthus annuus var. annuus* L.), the herbivore and natural enemy communities are well known (Chen and Welter 2003; Pilson 2000; Charlet 1992; Rogers 1992). The diversity of species feeding on flowers and associated plant parts is lower in the agricultural system (Aslam 1991), with one lepidopteran larvae (*Homoeosoma ellectellum* Hulst) considered the main herbivore pest of florets and seeds in California agricultural sunflower fields (Chen and Welter 2002; Aslam 1991; Charlet 1992). Work in Texas and California comparing wild and agricultural sunflower habitats has shown that 1) adult sunflower moth is more abundant in agricultural sunflowers while parasitism of this species is 6 to 10 times higher in the wild system (Chen and Welter 2002; Teetes and Randolph 1969); and 2) species richness of sunflower moth parasitoids is higher in wild than in agricultural sunflower fields (4 species found in agricultural fields, 7 species found in wild sites). Parasitism by two individual species of parasitoids, *Dolichogenidea homoeosoma* and *Pristomerus spinator*, was reduced by 90% in agricultural fields compared to wild sites (Chen and Welter 2002).

Larval densities per flower head are 10 times higher in agricultural sites but the parasitoid response is not strictly density dependent (Chen and Welter 2002). Clearly, *H. ellectellum* has successfully exploited the domesticated habitat of its ancestral host plant in ways that the other herbivores commonly found in wild sunflower have not (Chen and Welter 2002, 2003, 2007; Charlet 1992).

Factors that have been shown to influence the observed pattern of sunflower moth abundance in the agricultural setting include plant nitrogen fertilization and flowering phenology (Chen and Welter 2002), and escape from parasitism due to behavioral responses and seed characteristics (Chen and Welter 2007). An additional factor that is likely to influence abundance and fitness of sunflower moth in agricultural systems relative to wild systems is the difference in the herbivore community composition between the two systems. It is possible that an escape from its native herbivore guild has contributed to the success of sunflower moth in agricultural settings.

In the Central Valley of California the florivore guild in wild compared to agricultural sunflowers includes two additional commonly found lepidopteran species, *Plagiomimicus spumosum* Grote and *Suleima helianthana* Riley, as well as two tephritid species, *Neotephritis finalis* Loew and *Paracantha cultaris* Coquillet, that overlap at least partially in their feeding niche and life histories with *H. ellectellum* (Table 1). The impact of herbivore guild composition differences between the wild and agricultural sunflower habitats in terms of biological control and improved plant breeding has not been fully explored, though it has been noted (Seiler 1992; Charlet 1999). The effects of within guild predation or apparent competition mediated by shared natural enemies for biological control are being actively studied in other systems (Blitzer and Welter, in press; Alhmedi et al. 2011; Evans et al 1996; Rosenheim et al 1995; Karban et al 1994), but many questions remain. Species richness, species evenness and temporal variability within and amongst trophic levels are important to consider but often very difficult to document (Crowder et al 2010; Hazell and Fellowes 2009; Stevens and Stuart 2008). In orchards, increasing herbivore species diversity has been shown to be associated with an increase overall biological control (Brown and Tworkkoski 2006) while in other agricultural and laboratory settings phenology and omnivory within an herbivore guild have been shown to impact parasitism parameters in both positive and negative directions (Jonsson et al. 2009; Vanbergen et al. 2007).

In this study, we focus on the guild of herbivores that are commonly found feeding on the flowering parts of wild sunflowers in the Central Valley of California. The goal is to explore patterns of abundance of these herbivores and their parasitoids in wild sunflower patches. This will allow formulation of testable hypotheses about the influence of intra-guild interactions upon the parasitoid complex that exists in wild sunflower populations. A better understanding of the herbivore interactions in wild sunflowers, concurrent with studies that examine the parasitoid guild of sunflower moth in agricultural settings (Nerney Meyers et al. in preparation) will inform management recommendations for improving CBC strategies for this crop such as plant breeding and timing of planting.

Methods

We surveyed wild sunflower populations to assess the abundance of florivores, meaning flower and flowering parts consuming species, during the eight weeks of peak bloom in 2004, 2005 and 2006. We recorded herbivore species presence or absence at the flower-head level for each of the five species and noted the incidence of co-occurrence amongst the species. Next, we reared field collected florivore larvae and pupae from these surveys to assess the parasitoid species richness and total parasitism associated with these herbivores during each of the eight weeks of the survey. Finally, we compared our results for sunflower moth parasitism parameters with those found in agricultural surveys done in the same region during 2003, 2004 and 2005 to look for patterns in parasitoid activity across systems (Nerney Meyers et al. in preparation).

Florivore survey

In order to assess abundance of each of the herbivore species we surveyed a random sample of 100 flowers of similar age[1] once per week for eight weeks between 11 July and 3 October of 2004, 2005 and 2006. The blooming season for *Helianthus annuus* L. among these five sites in the San Joaquin Valley of California spanned 12 weeks (Table 1), each site was surveyed for the 8 week period of peak bloom for that particular patch determined by percent of flowers in R1 flowering stage as in Chen and Welter 2003. The total number of 100 flower surveys during the three year period at the 5 sites was 120, with a total of 12,000 flowers dissected. Temporal variation in peak bloom period is assumed to be caused by differential timing of seed establishment according to soil disturbance and water availability and varied by no more than 2 weeks on either end of the season. Sampling grids were not in the same exact location each year due to variability in annual sunflower population emergence, but they were within 0.5km. The five sites are a minimum of 4 kilometers apart. Each site was a self seeding continuous patch of wild type *H. annuus var. annuus* at least 0.5 km ^{2} in area (Table 2).

The florivores found in this study include: 1) *Plagiomimicus spumosum* Grote (Lepidoptera: Noctuidae); 2) *Homoeosoma ellectellum* Hulst (Lepidoptera: Pyralidae); 3) *Suleima helianthana* Riley (Lepidoptera: Tortricidae); 4)*Neotephritis finalis* Loew (Diptera: Tephritidae) and 5) *Paracantha cultaris Coquillet* (Diptera: Tephritidae). Voucher specimens are deposited with the U.C. Berkeley Essig Museum of Entomology and were identified with assistance from Yolanda Chen, University of Vermont. The feeding niches and basic natural history of these herbivore species are summarized in Table 1.

Each flower was scored for total number of larvae or pupae of each of the above herbivores. To ascertain the rates of co-occurrence at the flower level, we assigned each florivore species a

[1] Flowers were chosen randomly at each site in the following manner: Using a random number table, the number of paces to the first collection site from the South East Corner of the plot was chosen, random numbers were assigned to eight directions (N, NE, E, SE, S, SW, W, NW), and a random number between 0 and 150 was the height off the ground from which the nearest sample flower was picked (in cm). Flowers that did not have fully open outer florets were rejected from the sample to control for flower age.

number and recorded which of the 31 possible unique combinations of the five species were present in each flower. This data was also used to create a presence-absence binary matrix for the five species at each of the 4,000 flowers surveyed each year. Each insect larva or pupa was placed in an individual 8ml plastic rearing cup with 10z of sunflower moth diet (Jyoti et al., 1998) and reared to the next life stage (adulthood or pupa) or parasitoid emergence. Rearing outcomes for the five species are summarized in Table 3. While *H. ellectellum* larvae and the two tephritid species did relatively well on the laboratory diet, for *S. helianthana* and *P. spumosum* survival to adulthood, pupal stage or the emergence of an adult parasitoid was relatively less common indicating that the diet and laboratory conditions were not ideal. Unexplained laboratory mortality for *P. spumosum* was 71.7% and that for *S. helianthana* was 33.5% compared to 17.9% for *H. electelllum*, 26.8% for *N. finalis* and 19.3% for *P. cultaris* (Table 3). We were not able to find a more appropriate diet or rearing regime within the constraints of this study, thus we took this into account in interpreting resulting parasitism parameters.

Statistical test of species co-occurrence in flower-heads

Computing all pairwise combinations of species association would not allow us to assign a probability to the distribution of outcomes due to the lack of independence, so we considered the significance of associations among the species taken simultaneously (Schluter 1984). We used a variance ration (VR) derived from a null association model to test simultaneously for significant associations amongst the group of 5 species (Schluter 1984). We derived the VR index of association from our presence-absence data at the flower level for each year (N= 4,000) and used the W test statistic to test for significance departures from the expected value of no association, where W approximates a chi-square distribution (Schluter 1984). The null hypothesis that there is no association will be true when the species are independently distributed among samples, but may also result when positive and negative covariances cancel each other out. The alternative hypothesis is that there is a net positive or net negative association among species. The expected value of VR under H_0 is 1. A value less than 1 indicated that the overall species covary negatively in presence/ absence. We also computed a pairwise 2X2 table analysis followed by a chi-square test with one degree of freedom and Yate's correction formula to avoid the biased values resulting from low cell expectations for the association of of *P. spumosum* and *H. electellum* only in flower heads (Pielou 1974). The null hypothesis is that the species are independent (ie: no association) (Table 4). We performed the same test to investigate the association between *H. electellum* and *P. cultaris.* In this case, we were interested in testing the hypothesis that the two species are positively associated since they were found in flower heads together more often than any other combination of species.

Parasitism

The parasitoids reared from each species of florivore were removed from the diet cups and stored in 95% EtOh. Voucher specimens were identified with help from Yolanda Chen, University of Vermont; Michael Sharkey, University of Kentuky; and Norman Woodley, USDA. Several parasitoids were not identified to species, but grouped as a species complex based on characteristics of the genus.

Percent parasitism was calculated as (P/ [A + P]) * 100; where P is the number of parasitoids emerging and A is the number of adults (or pupa in the case of *P. spumosum*). Dead individuals were not included in the calculation, though as noted above the number of inexplicable deaths was proportionally much higher for *P. spumosm* and to a lesser degree for *S. helianthana* and the two tephritid species relative to *H. electellum* (Table 3).

Results

Florivore abundance

The mean number of flowers per 100 flower survey that had signs of damage from at least one of the five florivore species per site ranged from 29.1 at the Consumnes site to 56.4 at the Yolo Bypass #2, with a mean amongst all sites of 45.06 flowers, reported in Table 2.

P. spumosum was the most abundant florivore at all of the sites (Figures 1-15), with a mean number of 25.7 individuals found per 100 flower survey (Table 5). At most sites, the abundance of *P. spumosum* was high early in the season and then decreased from mid July to mid September, often peaking about two weeks after the 8 week peak blooming period for sunflower begins. Notably, we did not have a single 100 flower survey without at least 6 individual *P. spumosum* larvae (Table 5).

H. ellectellum was the second most abundant herbivore. At 11 of the 15 site/year combinations the abundance of this species surpassed that of *P. spumosum* for at least part of the season, and tended to build up over time; *H. electellum* abundance peaked between weeks 6 and 8 of the survey *(*Figures 1-15). The overall mean was 13.5 individuals of *H. electellum* per 100 flowers surveyed with a minimum of 0 and a maximum of 41 (Table 5).

Abundance of the two tephritid species was very similar relative to each other across the 15 site/year combinations. Overall numbers were consistently low across sites and years (Table 5), so to simplify graphical representation of the abundance data we pooled the two species into the line "Tephritids" (Figures 1-15). The two tephritid species generally exhibited stable abundance throughout the flowering period sampled, with a combined mean of 8.5 individuals, S.D. – 2.3) per 100 flower survey (Table 5, Figures 1-15).

S. helianthana had notably high abundance in the Yolo Bypass site #1 for both years 2004 and 2005 and at Cosumnes in 2005 during the first weekly survey, with 32, 36 and 37 individuals per 100 flower survey respectively, but then dropped down to comparable levels with other sites and years following that, and generally was similar to that of the tephritid species, with an overall average of 7.6 individuals per 100 flowers (Table 5, Figures 1-15).

Inter year patterns within the five study areas were remarkably similar, especially at the Yolo Bypass site #1 (Figures 1-3) and Stone Lakes site #1 (Figures 7-9). Peaks in the abundance of the two most common herbivores, *H. electellum* and *P. spumosum*, tended to happen about the same number of weeks into the peak bloom across the survey years at the two sites in the Yolo Bypass, the 5^{th} or 6^{th} week for *H. electellum* and the 3^{rd} or 4^{th} week for *P. spumosum*. There was more inter year and inter site variability in peak abundance for the two species at the Stone Lakes sites, but with the exception of the Stone Lakes # 2 in the year 2005, the abundance of *H. electellum* surpassed that of *P. spumosum* at about week 4 or 5 (Figures 7-12) at all of the Stone Lakes sites and years.

Cosumnes survey years 2004 and 2006 had similar herbivore abundance trends with all species gradually building up in abundance in parallel, but the patterns in 2005 were more similar to the "boom and bust" trend in *P. spumosum* and later season peaking of *H. electellum* seen in the Yolo Bypass sites (Figures 14, 1-6).

The abundance patterns found in our three year survey are similar to those found for *H. ellectellum* at wild sunflower sites within the same regions by Chen and Welter, who found a peak in larval abundance around the middle of August (Chen and Welter 2002).

Florivore intraspecific co-occurrence per flower

The percentage of flowers with one or multiple individuals of the same species is summarized in Table 6. Of the *P. spumosum* larvae found, 93.1% were in the flower with no other conspecifics, and no flowers with more than two *P. spumosum* larvae were found in the survey. In contrast, *H. electellum* was accompanied in the flower head by at least one other conspecific 54% of the time, with 37% of flowers surveyed containing *H. electellum* having two individuals, 9.4% having 3 and 7.7% having more than three. *S. helianthana* larvae were found alone in 40% of flowers containing this species, while 50.9% and 9.4% of flowers had two and three individuals respectively. No flowers were found with more than three individuals of this species. For *N. finalis* only 4.3% of flowers containing the species had only one individual, with 35% having two, 23.3% having three and 37.4% having more than three. *P. cultaris* showed a similar trend, with 6.3% of flowers containing one individual alone and 25.2%m 18.5% and 50% containing 2, 3, and more than 3 respectively (Table 6).

Florivore species interspecific co-occurrence per flower

The average incidence of co-occurrence of the five florivores in one flower head in the full field survey is summarized in Figure 16. The majority of flowers with herbivores present across all survey sites and years contained *P. spumosum* larvae and no other herbivore: on average 19.0 of the 100 flowers surveyed; standard deviation = 6.9. The next most common flower occupancy was that of *H. electellum* larvae alone, average 12.8, S.D. = 7.8. The most common combinations of two herbivore species in one flower was that of *H. electellum* and *P. cultaris* followed by that of *H. eleltellum* and *N. finalis* (2.4, S.D.= 2.1 and 2.2 S.D.= 1.7 respectively). Flowers with the two tephritid species alone were also relatively common (1.8, S.D. = 1.2), as were those with *S. heliantha* and *N. finalis* (1.6, S.D. = 1.5). Notably, the combination of three or more herbivores in one flower head was extremely rare, with a combined total average of 0.5 flowers per survey having 3, 4, or 5 species. The co-occurrence of *H. electellum* and *P. spumosum* in one flower head was also rare with an average of 0.3 flowers per survey; S.D. = 0.2 (Figure 16). In fact, any combination of *P. spumosum* and any other species was rare compared to combinations of other species. Of the 13 possible combinations of species involving *P. spumosum*, the highest average number of flowers per 100 flower survey containing it and another species was 0.38, with only *P. cultaris* (Figure 16).

Tests of species co-occurrence in flower heads

The VR derived from species presence-absence data in flower heads for all sites during 2003, 2004 and 2005 (N= 9,000 flowers) respectively was 0.87 ($p < 0.01$), 0.76 ($p < 0.001$), 0.69 ($p< 0.001$). These significant VR values bellow 1 indicate that there is a net negative association amongst the species at the flower level in all three years of the survey. The contingency table analysis for *H. electellum and P. spumosum* including the 12,000 flowers surveyed during all three years is summarized in Table 4. The chi-squared value for the negative association between the two species with the Yates correction is 353.89, $p<0.0001$. While neither of these tests can indicate the nature of the process, there is evidence that the distribution of co-occurrence of the species as a group and in particular the two most common, *H. electellum* and *P. spumosum,* as a pair is less than what would be expected by chance. This finding warrants further exploration of the nature of the interactions between these species.

While visual inspection of the data led us to believe there could be a positive association between *H. electellum* and *P. cultaris*, a contingency table analysis followed by a chi-squared test for this association gives a non significant value for the positive association between the two species of 124.7, $p = 0.13$, using the Yates correction.

Parasitism

Table 7 summarizes the percent parasitism, contribution to total parasitism by each parasitoid species and the total parasitoid species richness found in the survey. The parasitoid complex for *H. electellum* was the most species rich, with 10 total species represented from three families and two orders. This finding represents an additional 3 species not found in wild sunflower surveys in the same region in 1999 and 2000 (Chen and Welter 2003): *Macrocentrus ancylivorous*, *Parania geniculata* and *Lixophaga variabilis*. In our survey these three species were responsible for 4.3%, 6.3% and 5.2% of *H. electellum* parasitism. We did not, however, observe any parasitism of *H. electellum* in our survey by the chalcid, *Perilampus spp.,* which was responsible for less than 5% of *H. electellum* parasitism in wild habitats in Chen and Welter's 1999 and 2000 surveys (Chen and Welter 2003).

A total of six parasitoid species were reared from *P. spumosum*, with three families and two orders represented. Three species of parasitoid were reared from the tortricid moth *S. helianthana*, and a total of three species attacked the two tephritid flies (Table 7).

Overall parasitism was highest for *H. electellum*, at 33.6% (S.D. = 5.1; n=347), following was *N. finalis* with 22.2% parasitism (S.D. = 4.2; n=68), then *P. cultaris* with 20.9% parasitism (S.D. = 3.6; n=71), *S. helianthana* with 18.6% parasitism (S.D. = 3.2; n=97) and finally *P. spumosum* with 12.2% parasitism (S.D. = 4.2; n=96) (Table 7). A visual comparison of the total number of each species reared and the percent parasitism calculated from this number is provided in Figure 17.

Shared generalist parasitoid species

Four species of parasitoid attacked more than one of the florivore species in our survey: *Bracon nuperus* and *Erynnia tortricis* attacked both *P. spumosum* and *H. electellum; Pristomerus spinator attacked both H. electellum* and *S. helianthana;* and *Pteromalus sp.* attacked both of the tephritid species. *Pteromalus sp.* was the most important parasitoid of both of the tephritid species, responsible for 72.1% of the parasitism events for *N. finalis* and 78.9% of those for *P. cultaris*. *B. nuperus* was the most important parasitoid *of P. spumosum*, attacking 60.4% of hosts collected, but it was a relatively minor parasitoid of *H. electellum* , attacking 6.6% of these. *E. tortricis* was a minor parasitoid for both *H. electellum* and *P. spumosum*, at 6.6% and 6.3% of parasitism for these species. *P. spinator* was responsible for a large part of the parasitism for *S. helianthana*, at 44.3%, but was only a minor player for the *H. electellum* complex at 6.0% of parasitism (Table 7).

Patterns in parasitoid attacks of H. electellum and P. spumosum

Parasitism of *H. electellum* showed a temporal pattern across sites, peaking during weeks 4 and 5 of the 8 week blooming period during all three years and at all 5 sites (Figure 18). During these three years, the Cosumnes River Preserve site had the overall lowest numbers of parasitoids reared from *H. electellum*; followed by the two sites in the Stone Lakes and those at the Yolo Bypass (Figure 18). These peaks in parasitoid numbers coincide with the peaks in the *H. electellum* abundance numbers (Figures 1-15, Figure 18).

In contrast, parasitism of *P. spumosum* did not exhibit a clear increasing or decreasing pattern during the 8 week peak blooming period for sunflower. The 96 total parasitism records for this species are distributed evenly across the weeks and locations, although overall numbers were higher during the first four weeks of the survey in 2006 at all of the sites (Figure 19).

Discussion

While plant architecture mediated enemy release and field level nitrogen availability have been shown to impact the greater abundance of sunflower moth, *H. electellum*, in agricultural fields (Chen and Welter 2002), our survey data from wild sunflower sites in California indicates that there are complex patterns of herbivore interactions in the wild system that could also influence the differing community structure between the two systems.

Florivore abundance and co-occurrence

The number of flowers that exhibited damage from herbivores in the wild system was quite high, with an overall mean of 45.1 flowers per 100 flowers surveyed damaged by herbivores (Table 2). We found *P. spumosum* to be the most abundant florivore in the 5 sunflower patches surveyed, with an average of 25.7 individuals found per 100 flowers (Table 5). This species was negatively associated with the other four species in flower heads during all four years of the survey ($p < 0.01$, $p< 0.001$, $p < 0.0001$ respectively) and there was a significant negative association between it and *H. electellum* in particular ($p < 0.0001$). The abundance of *P. spumosum* tended to peak within the first three weeks of the 8 week peak flowering period surveyed while the abundance of *H. electellum* peaked in weeks 5 and 6 (Figures 1-15). The other four species, *S. helianthana*, *P. cultaris* and *N. finalis* had low and stable abundance across the 8 week period (Figures 1-15).

Larvae of *P. spumosum* are very aggressive and have been observed to be antagonistic towards and consume conspecifics and other larvae in the laboratory and in the field (Nerney Meyers unpublished data, Pislon 2000, pers. comm.). These larvae could be exerting active or passive competitive pressure for protected space and food resources inside the flower capitulum. One possibility that should be experimentally pursued is the relationship between the abundance of *P. spumosum* and the parasitism of *H. electellum* , which from our observations appears to be negative.

Parasitism and parasitoid species richness

The greatest diversity of parasitoids was reared from *H. electellum*, totaling 10 species. This species also had the highest overall parasitism rate, averaging 33.6% across sites and years. We reared 6 species of parasitoids from *P. spumosum*, with an overall parasitism rate of 12.2%. We expect that these are low estimates of actual parasitoid species diversity and parasitism due to the low survival rate of the larvae in the laboratory. Parasitism rates of *S. helianthana*, *P. cultaris* and *N. finalis* were 18.6%, 22.2% and 20.9% respectively, with three species of parastioids reared from each (Table 7).

The two most abundant herbivore species shared two parasitoid species in our survey, but while *B. nuperus* was an important parasitoid of *P. spumosum*, responsible for 60.4% of overall parasitism, it was only a minor parasitoid of *H. electellum*, responsible for 6.6% of overall parasitism.

The second parasitoid in common was responsible for similarly small proportions of overall parasitism, 6.6% and 6.3% respectively. *S. helianthana* and *H. electellum* also shared one parasitoid species, *P. spinator*, which was responsible for a large part of the *S. helianthana* parasitism, 44.3% but only a small part of that of *H. electellum*, 6.0%.

Temporal patterns in parasitism of *H. electellum* showed a marked peaking of parasitism at all sites and years during weeks 4 and 5 of the survey. In contrast, there was no discernable pattern of parasitism for *P. spumosum* (Figures 18, 19).

Many other indirect or direct interactions between the florivores are also possible, given that 1) the overall rate of flowers damaged by at least one herbivore is very high with an average of 45.1, S.D. = 3.2 (Table 1); 2) the there are four parasitoids that attack more than one of the florivore species (Table 6); 3) there is strongly negative co-ocurrence of the species in the flowers and 4) the abundance surveys at the five sites displayed remarkable similarities in abundance patterns and in particular a shift between dominance by *P. spumosum* and *H. electellum* in the last one third of the flowering season (Figures 1-15).

Parasitism parameters for H. electellum in wild survey compared to agricultural surveys

In the agricultural setting, the flowering phenology allows for only one full generation of sunflower moth to develop within a field (Chen and Welter 2003), and our results show that parasitism of sunflower moth in wild settings increases during the second generation of larvae. Along with this parasitism response, there may be intra-guild interactions among *H. electellum*, *P. spumosum, S. helianthana, P. cultaris* and *N. finalis* that impact the *H. electellum* population in wild sunflower settings.

The role of the specialist parasitoid, *D. homoeosomae*, in overall parasitism of sunflower moth is comparable in agricultural and wild settings. Parasitism of late larval stages of *P. spumosum*, on the other hand, seems to be caused by a guild of generalist parasitoids (Figure 20).

Four of the 10 species of parasitoids of *H. electellum* found in our survey were not present in agricultural fields surveyed during 2003, 2004 and 2005: *Bracon nuperus*, *Trichomma maceratum*, *Parania geniculata* and *Lixophaga variabilis* (Nerney Meyers in preparation). The specialist parasitoid *D. homoeosomae* was the dominant parasitoid in both the wild and agricultural fields, responsible for 45% of parasitism in the wild and 52.2% in the agricultural habitat (Table 6 and Nerney Meyers, in preparation). The generalist parasitoid *Macrocentrus ancylivorous*, was a much more important parasitoid in the agricultural habitats surveyed, on average responsible for 16.1% of parasitism compared to 4.3% in the wild.

The ecological effects of changes in herbivore species diversity and in particular evenness in agricultural relative to natural systems are likely many fold (Crowder et al. 2010), and future planning of agroecosystems should be informed by an improved understanding of the intended and unintended consequences of plan and habitat domestication. This study provides a baseline for future work detailing the herbivore-herbivore interactions in wild sunflower and their tri-trophic consequences. Experimental evidence of direct or indirect interactions amongst these florivores will help to guide the conservation of a native parasitoid guild for pests of agricultural sunflower.

Figure 1. Herbivore abundance during 2004 florivore survey at Yolo Bypass site 1

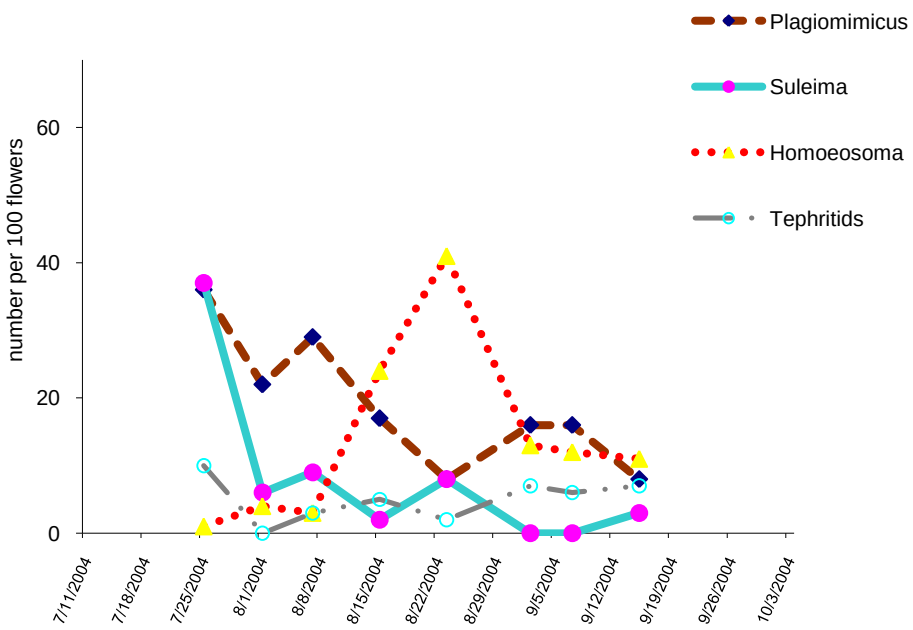


Figure 2. Herbivore abundance during 2005 florivore survey at Yolo Bypass site 1

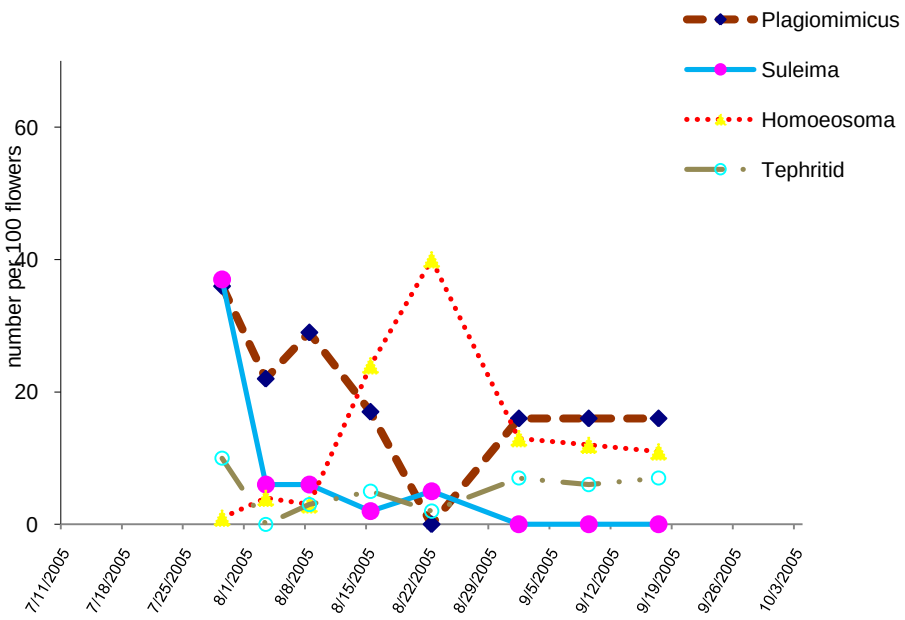


Figure 3. Herbivore abundance during 2006 florivore survey at Yolo Bypass site 1

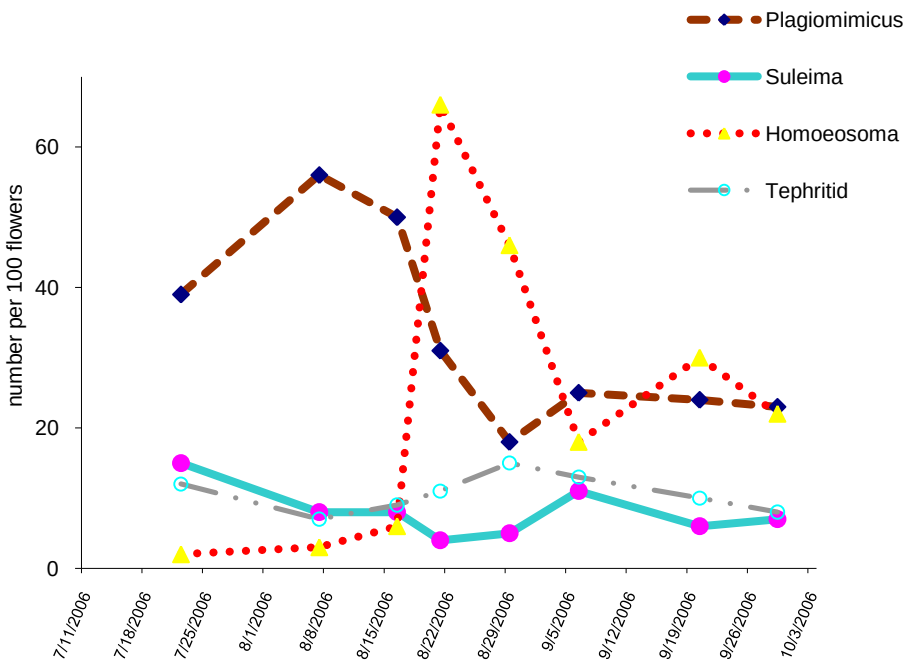


Figure 4. Herbivore abundance during 2004 florivore survey at Yolo Bypass site 2

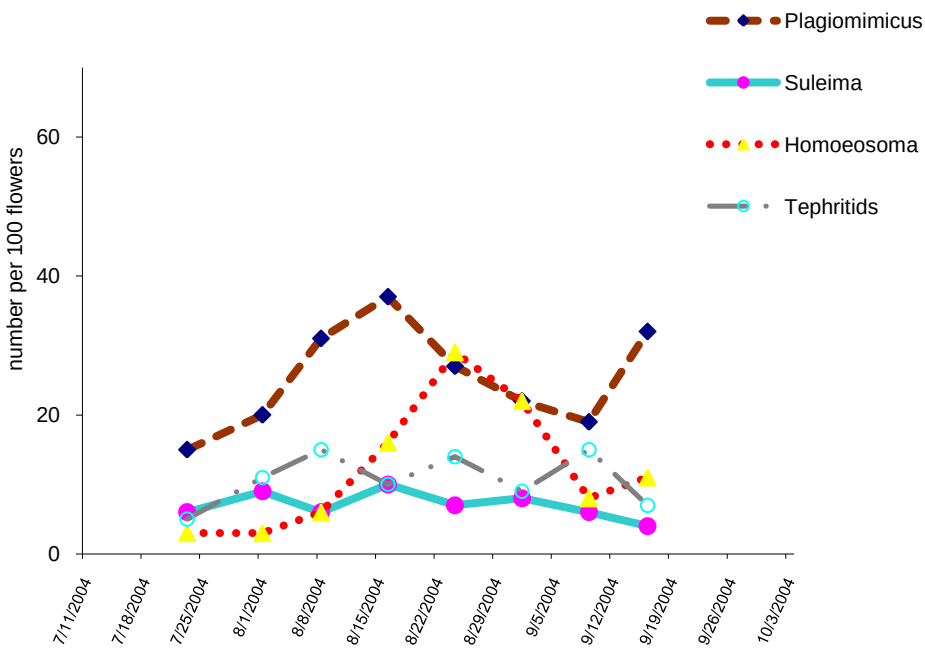


Figure 5. Herbivore abundance during 2005 florivore survey at Yolo Bypass site 2

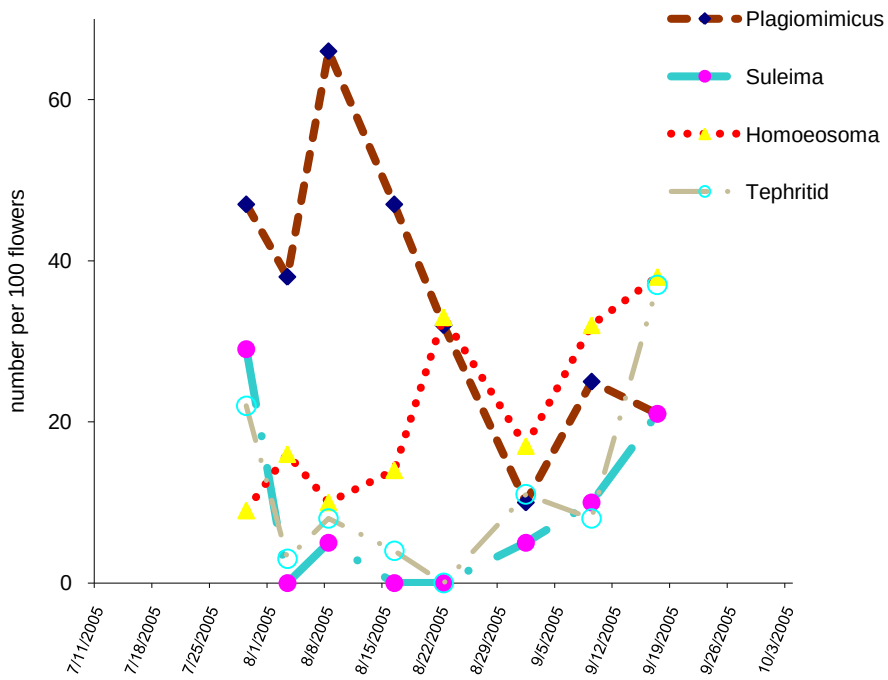


Figure 6. Herbivore abundance during 2006 florivore survey at Yolo Bypass site 2

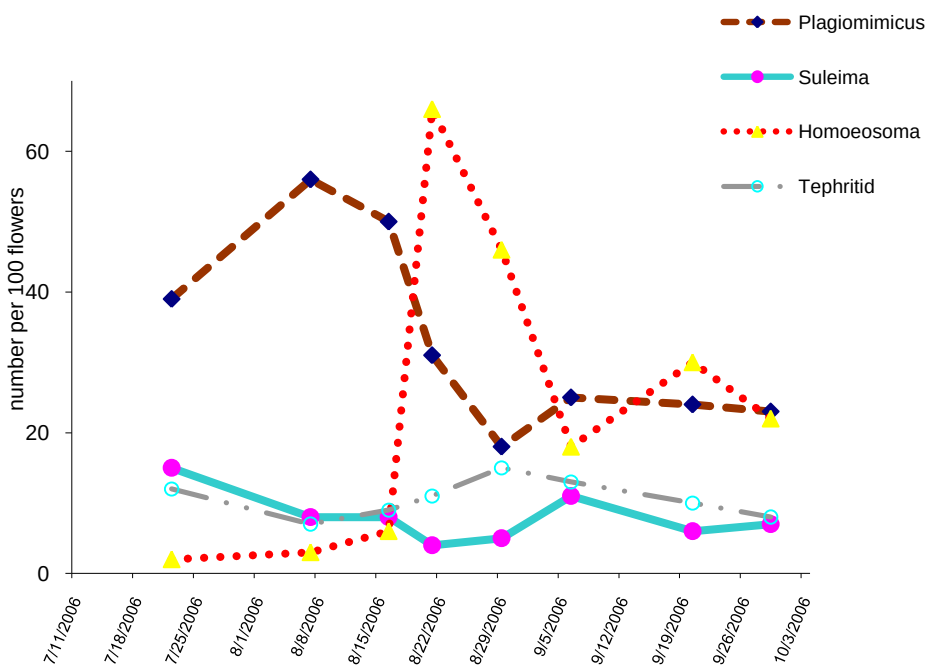


Figure 7. Herbivore abundance during 2004 florivore survey at Stone Lakes site 1

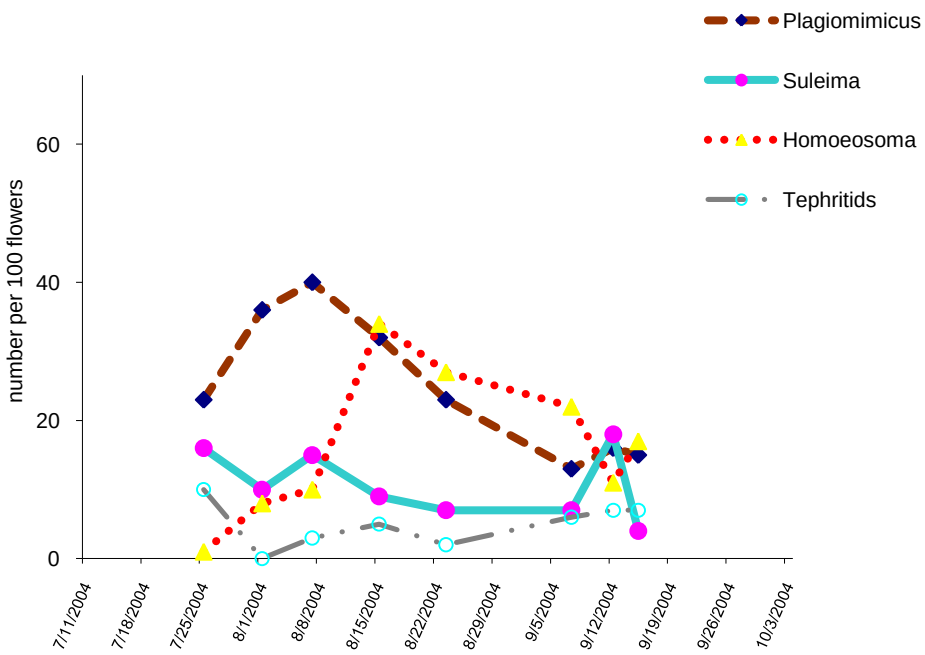


Figure 8. Herbivore abundance during 2005 florivore survey at Stone Lakes site 1

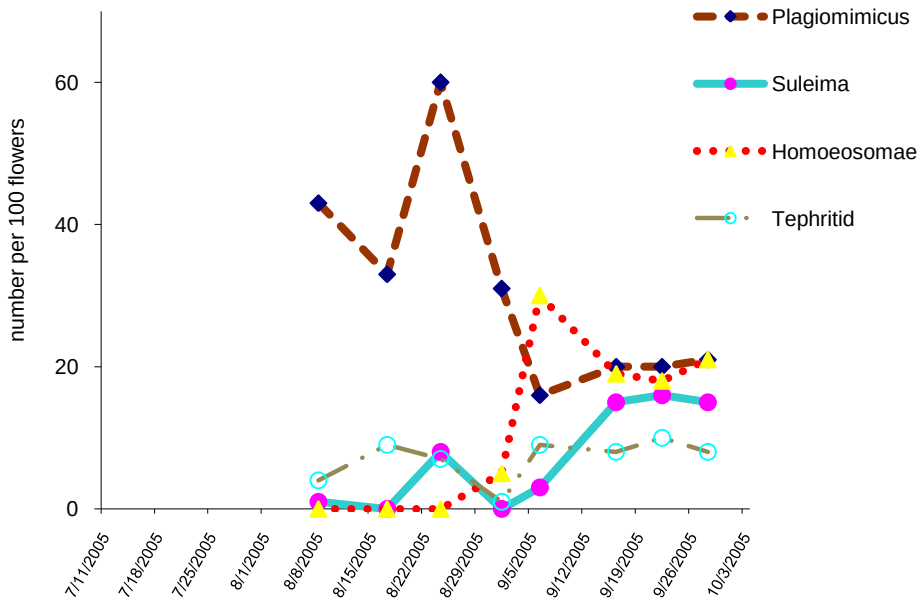


Figure 9. Herbivore abundance during 2006 florivore survey at Stone Lakes site 1

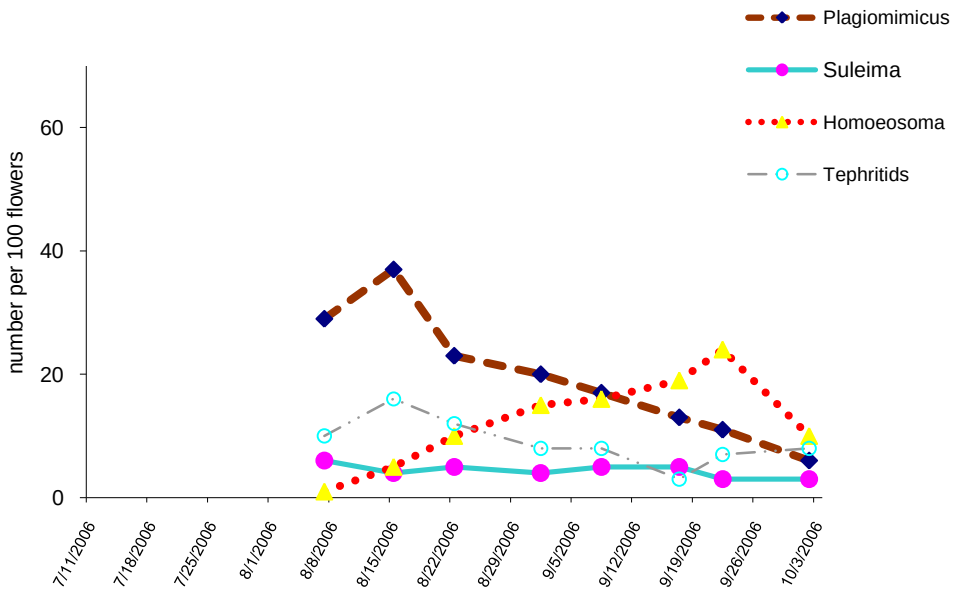


Figure 10. Herbivore abundance during 2004 florivore survey at Stone Lakes site 2

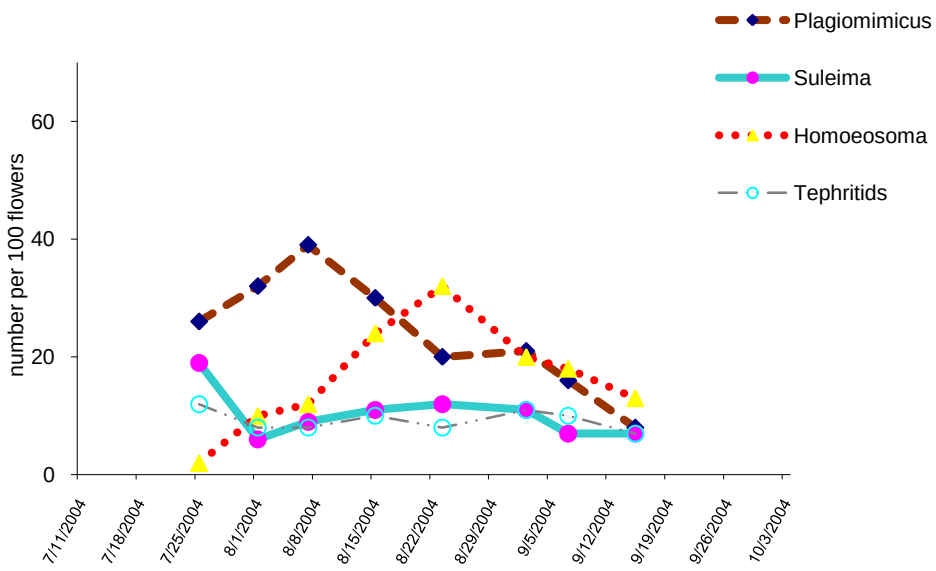


Figure 11. Herbivore abundance during 2005 florivore survey at Stone Lakes site 2

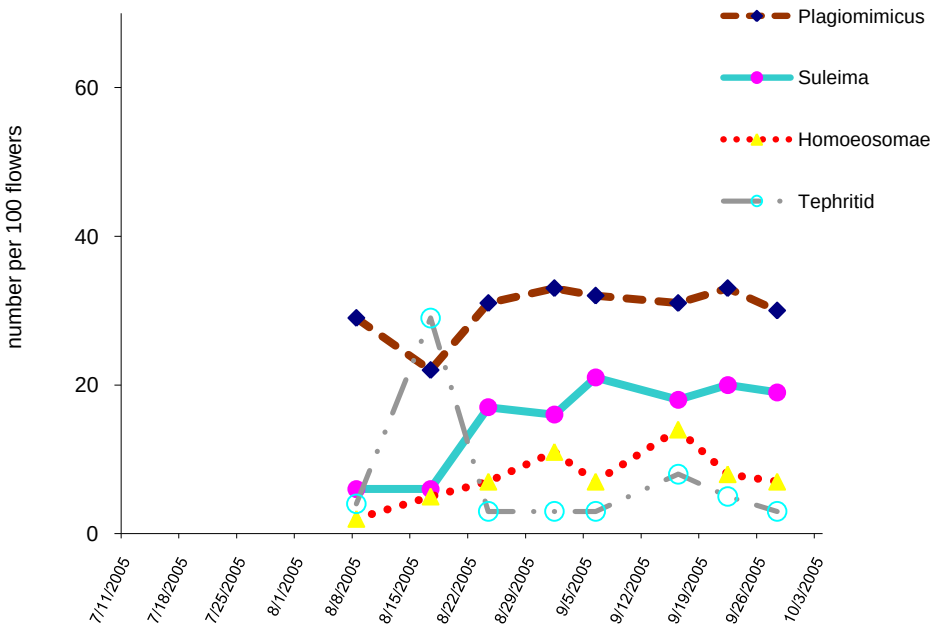


Figure 12. Herbivore abundance during 2006 florivore survey at Stone Lakes site 2

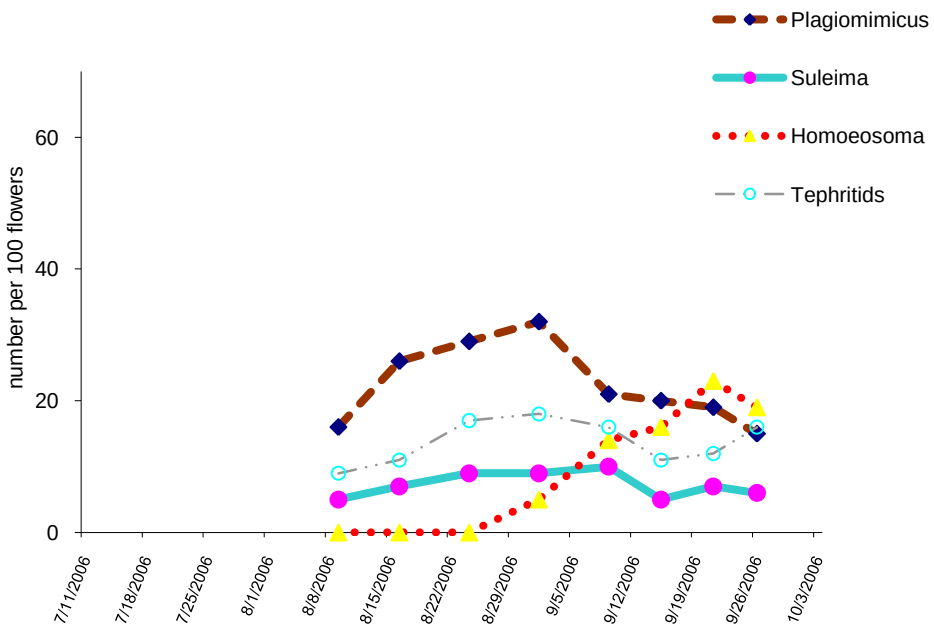


Figure 13. Herbivore abundance during 2004 florivore survey at Cosumnes site

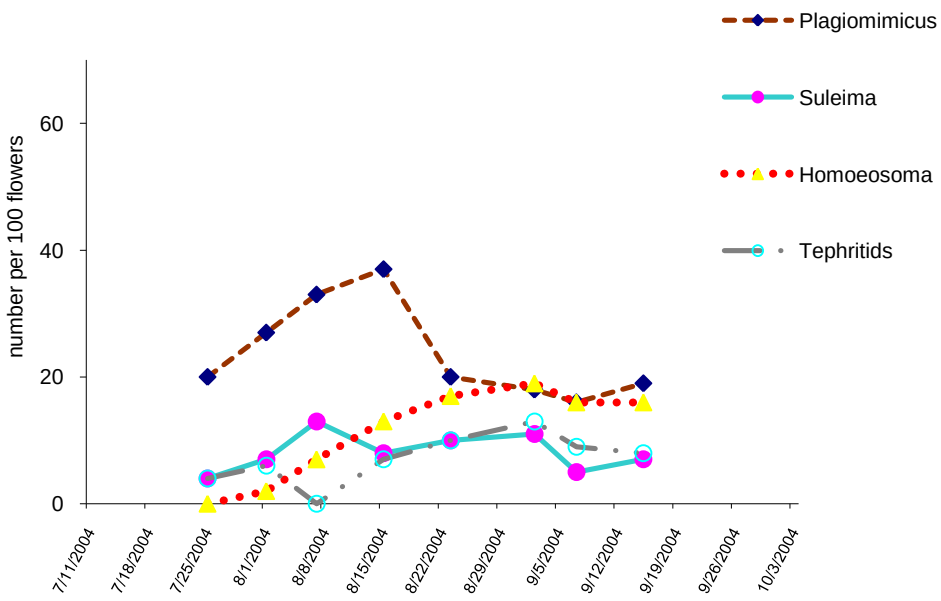


Figure 14. Herbivore abundance during 2005 florivore survey at Cosumnes site

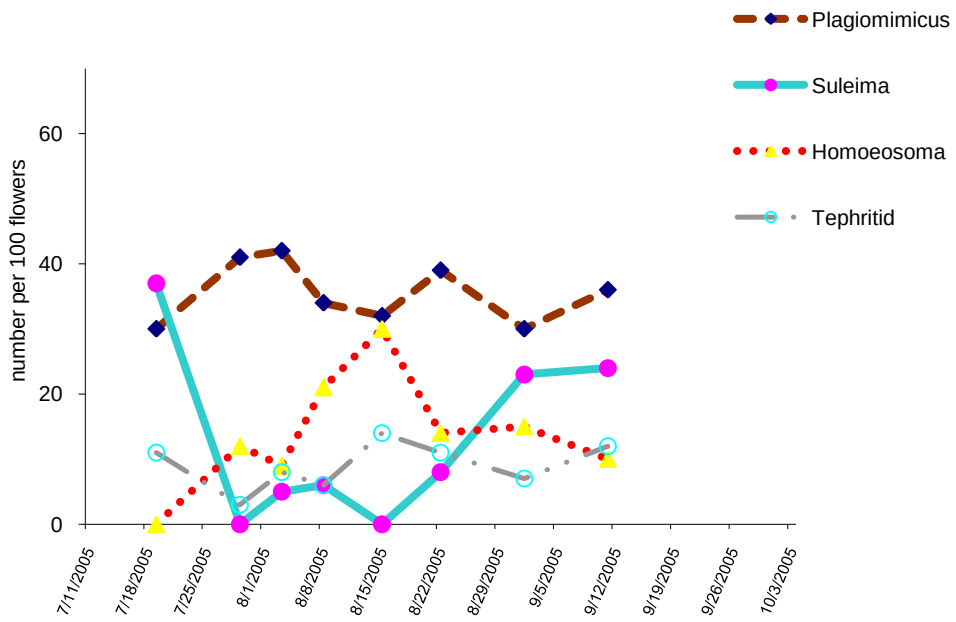


Figure 15. Herbivore abundance during 2006 florivore survey at Cosumnes site

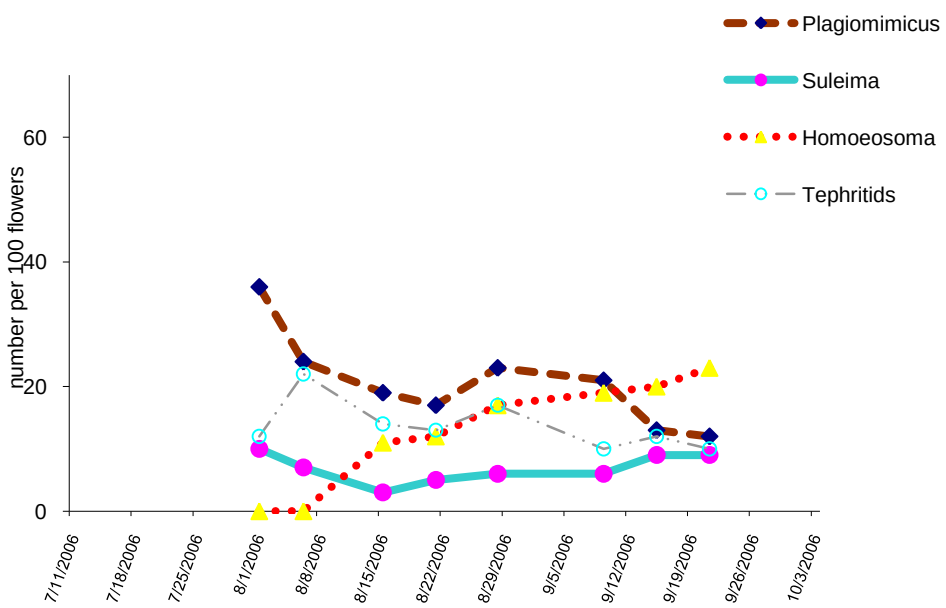


Figure 16. Average number of flowers out of 100 surveyed containing each of the 5 florivores and each possible co-occurrence combination across survey sites and years (total of 120 surveys)

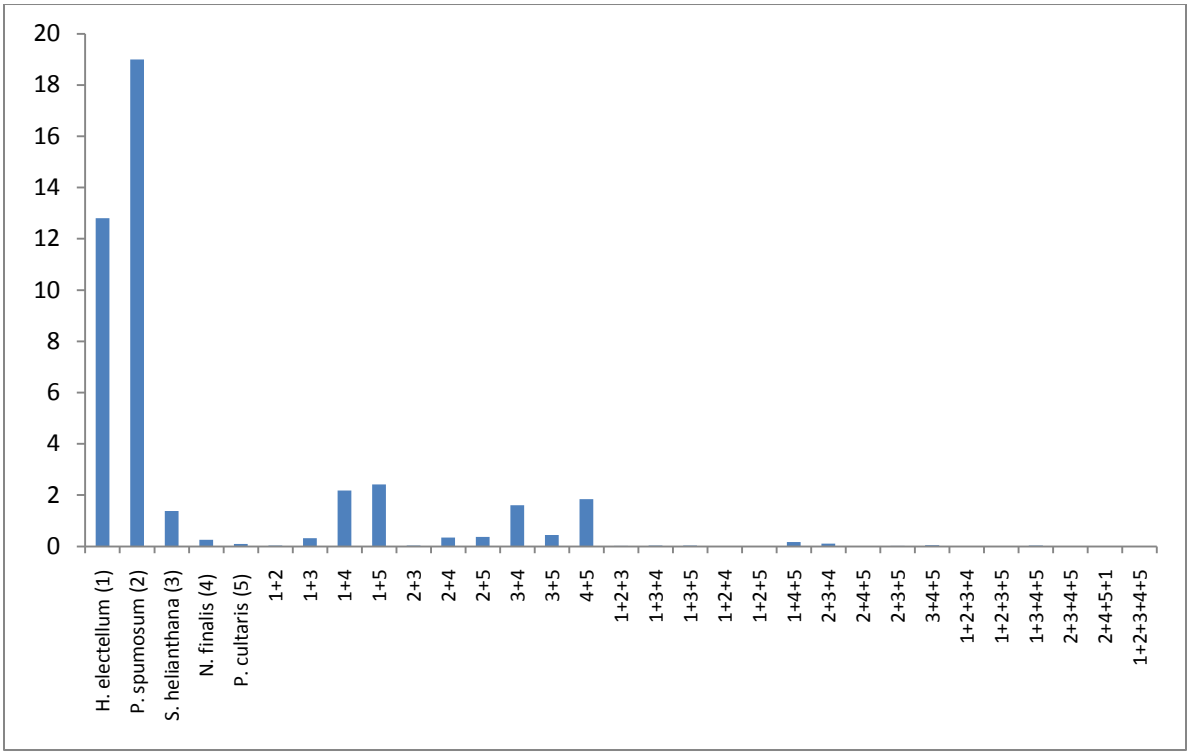


Figure 17. Comparison of total number of each species of florivore reared in the laboratory and the percent parasitism calculated using this number

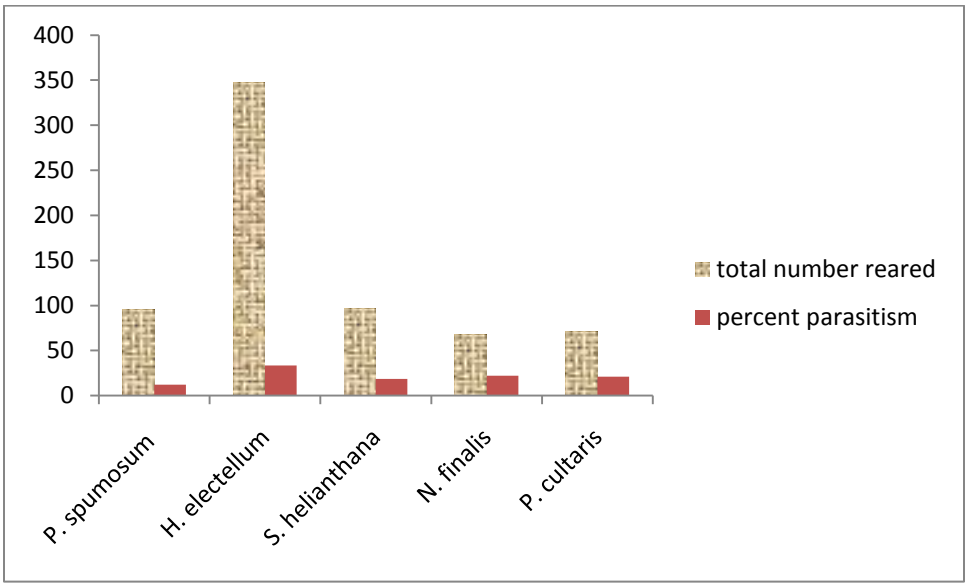
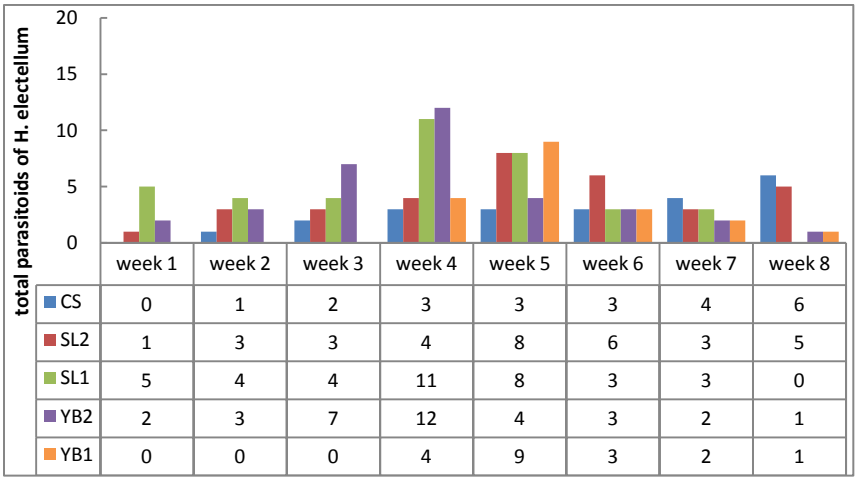


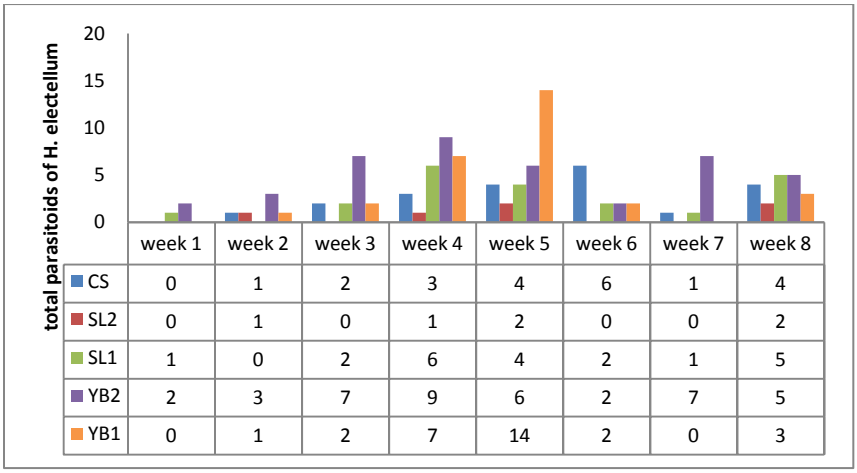
Figure 18. Number of *H. electellum* parasitoids reared each week during survey from each site

2004



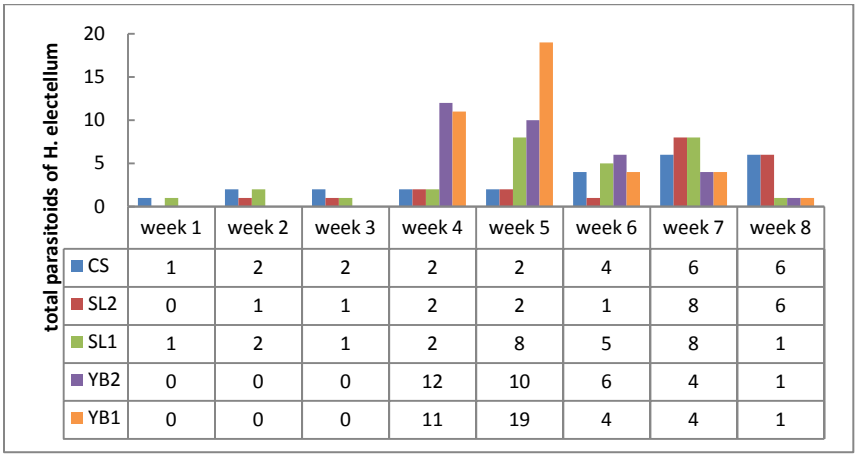
	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
CS	0	1	2	3	3	3	4	6
SL2	1	3	3	4	8	6	3	5
SL1	5	4	4	11	8	3	3	0
YB2	2	3	7	12	4	3	2	1
YB1	0	0	0	4	9	3	2	1

2005



	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
CS	0	1	2	3	4	6	1	4
SL2	0	1	0	1	2	0	0	2
SL1	1	0	2	6	4	2	1	5
YB2	2	3	7	9	6	2	7	5
YB1	0	1	2	7	14	2	0	3

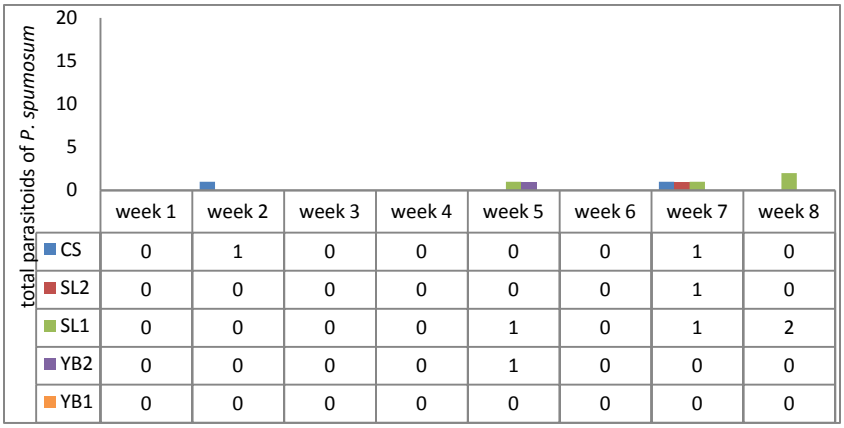
2006



	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
CS	1	2	2	2	2	4	6	6
SL2	0	1	1	2	2	1	8	6
SL1	1	2	1	2	8	5	8	1
YB2	0	0	0	12	10	6	4	1
YB1	0	0	0	11	19	4	4	1

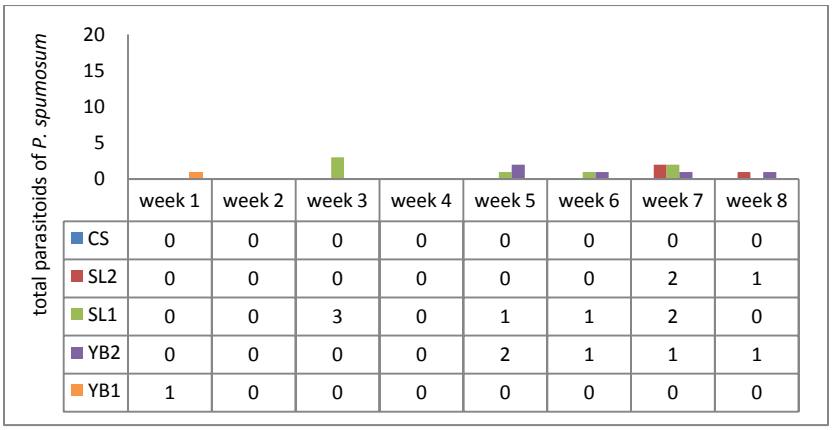
Figure 19. Number of *P. spumosum* parasitoids reared each week during survey from each site

2004



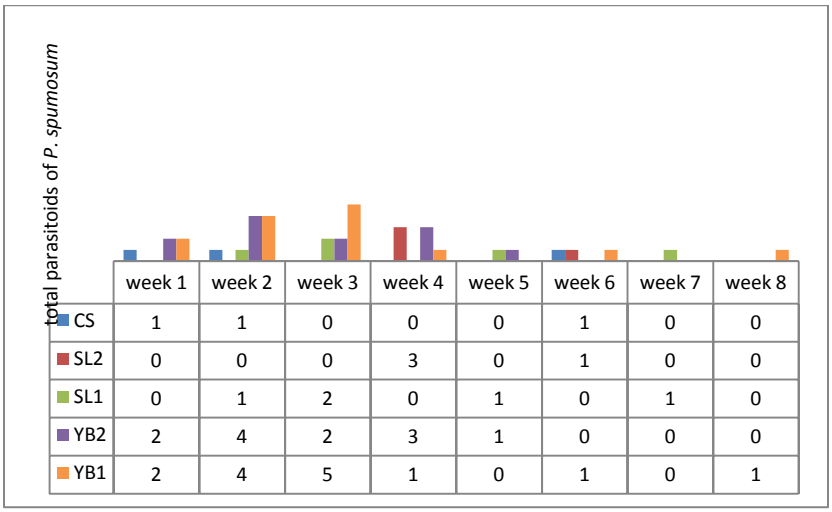
	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
CS	0	1	0	0	0	0	1	0
SL2	0	0	0	0	0	0	1	0
SL1	0	0	0	0	1	0	1	2
YB2	0	0	0	0	1	0	0	0
YB1	0	0	0	0	0	0	0	0

2005



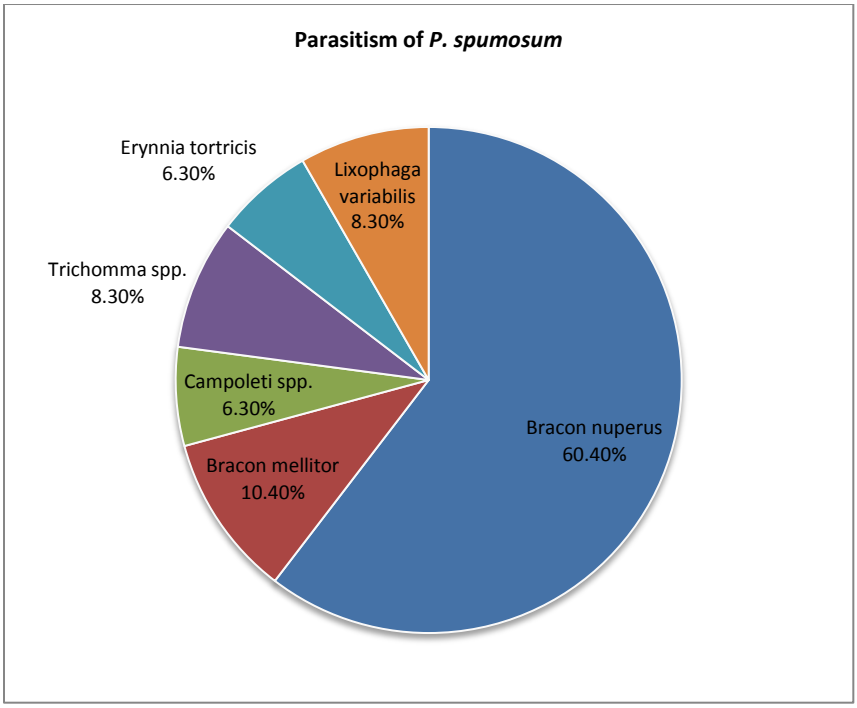
	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
CS	0	0	0	0	0	0	0	0
SL2	0	0	0	0	0	0	2	1
SL1	0	0	3	0	1	1	2	0
YB2	0	0	0	0	2	1	1	1
YB1	1	0	0	0	0	0	0	0

2006



	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
CS	1	1	0	0	0	1	0	0
SL2	0	0	0	3	0	1	0	0
SL1	0	1	2	0	1	0	1	0
YB2	2	4	2	3	1	0	0	0
YB1	2	4	5	1	0	1	0	1

Figure 20. Comparison of contribution to overall parasitism by each parasitoid species for *P. spumosum* and *H. electellum*



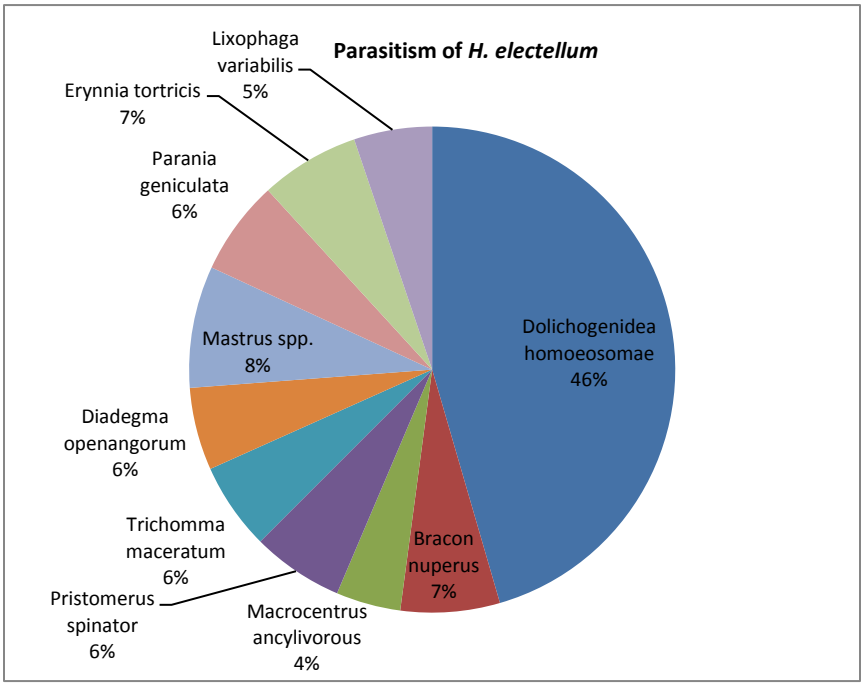


Table 1. Common florivores of self seeding sunflower in the Sacramento Valley of California with feeding niche and natural history notes

Seed Herbivore Species	**Order: Family**	**Feeding niche**	**Generations observed during summer**	**notes**
Plagiomimicus spumosum Grote	Lepitoptera: Noctuidae	Larvae feed on developing achenes and hollow out a space under the seeds. First instar found under the petals of unopened buds, later instars tunnel through the back of the flower head and hollow out a space in the receptacle (Charlet 1987).	At least two (Pilson, unpublished data)	Intraspecific predation observed in the field and the lab: several larvae enter the flower bud but only one survives to pupation. Damage to the flower extensive, often more than 50% of seeds destroyed (Charlet 1987)
Homoeosoma electellum Hulst	Lepidoptera: Pyralidae	Early instars feed on pollen, later instars feed and tunnel within developing achenes (Charlet 1987).	3-4 (Chen and Welter 2003; Charlet 1997)	Larvae are very mobile and move between flowers and plants, often leaving silk trails and coating on the flower faces. This is the most important herbivore pest of crop sunflower in this area (Chen and Welter 2003).
Suleima helianthana Riley	Lepidoptera: Tortricidae	Larvae develop in and consume tissues within the stems and buds (Knodel et al 2010).	At least 2 reported in the Central States (Knodel et al. 2009)	Stem and bud boring larvae cause malformations, loss of the leader stem and multiple side branching; damaged buds do not flower. Considered a pest in the Northern Plains (Knodel et al. 2009)
Neotephritis finalis Loew	Diptera: Tephritidae	Larvae feed within undeveloped ovaries, often completely consuming the floret before the seed is fertilized (Knodel et al 2010).	2-3 (multivoltine in Souther California per Goeden et al 1987)	Several larvae can occur together in one flower head, but usually no more than 3 or 4 pupate within one flower. The species has been recorded from over 20 host plants in the Asteraceae (Goeden et al., 1987).
Paracantha cultaris Coquillet	Diptera: Tephritidae	Larvae feed on immature florets and the receptacle, pupate in a space excavated between seeds (Knodel at al 2010).	2-3 (Knodel et al. 2010)	Cavender and Goeden (1984) state that each larvae destroyed an average of 34% disk florets in wild sunflowers studied Southern California.

Table 2. Florivore survey wild sunflower sites

Site	Dates sampled	Patch size	Seasonally flooded	Location coordinates	Average number of flowers in a 100 flower survey damaged by at least one florivore
Yolo Bypass Wildlife Area#1	Jul. 25 to Sept. 19, 2004; Jul.29 to Sept.17, 2005; July.14 to Sep 09, 2006	3.2 km^2	Yes	38°29'51.75"N 121°36'6.98"W	48.3 (S.D. = 6.1)
Yolo Bypass Wildlife Area#2	Jul. 25 to Sept. 17, 2004; Jul. 29 to Sept 17, 2005; July 4 to Sep 3, 2006	2.7 km^2	Yes	38°25'28.53"N 121°38'2.78"W	56.4 (S.D. = 4.4)
Stone Lakes Wildlife Area#1	Jul. 27 to Sept. 14, 2004; Aug. 8 to Sept. 28, 2005; Aug. 1 to Oct. 1, 2006	2.4 km^2	Yes	38°22'41.27"N 121°29'14.39"W	54.3 (S.D. = 3.1)
Stone Lakes Wildlife Area#2	Jul. 26 to Sept. 14, 2004; Aug. 8 to Sept. 28, 2005; Aug. 4 to Oct. 1; 2006	2.3 km^2	No	38°20'25.94"N 121°29'49.53"W	37.2 (S.D. = 1.5)
Cosumnes River Preserve	Jul. 25 to Sept. 17, 2004; Jul. 19 to Sept. 17, 2005; Jul. 12 to Sept. 14, 2006	2.4 km^2	No	38°23'19.46"N 121°19'17.01"W	29.1 (S.D. =2.1)

Table 3. Florivore species laboratory rearing results

Species	**Total collected from flowers surveyed and placed in diet cups in the laboratory**	**Total dead as larvae in the lab/ *death rate***	**Total reared to next life stage***	**Total parasitoids emerged**	**Percent parasitism**
H. ellectellum	1, 680	301/ *17.9%*	1,032	347	33.6%
P. spumosum	3,111	2,231/ *71.7%*	784	96	12.2%
S. helianthana	928	310/ *33.4%*	521	97	18.6%
N. finalis	417	112/ *26.8%*	305	68	22.2%
P. cultaris	508	98/ *19.3%*	339	71	20.9%

**H. electellum, S. helianthana* and the two tephritid species were reared to adulthood, but *P. spumosum* never emerged from the pupal stage in the laboratory, so we considered this the end of the life cycle in the study

Table 4. Two by two species association table for *P. spumosum* and *H. electellum* in the 12,000 flowers surveyed.

		H. electellum		
		Present	Absent	
P. spumosum	Present	50	2,652	m= 2,702
	Absent	1,432	7,866	n= 9,298
		r= 1,482	s= 10,518	N= 12000

X^2 (with Yates correction) = 353.89; $p<0.0001$

Table 5. Florivore survey pooled data (from all five sites during the three years)

Species	**Average number found per 100 flower survey (based upon 120 total surveys of 100 flowers)**	**Standard deviation**	**Minimum number of individuals found**	**Maximum number of individuals found**
H. electellum	13.5	1.4	0	41
P. spumosum	25.7	2.3	6	56
S. helianthana	7.6	1.2	0	37
N. finalis	4.1	2.2	0	14
P. cultaris	4.4	2.3	0	16

Table 6. Intraspecific rates of co-occurrence within one flower head in the full survey

Florivore species	**Total number collected across survey sites and years**	**% of individuals found in flower with no conspecifics**	**% of individuals found with one other conspecific**	**% of individuals found with two other conspecifics**	**% of individuals found with three or more other conspecifics**
S. spumosum	3111	93.1%	6.9%	0%	0%
H. electellum	1680	46.0%	37.0%	9.4%	7.7%
S. helianthana	928	39.8%	50.9%	9.4%	0%
N. finalis	417	4.3%	35.0%	23.3%	37.4%
P. cultaris	508	6.3%	25.2%	18.5%	50%

Table 7. Parasitism parameters found in florivore survey across all sites and years

Sunflower florivore	Average Total % parasitism per survey N=120 surveys *n= total parasitism events recorded for the species*	Parasitoid species (% of total parasitism)		Total parasitoid species richness
P. spumosum	12.2% (S.D. = 4.2) *n = 96*	Hymenoptera: Braconidae	*Bracon nuperus* (60.4%) *Bracon mellitor* (10.4%)	6
		Hymenoptera: Ichneumonidae	*Campoletis spp.* (6.3%) *Trichomma spp.* (8.3%)	
		Diptera: Tachinidae	*Erynnia tortricis* (6.3%) *Lixophaga variabilis* (8.3%)	
H. electellum	33.6% (S.D. = 5.1) *n = 347*	Hymenoptera: Braconidae	*Dolichogenidea homoeosomae* (45.5%) *Bracon nuperus* (6.6%) *Macrocentrus ancylivorous* (4.3%)	10
		Hymenoptera: Ichneumonidae	*Pristomerus spinator* (6.1%) *Trichomma maceratum* (5.8%) *Diadegma openangorum* (5.5%) *Mastrus sp.* (8.1%) *Parania geniculata* (6.3%)	
		Diptera: Tachinidae	*Erynnia tortricis* *(6.6%)* *Lixophaga variabilis* *(5.2%)*	
S. helianthana	18.6% (S.D. = 3.2) *n = 97*	Hymenoptera: Braconidae	*Apanteles sp.* *(37.1%)* *Bracon caulicola* (17.5%)	3
		Hymenoptera: Ichneumonidae	*Pristomerus spinator* *(44.3%)*	
N. finalis	22.2% (S.D. = 4.2) *n = 68*	Hymenoptera: Pteromalidae	*Pteromalus sp.* (72.1%)	2
		Hymenoptera: Chalcidae	*Dirhinus sp.* *(26.4%)*	
P. cultaris	20.9% (S.D. = 3.6) *n = 71*	Hymenoptera: Pteromalidae	*Pteromalus sp.* (78.9%)	2
		Hymenoptera: Braconidae	*Diacasmimorpha* sp. (19.7%)	

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