

ECOLOGICAL MANAGEMENT OF INSECT PESTS USING COVER CROPS  
IN FIELD CROPS AND VEGETABLES

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## ABSTRACT

This dissertation researched diversified cropping systems created through habitat management techniques, exploring whether these habitats would alter pests' behavior and/or enhance beneficial insects, and contribute to suppress pest and disease complexes in corn production systems.

An exploratory study through small-scale field experiments determined sunn hemp (*Crotalaria juncea*) as the potentially the most suitable species for further study. In the preliminary studies, sunn hemp intercropping reduced incidence of hopperburn and *Maize mosaic virus* (MMV) symptomatic plants caused by *Peregrinus maidis* feeding, and increased parasitism of *Helicoverpa zea* eggs by *Trichogramma* spp. on the corn silks. The reduced incidence of hopperburn and MMV was attributed to an increase in *P. maidis* within-field activity, resulting in reduced initial colonization on corn plants. The results showed a suitable intercrop might be useful for management of persistent viruses, which are usually considered unmanageable by habitat management to cropping systems.

On the basis of these preliminary studies, experiments were conducted in large-scale field with a higher corn- to sunn hemp intercropping ratio to validate results and possibly contribute new pest management options for large-scale corn production systems. Increase in within-field *P. maidis* activity with resulting in lower incidence of MMV symptomatic plants in the sunn hemp-intercropped treatments were consistently similar to results that were obtained from small-scale field experiments. This strategy may contribute an important component of integrated pest management for reducing spread of persistently transmitted viruses in large-scale corn production systems.

Greater parasitism of *H. zea* eggs by *Trichogramma* spp. in sunn hemp-intercropped treatments was consistently similar to that obtained from small-scale field experiments. These results suggested growing strips or patches of suitable cover crop may help in sustaining the populations of beneficial insects at the time of pest outbreaks. Augmentative biological control (releases of *Trichogramma pretiosum* in corn monoculture) resulted in a greater parasitism of *H. zea* eggs, and increased ear yield compared to habitat management (sunn hemp-intercropped). This result suggested *H. zea* management is important component to achieve economic yield and augmentative biological control is a more effective tool than the habitat management in cornfields.

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## **GENERAL INTRODUCTION AND DISSERTATION STRUCTURE**

### **Ecological management of pests**

Ecological management of insect pests is based on a thorough understanding of pest ecology as it relates to the crop in production (Pedigo and Rice 2006). In general, pests have ecological requirements such as food resources, and shelter from adverse weather and natural enemies in order to sustain populations in agro-ecosystems. Pest outbreaks occur when these basic requisites are all accessible within the cropping area or supplemented from source nearby, as is often the case with monoculture farming. The idea behind ecological management is to find “weak links” in the insects’ seasonal- or life cycle and exploit them (Pedigo and Rice 2006). These weak links may be found within behaviors executed to complete development, limited availability of food sources, and exposure to activity of natural enemies. Agro-ecosystems which increase the exposure of weak links in pest biology can be created in several ways, diverse cropping systems achieved through cultural management is one of them.

### **Crop diversification**

Combining different species of plants in spatial or temporal arrangements creates diverse agricultural systems, which are potentially important components of ecological management of pests, an area of research that deserves attention. Diverse cropping systems provide environmentally benign and sustainable pest management options that

can reduce pesticide dependence in agricultural systems. Cover cropping, the process of growing a non-harvested crop to reduce erosion, suppress weeds etc., provides appropriate opportunities to study effects of diverse cropping systems on insect pests and their natural enemies. The increased diversity created by cover cropping, contributes to suppress herbivores either by enhancing natural enemies (Andow 1991, Altieri 1999, Hooks and Johnson 2003) or creating a physical environment that may alter pest behavior (Finch and Collier 2000).

### **General hypotheses**

The philosophy of pest management in diverse crop habitats is based on the resource concentration and natural enemy hypotheses (Root 1973). Root's hypotheses propose that monoculture systems will support higher pest numbers owing to the readily available resources, and that natural enemies are less effective for various reasons in monoculture systems. Various other mechanisms responsible for lower incidence of herbivores in diversified habitats had been elucidated with specific habitat and pest combinations. These mechanisms include crop background, physical obstruction, visual camouflage, host odor masking, and attraction or repellency (Finch and Collier 2000 and references therein). However, some general theory has been developed, based on detailed observations of insect behavior on host plant finding. The "appropriate and inappropriate landing" theory suggests host plant selection involves a three-linked chain of events in which the first link is governed by volatile plant chemicals (host plant finding), the central link by visual stimuli (landing on host plant) and the final link by cues from non-

volatile plant chemicals (host plant acceptance) (Finch and Collier 2000). This is why insects, flying over plots of plant mixtures will likely have several inappropriate landings on non-host plants before finally land on the host plant.

### **The study systems**

Strip-tilled cover cropping systems: This dissertation introduces a new approach, the strip-tilled cover cropping system, where a cucurbit crop is grown in alternate strip-tilled rows of cover crop. This system provides diverse above- and below-ground habitats and hence may result in regulating weeds, insect pests, beneficial insects, and fossorial or subterranean (e.g. plant parasitic nematodes) pests. This dissertation will examine effects of strip-tilled cover cropping on above-ground insect pests and beneficial insects in a cucurbit production system.

Cover crop intercropping systems: Cover crops are used as intercrops with an adequate inter-row space between cash crop and cover crop rows to reduce interspecific competition. The cash crop, corn in this dissertation, is grown in between cover crop rows by optimizing intercropping densities. This system provides diverse above-ground habitats, and may regulate herbivores either by creating a physical environment that alters pest insect behavior or by enhancing beneficial insects.

## **Target Pests**

Among the pests of cucurbits, a mixed species assemblage of thrips, primarily western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae) is studied in this dissertation. The importance of these insects as pests is for the result of their feeding damage on immature fruits which generates silvery or streak-like scars accompanied by fruit malformation (Rosenheim et al. 1990, Welter et al. 1990), resulting in downgrading of fruits at harvest.

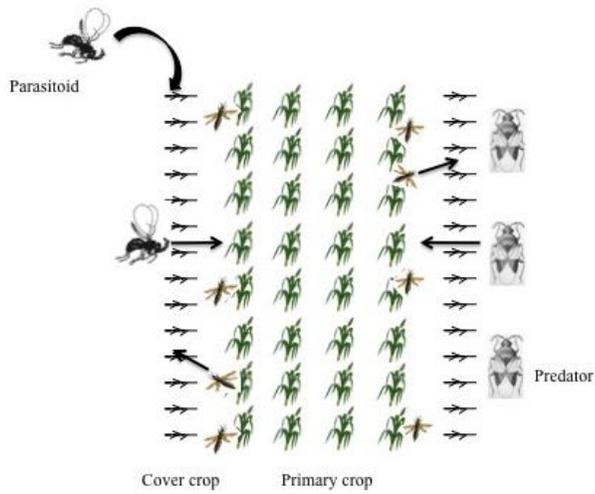
Pests and diseases are the most important production constraints in the corn production in Hawaii (Brewbaker 2003). Among them, corn planthopper, *Peregrinus maidis* Ashmead (Hemiptera: Delphacidae), corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), western flower thrips, *F. occidentalis* and corn thrips, *F. williamsi* (Thysanoptera: Thripidae) are the most important insect pests. Feeding activities of *P. maidis* and *F. williamsi* on corn plants result in transmission of *Maize mosaic virus* (MMV) and *Maize chlorotic mottle virus* (MCMV), respectively. *H. zea* feeding on kernels within corn ears results in destruction of seeds, and along with *F. occidentalis* provides access to ear rot fungi, *Fusarium verticillioides* (Sacch.) through the feeding tunnels produced.

## **Mechanisms whereby the study system may suppress pest and disease complexes**

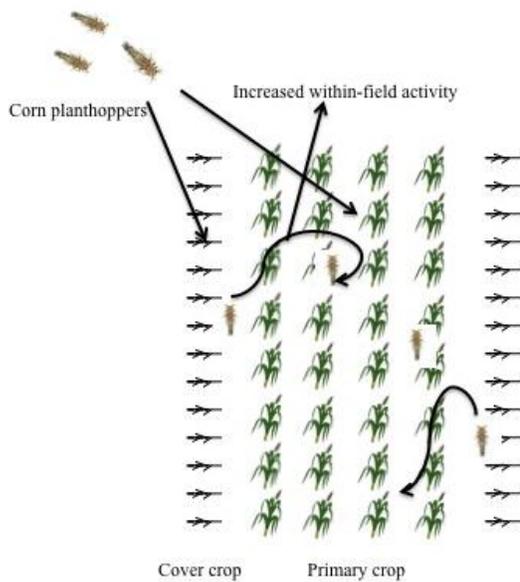
On the basis of hypotheses described above and considering previous work by various authors, the mechanisms which may suppress pest and disease complexes in the

proposed study systems, are listed below. These potential mechanisms formed the basis for the formulation of hypotheses tested in this study.

1. Diversity in cropping systems (e.g. intercropping) provides resources such as food (e.g. nectar, pollen etc.) for adult natural enemies (e.g. parasitoid and predators), alternative prey and hosts, and shelter from adverse conditions that result in enhanced activity of natural enemies and pest suppression (Landis et al. 2000, Jonsson et al. 2008) (Figure 1.1).
2. Intercrops with attractive flower colors (e.g. cover crops) attract populations of insects to cover crops and result in lower pest densities on the primary crops (Teulon et al. 1999, Blumthai et al. 2005, Buitenhuis et al. 2007) (Figure 1.1).
3. Diversity in cropping systems alters movement behavior of vector insects, resulting in reduced tenure time on the crop plants that ultimately results in reduced incidence of persistence viruses (Power 1987, 1988 and 1991) (Figure 1.2).
4. A typical dispersion pattern of insects has been found in crop-field through change in their movement behavior (dispersal or migration) as influence by adjacent crop or non-crop habitats. (Decante et al. 2009, Bressan et al. 2010).
5. Indundative releases of *Trichogramma* spp. in a monocrop (e.g. corn monoculture) at the susceptible stage of pest, such as *Helicoverpa* egg stage, results in greater parasitism of *Helicoverpa* eggs (Smith 1996).



**Figure 1.1:** A schematic diagram showing mechanisms that may reduce pest density on the primary crop in intercropping systems either by (i) providing sources of generalist predators and parasitoids or (ii) attracting populations of insects to the intercrop.



**Figure 1.2:** A schematic diagram showing mechanisms that may reduce disease incidence on the primary crop through change in movement behavior of vector insect as interfered by intercropping.

### **Aims of this dissertation**

The overall aims of this dissertation are to examine the impacts habitat management in the form of cover crops on pest- and beneficial insect populations, and plant pathogenic viruses vectored by the study insects. To this end, the following broad topics will be addressed:

- 1) Quantify effects of strip-tilled cover cropping on densities of thrips on cucurbit plants,
- 2) Identify a potential pest suppressive cover crop and examine its effect on multiple pest and disease complexes of corn using a corn - cover crop intercropping system, and

3) Compare habitat management techniques with augmentative biological control for their ability to manage corn earworm populations in sweet corn.

### **Outline of the dissertation**

This dissertation is set out in six chapters, each one with specific objectives, and providing foundational information for subsequent chapters.

In Chapter I, the strip-tilled cover cropping system, which is designed for multiple pest suppression (nematodes, weeds) and soil health management as part of a larger project, examines its effect on densities of mixed species of thrips on cucurbit crops.

In subsequent chapters, corn - cover crop intercropping systems are explored to optimize intercropping densities with the aim of minimizing inter-specific competition between corn and cover crops, yet providing modified pest behaviors and enhancing natural enemy densities. In Chapter II, three cover crops (buckwheat, cowpea, and sunn hemp) are examined for their effects on within-field activity of *P. maidis* and their populations on corn plants. Further, changes in *P. maidis* activity and populations on corn plants as influenced by intercropping cover crops is explored on incidence of hopperburn and MMV on corn plants. The results of Chapter II were used to select a suitable intercropping plant for further studies, reported in chapters IV and V.

Also in an exploratory manner, Chapter III considers the effects of intercropping cover crops on parasitism and predation of *H. zea* eggs and predation of thrips, and examined to explore how natural enemies benefit from floral resources in these systems.

In each cropping system, parasitism *H. zea* eggs by *Trichogramma* spp. is quantified, and relationship between predators (*Orius* spp.) and prey (*H. zea* eggs and thrips) is examined.

Prompted by the results of Chapters II and III, the potentiality of sunn hemp intercropping is examined for effects on within-field activity of *P. maidis* that may associated with reduced severity of hopperburn and MMV symptoms (Chapter IV and V), and parasitism of *H. zea* eggs and predator–prey relationships (Chapter VI). In addition, habitat management through intercropping corn with sunn hemp is compared with inundative release of *T. pretiosum* in corn monoculture for parasitism of *H. zea* eggs (Chapter VI). These studies were conducted in large-scale field experiments with a higher corn- to sunn hemp intercropping ratio to validate results from Chapter II and III and possibly contribute new options for pest management for large-scale corn production systems.

In chapter IV, spatially explicit *P. maidis* counts and ratings for severity of hopperburn and MMV symptoms were measured on corn plants in each treatment (monoculture and intercropped with sunn hemp). In chapter V, incidence of MMV and MCMV in relation to within-field activity of their vector insects in each cropping system (corn monoculture and intercropped with sunn hemp) is examined. Incidence of viruses on their mode of transmission in each cropping system is discussed.

In chapter VI, habitat management through intercropping sunn hemp with corn as a potential means of increasing *H. zea* egg parasitoids, is compared with inundative releases of *T. pretiosum* in corn monoculture for their ability to suppress *H. zea*.

Furthermore, effect of intercropping sunn hemp on predation of *H. zea* eggs and thrips is compared with corn monoculture. The summary of findings, and contributions of this dissertation to pest management options in cucurbit and corn production systems are summarized in the General Conclusion Chapter.

## CHAPTER I

### **Effects of strip-tilled cover cropping on population density of thrips (Thysanoptera: Thripidae) on cucurbit crops**

#### **Abstract**

Field experiments were conducted to examine effects of strip-tilled cover cropping on population densities of thrips (primarily, *Frankliniella occidentalis* and *Thrips palmi*) and an arthropod predator (*Orius* spp.) on cucurbit crops on Oahu, Hawaii in spring and fall, 2008. Two cover crops, sunn hemp, *Crotalaria juncea* L. and French marigold, *Tagetes patula* were used, and cucurbit cash crops (either bitter melon, *Momordica charantia* or cucumber, *Cucumis sativus*) were planted in strip-tilled rows of cover crops creating diverse above-ground habitats. Results showed that strip-tilled cover cropping systems effectively reduced densities of mixed species of thrips on cucurbits compared to monocultures. The level of protection against thrips was greater in marigold- compared to sunn hemp strip-tilled treatments. No significant effect was observed on abundance of *Orius* spp. Plant competition between cucurbits and cover crops was evident, with impacts on rate of crop growth and fruit yield in the strip-tilled cover cropping systems. In the fall 2008 experiment, although strip-tilled cover cropping significantly reduced cucumber fruit yield through competition, their thrips suppressive effect resulted in reduced thrips damage fruits compared to monoculture. Possible mechanisms responsible for reduced thrips density in strip-tilled cover cropping systems either by providing sources of natural enemies or thrips attraction to the cover crop flowers are discussed.

## Introduction

Societal demand for environmentally friendly crop production has increased the demand for using pest suppressive cover crops or diversified intercropped approaches in cropping systems (Costello 1994, Costello and Altieri 1995, Hooks et al. 1998). However, most studies aimed at examining the benefits of cover crops have examined the effect on weeds, nematodes or insect pests separately (McSorley and Gallaher 1992, Hooks and Johnson 2002, Ngouajio et al. 2003). Additionally, cover-cropping technology used to suppress one pest may have a positive impact on other pests in the system, resulting in increased pest pressure. Populations of plant parasitic nematodes were reduced by incorporation of cover crops as organic matter (Wang et al. 2001), pre-planting an allelopathic cover crop (Ploeg 2000), or trapping pests (Gardener and Caswell-Chen 1994). Suppression of weeds by cover crops is mainly through allelopathy (Hutchinson and McGiffen, 2000) or establishment of surface mulch that physically suppresses weed growth (Ngouajio et al. 2003). On the other hand, populations of insect pests may be reduced when the cash crop is intercropped with leguminous cover crops such as strawberry clover, white clover and sunn hemp (Costello and Altieri 1995, Hooks et al. 1998, Hooks and Johnson 2002; 2004, Manandhar et al. 2009, Manandhar and Hooks 2011). Thus, an integrated approach is required in using cover-cropping technology to target multiple pests in the cropping systems.

To achieve suppression of multiple pests in a cover cropping system, a suitable cover crop must be chosen. Two cover crops, sunn hemp and marigold have been shown to suppress nematodes (Wang et al. 2002, Hooks et al. 2010) and may be useful in a multiple-pest guild (insect, plant parasitic nematodes and weeds) suppression system.

Sunn hemp when inter-planted with zucchini, effectively reduced densities of *Bemisia argentifolii*, which reduced severity of silverleaf disorder on zucchini plants (Manandhar et al. 2009). Sunn hemp can also be used as a virus-sink to minimize aphid-transmitted non-persistent viruses in zucchini (Manandhar and Hooks 2011). Although the effects of marigold on insect pests is not known, it is known to produce a volatile essential oil (Ogunwande and Olawore 2006, Babu and Kaul 2007) that may alter pests' olfactory responses. Plants with unique odors as a result of volatile essential oils have been used to suppress pests either by spraying extracts, or intercropping for their repellent or host-odor masking action (van Tol et al. 2006, Tongi et al. 2010). Therefore, sunn hemp and marigold cover crops could be used in a pest management package targeting multiple-pest guild in the system.

This chapter introduces a new approach of cover cropping as a strip-tilled cover cropping system, where a cash crop is grown in alternate strip-tilled rows of cover crop, resulting in an intercropped habitat. This system will provide diverse above- and below-ground habitats and hence may influence populations of above-ground (weeds, insect pests, beneficial insects), and below-ground (plant parasitic nematodes) organisms. As a part of a larger study targeting this multiple-pest guild, the strip-tilled cover crops have (i) generally suppressed plant parasitic nematodes, (ii) consistently increased the numbers of free living nematodes (bacterivores and fungivores) and soil mesoarthropods (collembolans, beneficial mites, isopods and amphipods), (iii) enhanced soil food web structure, an indicator of soil health, and (iv) suppressed broad leaf weeds during initial period of the first cropping cycle, in cucurbit cropping systems (Marahatta et al. 2010, Wang et al. 2011). These benefits improved cucurbit plant growth and produced higher

marketable fruit yield at the second cropping cycle (Marahatta et al. 2010, Wang et al. 2011).

Usually a mixed assemblage comprising various species of thrips has been reported on cucurbits. In cucumber, western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is most abundant, and melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae), the next most common in cucumber (Rosenheim et al. 1990, Welter et al. 1990). The densities of western flower thrips were found to be higher in flowers whereas densities of melon thrips were higher on leaves of cucumber plants (Rosenheim et al. 1990). Two types of damage were associated with these mixed species of thrips infestation: indirect damage is caused by feeding on foliage and stem, and direct damage results from feeding on developing fruits. Thrips feeding on immature fruits generate silvery or streak-like scars, which may be accompanied by fruit malformation (Rosenheim et al. 1990, Welter et al. 1990). Both scarring and malformation results in downgrading of fruits at harvest. A 10% reduction in cucumber yield was observed at the peak densities of mixed-species of thrips, indicating mixed infestations can also cause reduction in mass of fruit produced (Welter et al. 1990). Thus, this study aimed to examine the effect of strip-tilled cover cropping on a mixed species thrips (*F. occidentalis* and *T. palmi*) on cucurbit plants to establish potentiality of strip-tilled cover cropping for multiple-pest guild suppression system.

This study specifically examines the following hypotheses (i) thrips densities will be lower in a strip-tilled cover cropping system compared with a monoculture system; (ii) densities of an arthropod predator, *Orius* spp. (Hemiptera: Anthocoridae) will be higher in a strip-tilled cover cropping system compared with a monoculture system; and (iii)

plant size, and quantity and quality of fruit yield will be greater / improved in strip-tilled cover cropping system compared with a monoculture system.

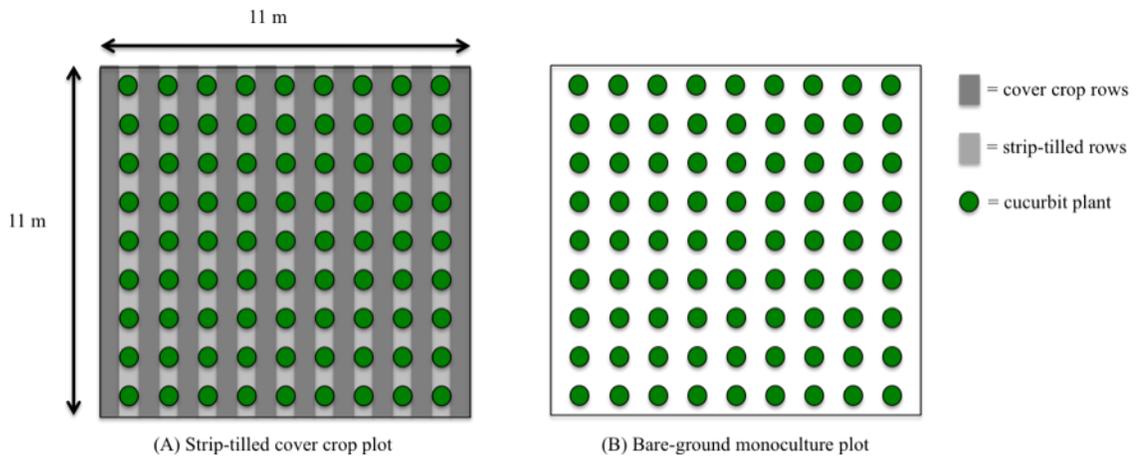
## **Methods**

Two field experiments were conducted to examine the effects of a strip-tilled cover cropping system on population densities of thrips and *Orius* spp. Experiments were conducted at a commercial grower field, Kamphoute Farms in Kunia, and the University of Hawaii at Manoa's experimentation station, Poamoho Research Station in Waialua on Oahu Island, Hawaii in spring and fall of 2008, respectively. A cash crop, bitter melon (*Momordica charantia*) (trailer type) was chosen for the Kamphoute Farms (spring) experiment, as it is an important vegetable crop in their existing farm production system. Bitter melon was replaced by cucumber (*Cucumis sativus*) (bush type) in the Poamoho Research Station (fall) experiment, for its simpler cultivation practices, and to avoid high fruit fly infestations such as those that occurred in bitter melon at the other site. Two cover crops, sunn hemp (SH), *Crotalaria juncea* L. (variety Tropic Sun) and French marigold (MG), *Tagetes patula* (variety Single Gold) were evaluated in both experiments.

### **Experimental Layout**

The experiment was set up in randomized complete block design with each treatment replicated four times. Each treatment plot was 11 m x 11 m, separated by minimum of 6 m in a block and each block was separated from others by a minimum of 11 m. Treatments within blocks included two strip-tilled cover crop plots as "dicultures"

and a bare-ground monoculture plot as “monoculture”. Nine rows of cucurbit plants were grown in strip-tilled cover crop rows in-between the standing or clipped cover crop rows in diculture plots, whereas nine rows of cucurbits were grown in monoculture (bare-ground) plots (Figure 2.1).



**Figure 2.1:** Field layout of experimental plots: (A) strip-tilled cover crop plot and (B) bare-ground monoculture plot.

### Crop planting

Cover crop seeds were sown in 19 rows at inter-row spacing of 61cm in cover crop plots. MG and SH were seeded at the rate of 2 kg / ha and 40 kg / ha, respectively and grown for approximately 3 months prior to planting the main crop. In the spring experiment, SH plants were mowed just below knee height level (~30 cm) using a lawn mower, while alternate rows of MG plants were mowed using a weed whacker. Each alternate row of mowed plants was tilled in a 61 cm wide strip and incorporated in the soil using a FRC tiller (Honda Motor Co., Ltd., Japan). Cover crops were mowed on Jan, 28-29 and incorporated in the soil on Feb 1-4 in the spring experiment. In the fall

experiment, alternate rows of SH and MG were cut at the base using Weed Whacker and/or sickle and incorporated in the soil using the FRC tiller. Additionally, remaining rows of SH were clipped using a sickle to maintain them at 1 m height just before the cucumber planting. Cover crops were cut on July 16 and incorporated in the soil on July 19-23 in the fall experiment. Approximately 25-32 days old, green house grown cucumber (variety Sweet Slice) (Source: University of Hawaii at Manoa, Seed Lab) or bitter melon (variety Local) (Source: Fukuda Seeds) seedlings were transplanted into the tilled strips at an intra-row spacing of 1.8 m. The seedlings were transplanted on February 11 and August 1 in spring and fall experiments, respectively. Borders and plot areas of the study sites were kept weed free by spot spraying of glyphosate (Roundup, Monsanto, St. Louis, MO) and hand weeding, respectively. A standard male annihilation technique was used to monitor fruit fly using traps baited with Cuelure (Amulate, Crop Care, Australia) throughout the cucurbit developmental time. Sudan grass (*Sorghum bicolor* variety *sudanese* Moench) was planted near the experimental sites and sprayed with GF 120 (Dow Agro-Science, Indianapolis, IN) weekly to attract and suppress melon fruit fly, *Bactocera cucurbitae* populations. *Bacillus thuringiensis* variety *kurtaski* (Crymax, Certis, Columbia, MD) was sprayed on cucurbit plants at the rate of 1.2 g / liter of water (35 kg a. i. / hectare) using a hand pumped knapsack sprayer to help manage pickleworm, *Diaphania nitidalis*. Cucurbit plants were fertilized with urea at a rate of 25 g per plant during experimental trial.

### **Foliar sampling**

On each sampling date, 10 plants were randomly selected from treatment plots. One leaf was chosen from the central part of selected plants and brought to the laboratory

(Castane et al. 1996). Leaves were washed with 70% ethanol and rinsed with distilled water to isolate insects. The ethanol water rinse was passed through a coffee filter and numbers of thrips (Rosenheim et al. 1990), and *Orius* spp. on the filter were counted using a microscope. Sampling was conducted weekly from February 29 (18 DAP) to May 30 (109 DAP) and August 26 (25 DAP) to October 21 (81 DAP) in spring and fall experiments, respectively.

### **Flower sampling**

One fully opened, non-senescent flower was collected from each of 10 randomly selected plants per plot on each sampling date. The collected flower was individually placed in a vial and returned to the lab where thrips and *Orius* spp. were counted using a microscope (Shipp et al. 2000). Sampling was conducted weekly from March 31 (49 DAP) to May 30 (109 DAP) and September 2 (32 DAP) to October 21 (81 DAP) in spring and fall, respectively.

### **Plant growth and fruit yield**

Five plants were randomly selected from each experimental plot for measuring plant growth parameters. Number of new leaves produced, leaf width of the fifth leaf from the terminal end (Hao et al. 2002) and stem diameter at base of the plant were measured repeatedly on the same sampled plant. Stem diameters were measured using a digital caliper (Digimatic Caliper, Mitutoyo Corporation, Kanagawa, Japan). Measurements were taken weekly from Feb 29 (18 DAP) to May 12 (91 DAP) and August 22 (21 DAP) to October 5 (65 DAP) in spring and fall, respectively. Fruits were harvested at maturity, normally two times a week from April 21 (70 DAP) to June 4 (114

DAP) and September 6 (36 DAP) to October 26 (86 DAP) in spring and fall, respectively.

In the spring experiment, all bitter melon fruits were stung by fruit flies during their development, causing premature fruit ripening and making fruits liable to be dropped from the plant. Therefore, all fruit fly infested premature fruits were picked at each harvest. Additionally, some of the developing fruits in each treatment plot were bagged to protect fruit fly from stinging them. The bagged fruits were harvested at marketable size and weighed to estimate exact fruit yield from the number harvested from each treatment plot. In the fall experiment, harvested fruit were graded individually to marketable, thrips only damaged and culled due to insect- and non-insect damage. Insect-damaged culled fruits were either infested by fruit fly, pickleworm or combination of both. Non-insect-damaged culled fruits were either due to bird damaged or infested by charcoal rot disease.

### **Data Analyses**

Data from arthropod counts were analyzed by using mixed model analysis of variance (PROC MIXED, SAS Institute). In all cases, the model was constructed to examine the main effect of treatment by date, with block designated as a random factor. Relationship between thrips and *Orius* counts either on cucurbit leaf or flower was examined using Pearson's correlation coefficient (PROC CORR, SAS Institute). The occurrences of *Orius* spp. in cucurbit leaves and flowers were analyzed using chi-square tests (PROC FREQ, SAS Institute). Data from plant measurements were analyzed using repeated-measures analysis of variance to examine between and within treatment effect

with plant as a repeated subject (PROC MIXED, SAS Institute). The yield data were analyzed by using mixed model analysis of variance (PROC MIXED, SAS Institute). Within each model, the following pre-planned orthogonal contrasts were conducted: diculture (cucurbits grown in strip-tilled cover crop) vs. monoculture (cucurbit grown in bare-ground), and cucurbits grown in strip-tilled SH vs. cucurbit grown in strip-tilled MG.

## Results

### Foliar sampling

The mean number of thrips on bitter melon leaves was lower ( $\sim 1.9$  / leaf) in the spring compared to cucumber leaves ( $\sim 12.5$  / leaf) in the fall experiment. The number of thrips on bitter melon leaves was found to be significantly lower in dicultures compared to monoculture on 39 DAP ( $F_{1,6} = 7.34$ ,  $P = 0.04$ ) in the spring experiment. The numbers were also significantly lower in MG compared to SH diculture from 18 - 25 DAP (18 DAP:  $F_{1,6} = 16.66$ ,  $P = 0.01$  and 25 DAP:  $F_{1,6} = 14.55$ ,  $P = 0.01$ ) (Figure: 2.2A).

Similarly in the fall experiment, the number of thrips on cucumber leaves were significantly lower in dicultures compared to monoculture from 46 - 81 DAP (46 DAP:  $F_{1,6} = 14.06$ ,  $P = 0.01$ ; 55 DAP:  $F_{1,6} = 10.18$ ,  $P = 0.02$ ; 62 DAP:  $F_{1,6} = 72.13$ ,  $P < 0.01$ ; 68 DAP:  $F_{1,6} = 19.00$ ,  $P < 0.01$ ; 81 DAP:  $F_{1,6} = 14.36$ ,  $P = 0.01$ ) (Figure 2.2B).

Low abundance of *Orius* spp. ( $\sim 0.16$  / leaf) was observed on bitter melon leaves in the spring experiment. The numbers of *Orius* spp. on bitter melon leaves did not differ significantly between the treatment habitats ( $F_{2,6} = 2.00$ ,  $P = 0.22$ ) nor were explicitly

correlated with the thrips densities ( $r = -0.01$ ,  $P = 0.77$ ). Additionally, the proportion of leaves with at least one *Orius* spp. did not significantly differ between treatments ( $\chi^2 = 3.25$ ,  $df = 2$ ,  $P = 0.20$ ).

### **Flower sampling**

Unlike thrips numbers on leaves, the mean number of thrips on cucumber flowers was lower ( $\sim 1.81$  / flower) in the fall compared to bitter melon flowers ( $\sim 2.42$  / flower) in the spring experiment. The number of thrips on bitter melon flowers were significantly lower in dicultures compared to monoculture on 63 DAP ( $F_{1,6} = 8.86$ ,  $P = 0.02$ ). The numbers were significantly lower in MG compared to SH diculture on different sampling dates (49 DAP:  $F_{1,6} = 9.44$ ,  $P = 0.02$ ; 63 DAP:  $F_{1,6} = 8.58$ ,  $P = 0.03$ ; 88 DAP:  $F_{1,6} = 7.78$ ,  $P = 0.03$ ; and 109 DAP:  $F_{1,6} = 22.06$ ,  $P < 0.01$ ) (Figure: 2.3A). In the fall experiment, the number of thrips on cucumber flowers was significantly lower in dicultures compared to monoculture from 62 - 68 DAP (62 DAP:  $F_{1,6} = 32.17$ ,  $P < 0.01$  and 68 DAP:  $F_{1,6} = 18.36$ ,  $P = 0.01$ ) (Figure: 2.3B).

The abundance of *Orius* spp. on bitter melon flowers did not differ significantly between the treatment habitats ( $F_{2,6} = 0.50$ ,  $P = 0.63$ ) nor were explicitly correlated with the thrips densities ( $r = -0.05$ ,  $P = 0.14$ ). The proportion of bitter melon flowers with at least one *Orius* spp. individual was not significantly different among the treatments ( $\chi^2 = 2.54$ ,  $d.f. = 2$ ,  $P = 0.28$ ). In the fall experiment, populations of *Orius* spp. were only observed on isolated occasions on cucumber leaves and flowers, and hence not analyzed statistically.

## Plant measurement and fruit yield

In the spring experiment, bitter melon plants produced on average 4.2 new leaves per week. The rate of leaf production during the development of the plants was similar among the treatments (treatment x time:  $F_{20,610} = 1.48$ ,  $P = 0.08$ ) (Figure: 2.4A). However, the rate of increase in leaf width was significantly different among the treatments (treatment x time:  $F_{20,616} = 1.95$ ,  $P = 0.01$ ). The leaf width was significantly lower in dicultures compared to monoculture on different sampling dates (63 DAP:  $F_{1,6} = 15.61$ ,  $P = 0.01$ ; 70 DAP:  $F_{1,6} = 8.71$ ,  $P = 0.03$ ; 84 DAP:  $F_{1,6} = 8.40$ ,  $P = 0.03$  and 91 DAP:  $F_{1,6} = 8.33$ ,  $P = 0.03$ ) (Figure: 2.4B). Similarly, the rate of increase in stem diameter was significantly lower in dicultures compared to monoculture (treatment x time:  $F_{20,617} = 3.06$ ,  $P < 0.01$ ). The stem diameter was found to be significantly lower in dicultures compared to monoculture from 63 - 91 DAP (63 DAP:  $F_{1,6} = 13.05$ ,  $P = 0.01$ ; 70 DAP:  $F_{1,6} = 6.78$ ,  $P = 0.04$ ; 77 DAP:  $F_{1,6} = 11.64$ ,  $P = 0.01$ ; 84 DAP:  $F_{1,6} = 9.07$ ,  $P = 0.02$  and 91 DAP:  $F_{1,6} = 14.68$ ,  $P = 0.01$ ) (Figure: 2.6A).

Similarly, cucumber plants produced on average 3.2 new leaves per week. The rate of leaf production during the development of the plants was significantly different among the treatments (treatment x time:  $F_{12,383} = 3.83$ ,  $P < 0.01$ ). The number of new leaves produced was significantly lower in dicultures compared to monoculture from 36 - 50 DAP (36 DAP:  $F_{1,6} = 19.54$ ,  $P < 0.01$ ; 43 DAP:  $F_{1,6} = 21.11$ ,  $P < 0.01$  and 50 DAP:  $F_{1,6} = 12.83$ ,  $P = 0.03$ ) (Figure: 2.5A). The rate of increase in leaf width was significantly different among the treatments (treatment x time:  $F_{12,382} = 1.82$ ,  $P = 0.04$ ). The leaf width was significantly lower in dicultures compared to monoculture on 36 and 50 DAP (36 DAP:  $F_{1,6} = 15.21$ ,  $P = 0.01$ ; 50 DAP:  $F_{1,6} = 6.60$ ,  $P = 0.04$ ). The leaf width was found to

be larger in SH compared to MG diculture on different sampling dates (30 DAP:  $F_{1,6} = 8.71$ ,  $P = 0.03$ ; 36 DAP:  $F_{1,6} = 8.71$ ,  $P = 0.03$ ; 57 DAP:  $F_{1,6} = 8.71$ ,  $P = 0.03$  and 65 DAP:  $F_{1,6} = 8.71$ ,  $P = 0.03$ ) (Figure: 2.5B). Similarly, the rate of increase in stem diameter was significantly different among the treatments (treatment x time:  $F_{6,218} = 30.87$ ,  $P < 0.01$ ). The stem diameter was found to be lower in dicultures compared to monoculture from 30 - 57 DAP (30 DAP:  $F_{1,6} = 27.08$ ,  $P < 0.01$ ; 43 DAP:  $F_{1,6} = 64.19$ ,  $P < 0.01$ ; 57 DAP:  $F_{1,6} = 87.81$ ,  $P < 0.01$ ) (Figure: 2.6B).

High densities of fruit fly were observed in the spring ( $\sim 228.3$  / trap / week) compared to the fall experiment ( $\sim 21.8$  / trap / week) on the basis of cue-lure baited trap catches. In the spring experiment, all bitter melon fruits were stung by fruit flies and the number of stung fruits harvested was significantly higher in monoculture compared to dicultures ( $F_{1,6} = 12.42$ ,  $P = 0.01$ ) (data not shown). The estimated mean weights of a bitter melon fruit, if not stung by fruit flies were 128.6 g (monoculture), 94.6 g (MG-diculture) and 146.1 g (SH-diculture). The estimated yield (t / ha) of non-infested fruits was significantly higher in monoculture compared to dicultures ( $F_{1,6} = 12.74$ ,  $P = 0.01$ ). Also, SH- produced a significantly higher yield than MG diculture ( $F_{1,6} = 10.24$ ,  $P = 0.02$ ) (data not shown). In the fall experiment, the total cucumber yield (kg / ha) was significantly higher in monoculture compared to dicultures ( $F_{1,6} = 9.36$ ,  $P = 0.02$ ), however marketable fruit yield was not significantly different between the treatments ( $F_{2,6} = 2.79$ ,  $P = 0.14$ ). Fruit yield damaged by thrips alone was significantly lower in dicultures compared to monocultures ( $F_{1,6} = 39.93$ ,  $P < 0.01$ ). Insect- and non-insect-damaged culled fruits were significantly lower in dicultures compared to monoculture

(insect-damaged:  $F_{1,6} = 8.15$ ,  $P = 0.03$ ; non-insect-damaged:  $F_{1,6} = 10.46$ ,  $P = 0.02$ )

(Figure: 2.7).

## Discussion

Experimental results showed that thrips densities were effectively reduced on foliage and flowers of cucurbit crops in the strip-tilled cover cropping system compared to a monoculture system. This has previously been demonstrated in other systems. Undersown clover had a suppressive effect on *Thrips tabaci* Lindeman infestation in leeks (Theunissen and Schelling 1996 and 1998). Crimson clover or a ground cover of rye significantly reduced larval and adult thrips (*F. occidentalis* and *F. Fusca*) populations compared to no cover in cotton and peanut (Olson et al. 2006). Strip-tilled winter cover crops (crimson clover, wheat, or rye) reduced the density of adult thrips (primarily, *F. fusca*) on seedling cotton compared to a conventional till system (Toews et al. 2010). These studies have shown that thrips densities can be effectively suppressed when crops are grown with cover crops or in strip-tilled cover cropping systems.

Nicholls et al. (2000) accredited reduction in *F. occidentalis* density in vineyards grown with cover crops to greater abundance of generalist predators supported by resources provided by buckwheat and sunflower. However, the current study suggested that the cover crops did not influence the abundance of *Orius* spp., and hence did not contribute to any change in thrips densities on cucurbit plants grown in strip-tilled cover crops. On the other hand, preference of *F. occidentalis* to plants with yellow flowers, such as Transvaal daisy (*Gerbera jamesonii*) and chrysanthemum (*Dendranthema x*

*Grandiflorum*) was demonstrated in laboratory and green house studies (Teulon et al. 1999, Blumthai et al. 2005). Similarly, a greenhouse study showed that flowering chrysanthemum, *Dendranthema grandiflora* (Tzvelv) could be used as a trap plant in reducing numbers of adult *F. occidentalis* in a vegetative chrysanthemum crop (Buitenhuis et al. 2005). Preliminary observations on pests and beneficial insects of cover crops in the current study found abundant numbers of thrips on sunn hemp flowers. Therefore, reduced density of thrips on cucurbit plants grown with cover crops was likely attributable to the attraction of the insects to the yellow flowers of sunn hemp and marigold, and this might have diverted them from cucurbit plants.

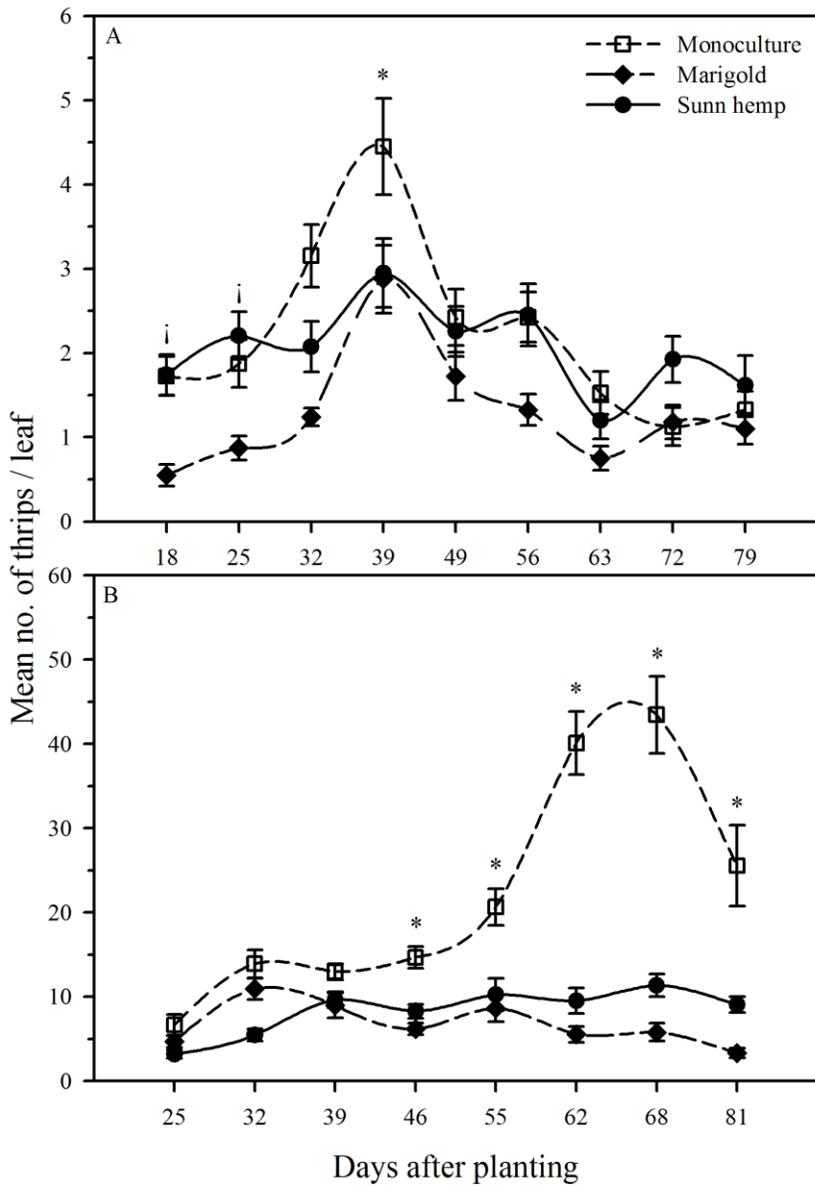
Densities of thrips on cucurbit plants were generally lower in marigold- compared to sunn hemp strip-tilled treatments. The higher degree of protection in marigold strip-tilled treatments is possibly due to their constitutive volatiles that may have caused host odor masking, resulting lower densities of thrips on cucurbit plants compared to sunn hemp strip-tilled treatments.

Improved crop growth is an important parameter to consider when addressing a pest suppressive cover cropping system (den Belder et al. 2000, Manandhar et al. 2009). In the current study, although strip-tilled cover cropping reduced the densities of thrips on cucurbit plants, plant growth was severely affected as a result of competition between cover crops and cucurbits. The competition was probably due to interception of sunlight by tall sunn hemp plants, and an increased basal area under marigold plants after the first flowers had senesced. As a result, the more vigorous plants in the monoculture increased fruit production compared to less production in the strip-tilled systems. Similar to this study inter-specific competition has been observed in the pest suppressive mixed or

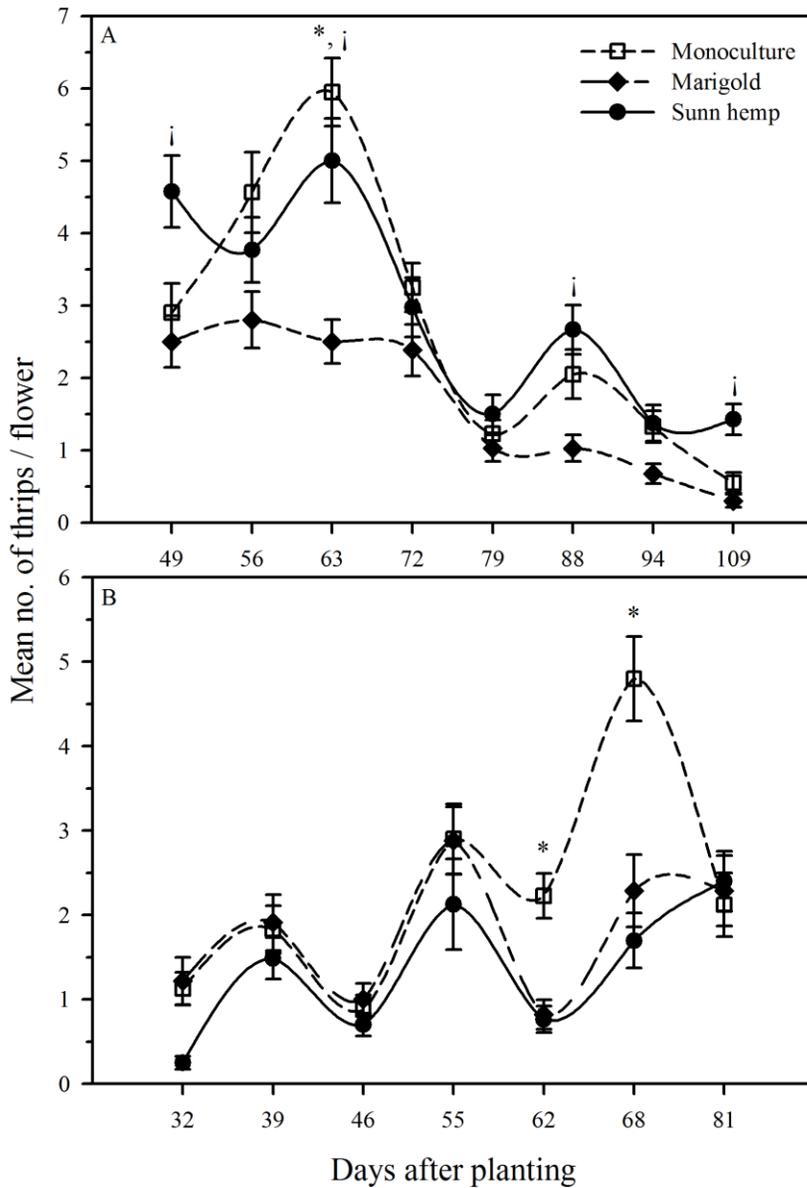
intercropping systems. Andow et al. (1986) found reduced populations of the flea beetle, *Phyllotreta cruciferae* Goeze and the aphid, *Brevicoryne brassicae* (L.) on cabbage interplanted with living mulches compared to monoculture, however the potential benefit of intercropping was neutralized by reduction in cabbage yield due to competition. Letourneau (1986) found that population densities of the melonworm, *Diaphania hyalinata* (L.) was lower in maize/legume/squash –polycultures compared to monocultures, however yield of each crop was higher when planted as monocultures compared to polycultures. The current study and above-mentioned studies showed the crops grown in diverse cropping systems may result in inter-specific competition impacting plant growth and yield of the targeted crop.

High infestations of fruit fly (*Bactocera cucurbitae*) and pickleworm (*Diaphania nitidalis*) were observed as major problems causing fruits to be damaged and thus unmarketable. Although there was no significant difference in marketable cucumber yield, fruits damaged due to thrips were significantly lower in the strip-tilled treatments compared to the monoculture. Theunissen and Schelling (1996) similarly found undersowing leeks with clover drastically reduced *Thrips tabaci* Lindeman infestation, which improved the quality of leeks at harvest. Similarly, although broccoli head weights were significantly lower in the broccoli intercropped with tomato or yellow sweet clover treatments compared to broccoli monoculture, the mean percentages of harvested broccoli heads were less infested with insects and associated frass (Hooks and Johnson 2002). These results suggested pest suppressive cover cropping results in the better quality yield even at the times when the quantity of yield is reduced due to inter-specific competitions.

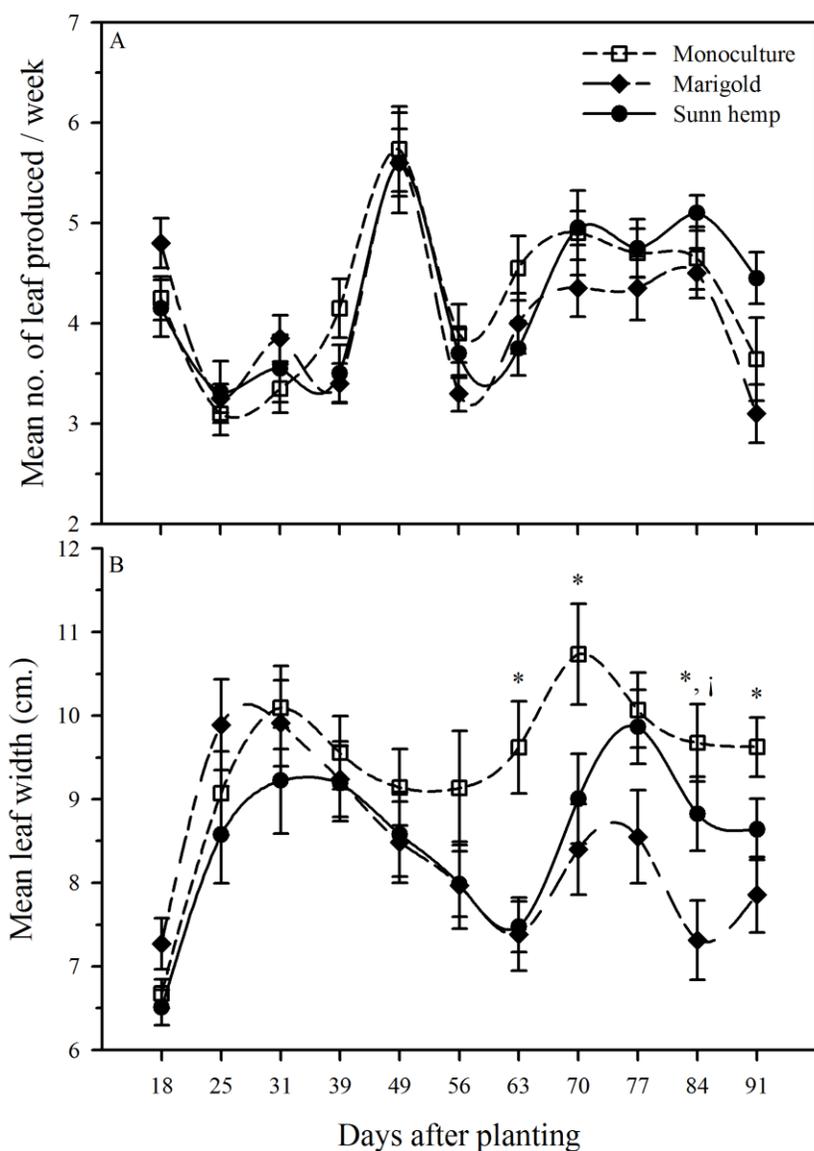
In conclusion, the strip-tilled cover cropping system had positive impacts by reducing densities of thrips on the cucurbit plants. The reduced incidence of thrips in the strip-tilled cover crop treatments is attributable to thrips attraction to the yellow flowers of sunn hemp and marigold. In addition, sunn hemp and marigold flowers may produce higher quality pollen than cucurbit flowers, which might have attracted thrips to cover crop flowers for nutritional benefit (Hulshof and Vanninen 2001). The inter-specific competition between cucurbits and cover crops were evident, impacting rate of crop growth and fruit yield in strip-tilled cover cropping systems. General suggestions to reduce competition between the crops and companion plants have been provided in Hooks and Johnson (2004). Among their suggestions, optimal spacing between the cash crop and cover crop as indicated by Bugg et al. (1991) may play a role in reducing competition between cash crop and cover crop. Modification in cropping patterns such as increasing the strip-tilled area to accommodate additional cash crop rows in between standing cover crop rows may provide enough space to grow cash crop and help minimize competition between them. Thus a suitable strip-tilled cover cropping pattern can contribute to effective pest management package targeting multiple-pest guild and simultaneously impact on growth and yield of a target crop in the system.



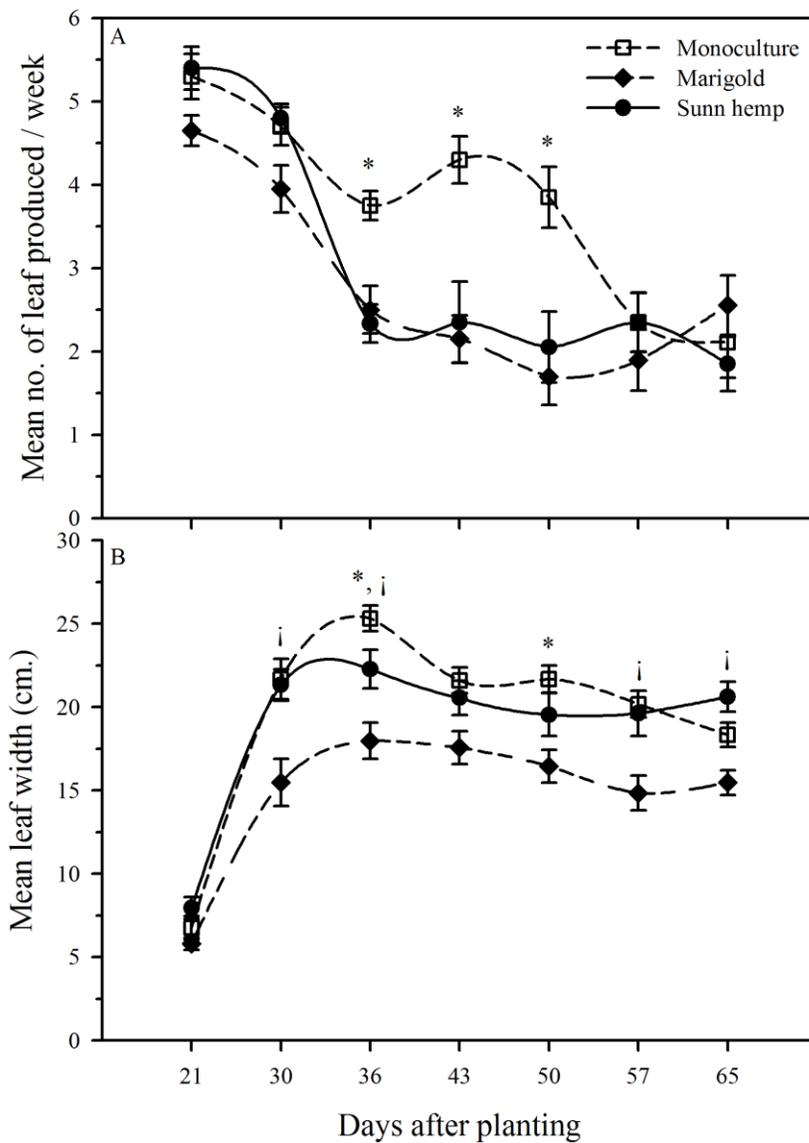
**Figure 2.2:** Mean numbers of thrips ( $\pm$  standard error, SE) per cucurbit leaf in different treatment habitats, (A) bitter melon in the spring experiment at Kamphoute Farms, (B) cucumber in the fall experiment at Poamoho Research Station. Monoculture represents cucurbit grown in bare-ground. Marigold and sunn hemp are two cover crops, representing diculture habitats with cucurbit grown in the stripped-tilled cover crop rows. \* Indicates diculture is significantly lower than monoculture and  $\bar{\bar{}}$  indicates marigold is significantly lower than sunn hemp.



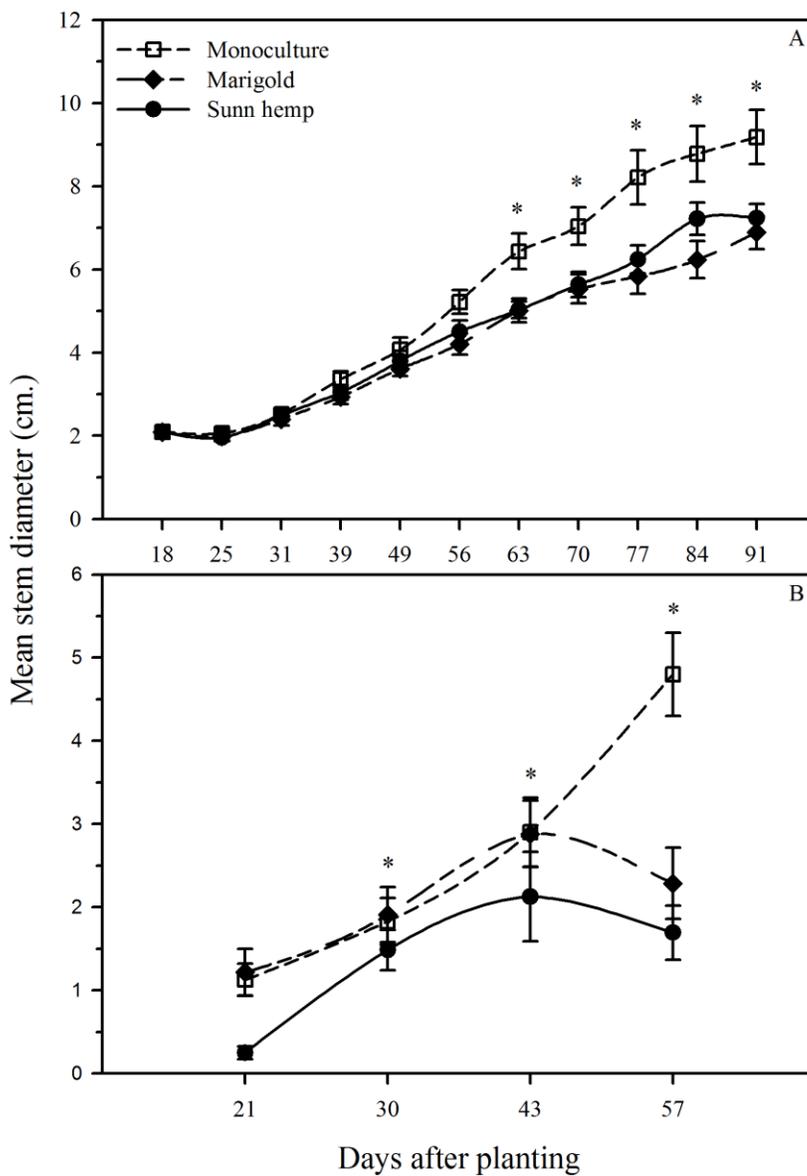
**Figure 2.3:** Mean numbers of thrips ( $\pm$  SE) per cucurbit flower in different treatment habitats, (A) bitter melon in the spring experiment at Kamphoute Farms, (B) cucumber in the fall experiment at Poamoho Research Station. Monoculture represents cucurbit grown in bare-ground. Marigold and sunn hemp are two cover crops, representing diculture habitats with cucurbit grown in the stripped-tilled cover crop rows. \* Indicates diculture is significantly lower than monoculture and † indicates marigold is significantly lower than sunn hemp.



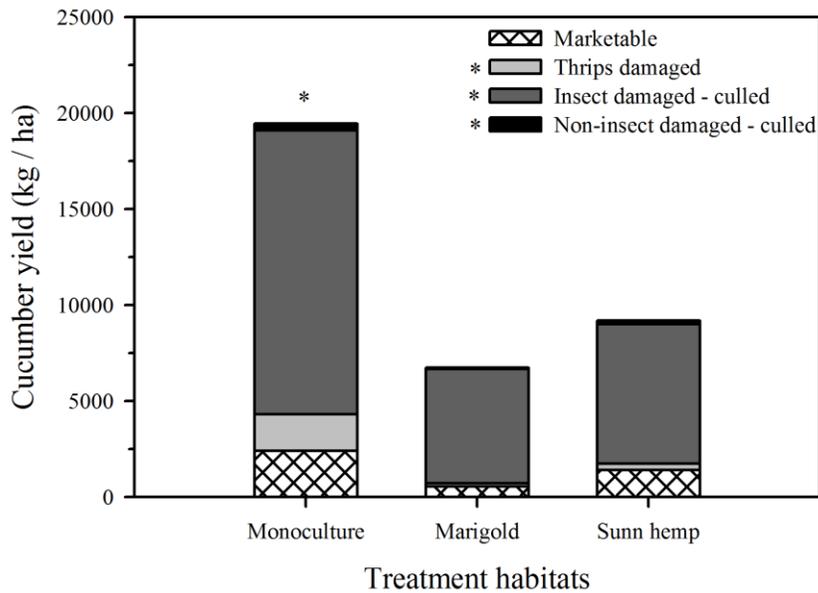
**Figure 2.4:** (A) Mean numbers leaf produced ( $\pm$  SE) per week and (B) mean leaf width in cm. ( $\pm$  SE) of bitter melon plant in different treatment habitats in the spring experiment at Kamphoute Farms. Monoculture represents bitter melon grown in bare-ground. Marigold and sunn hemp are two cover crops, representing diculture habitats with bitter melon grown in the stripped-tilled cover crop rows. \* Indicates diculture is significantly lower than monoculture. † indicates marigold is significantly lower than sunn hemp.



**Figure 2.5:** (A) Mean numbers leaf produced ( $\pm$  SE) per week and (B) mean leaf width in cm. ( $\pm$  SE) of cucumber plant in different treatment habitats in the fall experiment at Poamoho Research Station. Monoculture represents cucumber grown in bare-ground. Marigold and sunn hemp are two cover crops, representing diculture habitats with cucumber grown in the stripped-tilled cover crop rows. \* Indicates diculture is significantly lower than monoculture and ; indicates marigold is significantly lower than sunn hemp.



**Figure 2.6:** Mean stem diameter ( $\pm$  SE) of cucurbit plants in different treatment habitats (A) bitter melon plant in the spring experiment at Kamphoute Farm and (B) cucumber plant in the fall experiment at Poamoho Research Station. Monoculture represents cucurbits grown in bare-ground. Marigold and sunn hemp are two cover crops, representing diculture habitats with cucurbit grown in the stripped-tilled cover crop rows. \* Indicates diculture is significantly lower than monoculture.



**Figure 2.7:** Mean cucumber yield (Kg / ha) in different treatment habitats in the fall experiment at Poamoho Research Station. Monoculture represents cucumber grown in bare-ground. Marigold and sunn hemp are two cover crops, representing diculture habitats with cucumber grown in the stripped-tilled cover crop rows. Marketable indicates marketable fruit yield, thrips damaged indicates fruits damaged by thrips alone, insect damaged-culled indicates culled fruit due to damage caused by insects (fruit fly and pickleworms) and non-insect damaged-culled indicates culled fruits due to damage caused by birds and charcoal rot. \* Indicates diculture is significantly lower than monoculture.

## CHAPTER II

### **Effects of cover crops on population densities of corn planthopper, *Peregrinus maidis*, and incidence of hopperburn and *Maize mosaic virus* symptomatic plants in corn - cover crop intercropping systems**

#### **Abstract**

Field experiments were conducted in Hawaii to examine effects of agricultural diversification through intercropping corn with cover crops on (i) densities of colonizing corn planthoppers, *Peregrinus maidis* Ashmead and (ii) within-field activity of *P. maidis*, and (iii) incidence of hopperburn and *Maize mosaic virus* (MMV) symptomatic plants. Sweet corn, *Zea mays* L. was grown with three cover crops, buckwheat, *Fagopyrum esculentum* (Moench.); cowpea, *Vigna unguiculata*; and sunn hemp, *Crotalaria juncea* L. in 2:1 and 4:1 intercropping ratio in 2009 and 2010, respectively. Counts were made at weekly intervals of macropterous *P. maidis* on plants (colonists) and in traps (within-field movement). The number of corn plants showing hopperburn and MMV symptoms were quantified. Results showed that sunn hemp significantly lowered colonization by *P. maidis*, resulting in reduced incidence of hopperburn at the vegetative stages and incidence of MMV at the reproductive stages of corn plants in the year 2009. Cowpea did not suppress *P. maidis* densities and resulted in complete collapse of corn in the year 2009. Results suggest that a tall-stature sunn hemp intercrop increased within-field activity of *P. maidis* resulting in lower rates of colonization of corn plants, and reduced incidence of hopperburn and MMV symptomatic plants.

## Introduction

Chapter I of this dissertation addressed the pest suppressive services offered by habitats with diversified vegetation, through use of strip-tilled cover crops in cucurbit cropping systems. That study showed that the potential benefits of a pest suppressive environment were nullified due to competition between cucurbits and cover crops, impacting negatively on cucurbit growth and yield. In order to address this problem, the cropping pattern in the current study was modified to provide adequate space for crops to grow without competing with the cover crop. Cover crops were used as intercrops with an adequate inter-row space between cash crop and cover crop rows. The cash crop was grown in between cover crop rows in two different intercropping ratios to examine an appropriate inter-space between them. The strip-tilled cover cropping previously attempted was removed because of the difficulties it posed in agricultural operations. The current study examines the effect of intercropping on above-ground pests of corn and their effects on crop growth and yield.

In this study, corn; *Zea mays* L. was chosen as the target crop. Corn (or maize) is a staple crop in many countries and is an important component of US agriculture in general. Seed-corn production is currently the largest agricultural industry in Hawaii (NASS 2009), and pests and diseases are the most important production constraints (Brewbaker 2003). Corn planthopper, *Peregrinus maidis* Ashmead is a serious economic pest of corn and sorghum in many tropical and sub-tropical areas (Brewbaker 2003, Singh and Seetharama 2008). Adult *P. maidis* exhibit two distinct morphs: a long-winged form (macropterous), and a short winged form (brachypterous). These alternative forms develop depending upon population densities and host plant quality (Denno and

Roderick 1990). Brachypterous females are generally more fecund and reproduce earlier in their life and macropterous adults can disperse long distances (Denno and Roderick 1990).

Overwhelming numbers of planthoppers feeding on young corn plants often result a condition known as “hopperburn” (Takara and Nishida 1983). Corn plants with hopperburn show symptoms of extended necrosis, reduced plant vigor, stunting and are often pre-disposed to moisture stress (Backus et al. 2005, Singh and Seetharama 2008). High infestation levels on young corn plants can lead to early plant death or to significant yield loss (Nishida 1978). *Maize mosaic virus*, MMV (Family: Rhabdoviridae) among the most important economic diseases of corn in Hawaii (Brewbaker 2003). The virus is transmitted persistently, propagative in the insect vector and can also be transovarially transmitted to their offspring (Nault and Ammar 1989, Hogenhout et al. 2008). Symptoms of MMV include acute chlorosis with elongated white streaks over the veins of leaves, leaf sheath and ear husks (Ming et al. 1997). Plants may be severely or mildly stunted, bearing small ears with shortened husks (Brewbaker 2003). These effects can cause serious economic losses due to plant death or poor ear development, especially when infection occurs in early stage (Singh and Seetharama 2008). Crop loss attributable to *P. maidis* may result from either feeding damage or transmission of MMV disease.

The current approach to MMV management is solely dependent on resistant varieties. All economically important Hawaiian super-sweet, field-corn inbreds and hybrids carry the *Mv* gene for general resistance to this pathogen (Brewbaker 2003). Field- and sweet corn hybrids from other parts of world rarely show any tolerance of MMV. This virus replicates only on corn, lacks alternate plant hosts and is severe only in

regions where corn grows continuously around the year, e.g. Hawaii. Thus, it is challenging to grow other temperate corns from around the world in Hawaii, and MMV is a limiting factor for parent seed-corn production. Failure of current management strategies such as the use of insecticides, and an inability to target multiple pest and disease complexes, raises the need of alternative pest management strategies. Thus, agricultural diversification with cover crops may offer an alternative solution to current practices that are sustainable, reduce pesticide use and environment friendly for suppressing pest and disease complexes in corn.

Effects of crop diversification through intercropping or mixed cropping on population dynamics of *P. maidis* and incidence of MMV have never been studied. Studies on populations of leafhoppers (Hemiptera: Cicadellidae) in corn and vineyards showed lower densities in mixed cropping system, mainly due to increased abundance of natural enemies (Nichollas et al. 2000, Weiser et al. 2003, English-Loeb et al. 2003). Recent studies showed that population densities of rice planthoppers, *Nilaparvata lugens* (Stal.) and/or *Sogatella furcifera* Hawarth were significantly lower in rice polycultures, primarily due to resource limitation (Lin et al. 2011, Yao et al. 2012). Further, Power (1987, 1988 and 1991) showed increasing plant community or genetic diversity alters the behavior of vector insects, which in turn limits their within-field movements. Ultimately, this results in lower abundance and decreased disease incidence. Hence, diverse cropping systems may limit resource availability, thus change their behavior, and result in altered densities in intercropping systems. Therefore, the objective of this chapter is to examine the effect of cover crops on *P. maidis* densities, and their feeding damage (hopperburn) and MMV incidence in corn - cover crop intercropping systems. Three cover crops,

buckwheat, *Fagopyrum esculentum* (Moench); cowpea, *Vigna unguiculata*; and sunn hemp, *Crotalaria juncea* L. were chosen for an intercropping system. From here onwards, “diculture” indicates diverse habitat achieved through intercropping a cover crop with the corn, and “monoculture” indicates a simpler habitat with corn seeded into bare soil. This chapter proposes to examine following hypotheses: (i) the within-field activity of *P. maidis* will be greater in the diculture than the monoculture, (ii) the density of colonizing *P. maidis* will be lower in the diculture than the monoculture, and (ii) the incidence of hopperburn and MMV will be lower in the diculture than the monoculture.

## **Methods**

Two field experiments were conducted to examine the effects of cover crops on population densities of *P. maidis* and their damage symptoms in a corn - cover crop intercropping system. Experiments were conducted at a parent-seed corn farm (Pioneer Hi-Bred International International Inc., Waiialua, HI) and at the University of Hawaii Manoa’s Waimanalo Research Station (Waimanalo, HI) in summer, 2009 and spring, 2010, respectively.

### **Experimental layout**

The experiment was set up in a randomized complete block design with each treatment replicated four times. Treatment plots were 13.7 m x 13.7 m, separated by 9 m in a block and each block was separated by 12.2 m. Treatments within blocks included three intercropped plots and a monoculture. In the intercropped plots, cover crop seeds, sunn hemp and cowpea were sown approximately one month before corn planting and

buckwheat were broadcasted a week after corn planting. Two and four rows of corn were grown in between the rows of cover crops in 2009 and 2010, respectively. In 2010, the number of rows of corn between two cover crop rows was increased in order to minimize competition between the cover crop and corn. A total of 12 rows of corn were grown per plot between 7 and 4 rows of cover crops in 2009 and 2010, respectively. The monoculture plot had 19 rows of corn.

### **Crop planting**

Sunn hemp (SH) (variety Tropic Sun, Source: Crop Care Hawaii LLC) and cowpea (CP) (variety Iron and Clay, Source: Peaceful Valley Farm Supply, CA) seeds were sown at the rate of 40 g / row and 50 g / row, respectively. After seeding, plots were treated with a pre-emergence herbicide, Pendimethalin (Prowl H 20, BASF, NC) to check weeds during cover crop growth. A temperate corn variety Passion (Seminis Vegetable Seeds, CA), susceptible to MMV was seeded on 26 May and 4 Feb in 2009 and 2010 experiments, respectively. Wheeler (Precision Garden Seeder, Earthway Products Inc.) and Jab planters were used to seed corn in 2009 and 2010 experiments, respectively. At least two seeds per hole were planted at 15.2 cm intra-row and 76.2 cm inter-row spacing. Buckwheat (BW) (source: Peaceful Valley Farm Supply, CA) seeds were broadcasted in a strip enclosed by two drip tubes placed 30.5 cm apart at the rate of 200 g / strip. In the 2009 experiment, a selective herbicide to broad leaf weeds, Mesotrione (Callisto, Syngenta, NC) was applied to rows of corn at the V2 – V3 stage (early vegetative stage with initiation of 2 -3 leaves), followed by hand weeding at the V8 stage (vegetative stage with initiation of eight leaves) to suppress weeds. In the 2010 experiment, a spray of pre-emergence herbicide, Atrazine (AAtrax 4L, BASF, NC) was applied immediately after

corn seeding to suppress weeds until the final harvest. Corn plants were thinned out to one per hole at the V8 stage and fertilized following standard dosage and application schedules of each field station. Areas in between the field plots were cultivated to keep them weed free in both the years.

### ***P. maidis* counts on traps**

Macropterous *P. maidis* were monitored using clear plastic and yellow sticky traps in 2009 and 2010 experiments, respectively. A clear plastic soda bottle (1.5 liter capacity) with the top removed was made into trap by painting a central 12 cm band with Tangletrap, Insect trap coating (Gempler's Inc., WI). The clear plastic traps were replaced by yellow sticky traps in the 2010 experiment owing to difficulties in removing and repainting Tangletrap on the surface in subsequent trap counts. Yellow sticky traps (Seabright Laboratories, CA) were reverse folded to expose its 10.2 cm x 35.6 cm (~360 cm<sup>2</sup>) area coated with adhesive. A 1.2 m long PVC pipe elbowed with a 25 cm PVC pipe at the top was pounded into the soil to form a support upon which a trap was suspended. A total of four traps were placed in each plot, adjusting the height at each sampling to maintain traps at a level equal to the top of the corn canopy. Trapped *P. maidis* were counted at weekly intervals from June 17 (22 DAP) to July 22 (57 DAP) and Feb 17 (13 DAP) to April 14 (69 DAP) in 2009 and 2010, respectively. In the 2009 experiment, *P. maidis* were counted from 3 randomly assigned 5.1 cm x 5.1 cm (~25.8 cm<sup>2</sup>) areas in a clear plastic trap and mean of counts from 3 squares was reported as a trap catch. In the 2010 experiment, the total number of *P. maidis* trapped per yellow sticky trap was reported as a trap catch.

### ***P. maidis* counts on plants**

On each sampling date, 10 plants were randomly sampled from treatment plots for counting macropterous *P. maidis*. Planthoppers were counted in leaf whorls until the V8 stage, after which inspections were extended to leaf sheaths, tassels, flag leaves and the corn ear at the R stages (reproductive stages) on later sampling dates. Planthoppers were counted weekly from June 12 (17 DAP) to July 17 (52 DAP) and Feb 16 (12 DAP) to April 14 (69 DAP) in 2009 and 2010, respectively.

### **Hopperburn and MMV evaluation**

Sampling was initiated after plants produce first visual symptoms of MMV or hopperburn. Four rows of corn were randomly selected from each plot and plants in each row were evaluated for hopperburn and MMV on the basis of visual symptoms. Corn plants were evaluated weekly from June 17 (22 DAP) to July 16 (46 DAP) and March 22 (46 DAP) to April 12 (67 DAP) in 2009 and 2010, respectively. Four leaf samples, 2 each from plants showing hopperburn and MMV symptoms were sampled from each plot in 2009. At least two leaf samples from plants showing MMV symptoms were sampled from each plot in 2010. Leaf samples were diagnosed using Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay (DAS-ELISA) to confirm presence or absence of MMV. ELISA reagent kit for MMV (Agdia Inc., US) was used for diagnosis of MMV.

### **Data Analyses**

Data for *P. maidis* counts and percentage of plants showing hopperburn and MMV symptoms were analyzed by using a mixed model analysis of variance (PROC MIXED, SAS Institute). In all cases, the model was constructed to examine the main

effect of treatment by date, with block designated as a random factor. Within each model, the following pre-planned orthogonal contrasts were conducted: dicultures (corn + BW, corn + CP and corn + SH) vs. monoculture, (corn + SH) vs. (corn + BW and corn + CP) and corn + BW vs. corn + CP. All the statistical analysis procedures involving mixed models in SAS were followed as described by Littell et al. (2006).

## Results

### *P. maidis* counts on traps

Average weekly trap catch corrected to similar sticky area in traps was found to be higher (1.59 / 25.08 cm<sup>2</sup>) in the 2009 than in the 2010 experiment (0.04 / 25.08 cm<sup>2</sup>). In the 2009, trap counts of macropterous *P. maidis* were significantly higher in the diculture compared to the monoculture ( $F_{1,9} = 7.48$ ,  $P = 0.02$ ), and sunn hemp (SH) compared to buckwheat (BW) and cowpea (CP) dicultures on the first sampling date, 11 DAP ( $F_{1,9} = 7.53$ ,  $P = 0.023$ ). Conversely, trap counts were significantly lower in SH compared to BW and CP dicultures on 43 - 51 DAP (43 DAP:  $F_{1,9} = 14.17$ ,  $P < 0.01$ ; 51 DAP:  $F_{1,9} = 12.82$ ,  $P = 0.01$ ) (Figure 3.1A).

Similar results were found in the 2010 experiment, trap counts were higher in the diculture compared to the monoculture on 28 DAP ( $F_{1,9} = 9.05$ ,  $P = 0.01$ ), and SH compared to BW and CP on 21 - 36 DAP (21 DAP:  $F_{1,9} = 6.97$ ,  $P = 0.03$ ; 28 DAP:  $F_{1,9} = 22.55$ ,  $P < 0.01$ ; 36 DAP:  $F_{1,9} = 12.88$ ,  $P = 0.01$ ). Trap counts were significantly higher in CP compared to BW on 21 - 28 DAP (21 DAP:  $F_{1,9} = 6.98$ ,  $P = 0.03$ ; 28 DAP:  $F_{1,9} = 5.24$ ,  $P = 0.04$ ) (Figure 3.2B).

### ***P. maidis* counts on plants**

Average number of *P. maidis* present on the plants was higher ( $13.8 \pm 0.35$ ) (mean  $\pm$  SE) in the 2009 compared to the 2010 experiment ( $0.35 \pm 0.03$ ). In the 2009, number of *P. maidis* was significantly lower in SH compared to BW and CP on 17 - 31 and 55 DAP (17 DAP:  $F_{1,9} = 14.78$ ,  $P < 0.01$ ; 24 DAP:  $F_{1,9} = 34.57$ ,  $P < 0.01$ ; 31 DAP:  $F_{1,9} = 16.43$ ,  $P < 0.01$ ; 55 DAP:  $F_{1,9} = 11.18$ ,  $P = 0.01$ ) (Figure 3.2A). Unlike the 2009 experiment, *P. maidis* numbers were not significantly different between treatments ( $F_{3,9} = 1.30$ ,  $P = 0.33$ ) in the 2010 experiment, except at one occasion, when numbers were significantly higher in BW compared to CP on 34 DAP ( $F_{1,9} = 16.43$ ,  $P < 0.01$ ), and BW and CP compared to SH ( $F_{1,9} = 5.51$ ,  $P = 0.04$ ) (Figure 3.2B).

### **Hopperburn and MMV incidence**

Overall percentage of plants showing MMV symptoms irrespective of treatment habitats was high (90.74%) in the 2009 compared to low (1.15%) in the 2010 experiment on the final sampling date. The difference in plants showing MMV symptoms in experiments was mainly attributable to differences in *P. maidis* densities with respect to locality and seasonality. In 2009, percentage of plants showing hopperburn symptoms was significantly lower in the diculture compared to the monoculture on 22 DAP ( $F_{1,9} = 8.05$ ,  $P = 0.02$ ), and SH compared to BW and CP on 22 - 33 and 46 DAP (22 DAP:  $F_{1,9} = 105.00$ ,  $P < 0.01$ ; 29 DAP:  $F_{1,9} = 22.32$ ,  $P < 0.01$ ; 33 DAP:  $F_{1,9} = 5.49$ ,  $P = 0.04$ ; 46 DAP:  $F_{1,9} = 11.70$ ,  $P = 0.01$ ). The percentage was also significantly lower in BW compared to CP on 39 DAP (39 DAP:  $F_{1,9} = 5.63$ ,  $P = 0.04$ ) (Figure 3.3A). Similarly, percentage of plants showing virus symptoms was significantly lower in SH compared to

BW and CP on 40 - 46 DAP (40 DAP:  $F_{1,9} = 8.91$ ,  $P = 0.01$ ; 46 DAP:  $F_{1,9} = 15.18$ ,  $P < 0.01$ ) (Figure 3.3B). In the 2010 experiment, symptoms of hopperburn were not seen on any of the plants. The incidence of MMV among treatments was not significantly different at any sampling dates ( $F_{3,9} = 0.67$ ,  $P = 0.59$ ).

Effectiveness of visual scoring was validated using DAS-ELISA, which showed 10% and 100% of leaf samples collected from hopperburn and MMV symptomatic plants, respectively were positive for MMV in the 2009 experiment. All leaf samples with virus symptoms tested positive for MMV in the 2010 experiment.

## Discussion

The results of field experiments showed that the two experimental sites varied greatly in *P. maidis* population densities and their associated hopperburn and MMV symptoms on the corn plants. *P. maidis* densities were correlated with MMV incidence. High population densities of *P. maidis* at the Waialua site (2009) resulted in a greater percentage plants with MMV symptoms. Low population densities of *P. maidis* at the Waimanalo site (2010) produced a negligible percentage of plants with MMV symptoms. Despite these contrasting differences at the study sites, population densities of *P. maidis* and incidence of MMV were substantially lower in sunn hemp compared to cowpea and buckwheat habitats at the Waialua site, whereas the effect of treatment habitats on *P. maidis* densities and MMV incidence were negligible at the Waimanalo site.

### Effects on *P. maidis* densities

A recent study showed that population densities of rice planthoppers, *Nilaparvata lugens* (Stal.) and *Sogatella furcifera* Hawarth were significantly lower in rice polycultures with chili peppers, ginger, maize and peanut plants than monoculture rice plots (Lin et al. 2011). Similarly, Yao et al. (2012) showed that rice fields with corn intercrops had 26-48% fewer rice planthoppers, *N. lugens* (Stal.) than rice monoculture. Both of these studies illustrated that reduced densities of planthoppers was attributable to resource limitation, and visual- and odor- masking by non-rice plants in rice polycultures. Unlike these studies, the current study did not show remarkable effects of intercropping on *P. maidis* densities. However, results of the 2009 experiment showed that the colonizing populations of *P. maidis* were lower in sunn hemp than buckwheat and cowpea on the initial sampling date, while their within-field activity was lower in sunn hemp than buckwheat and cowpea by the final sampling date. Power (1987) showed that movement of corn leafhopper, *Dalbulus maidis* was higher in corn plots intercropped with bean or weeds, resulting in lower population densities than corn monoculture. There may therefore have been a change in within-field movement of macropterous *P. maidis*, possibly resulting in altered densities influenced by treatment habitats.

Counts of macropterous *P. maidis* on sticky traps provide an indication of *P. maidis* within-field activity during the growth of the corn plants. Results showed increased activity of *P. maidis* during V2 - V8 (early vegetative) stages and R (reproductive) stages, suggesting bimodal periodicity. Results of trap catches during V2 – V8 stages showed increased *P. maidis* activity in the dicultures compared with the monocultures. This suggests immigrating *P. maidis*, which randomly land on cover crops,

need to find their host plant through subsequent short distance flights, perhaps governed by host plant volatile cues (Liu et al. 1994), resulting in increased activity in the dicultures.

Reduced activity of macropterous *P. maidis* as indicated by trap counts during V12 to VT (tassel initiation) stages corresponded with colonization on the corn plants. Colonized planthoppers feeding on healthier plants normally produced brachypterous adults, followed by rapid population growth (overcrowding) (Denno et al. 1986, Denno and Roderick 1992). During colonization on plants, extensive feeding by planthoppers causes hopperburn and/or transmits viruses to host plants, resulting in reduction in the host plant nutritional quality. The overcrowding and reduced host plant nutritional quality triggers the production of macropterous offspring, which results in dispersal of the planthoppers (Denno et al. 1986, Denno and Roderick 1992). Similarly to these conditions, *P. maidis* activity increased at the R stage of corn plants indicating dispersal in the treatment plots. The results showed that sunn hemp reduced activity of *P. maidis* more than the other intercropping treatments during the R stage. The reduced activity of *P. maidis* in the sunn hemp treatment may be attributable to lower production of macropterous adults during the R stages aided by reduced colonization and less hopperburn incidence during the vegetative stages of corn plants.

Numbers of macropterous *P. maidis* encountered on corn plants were higher at the V12 to VT stages, which indicate the colonizing stage. Subsequently, newly emerging macropterous adults leave the plants resulting in reduced colonization, which coincide with increased activity in the field as indicated by trap catches during the R stage. In this experiment, sunn hemp reduced *P. maidis* colonization during the V2 – V8 stages, as a

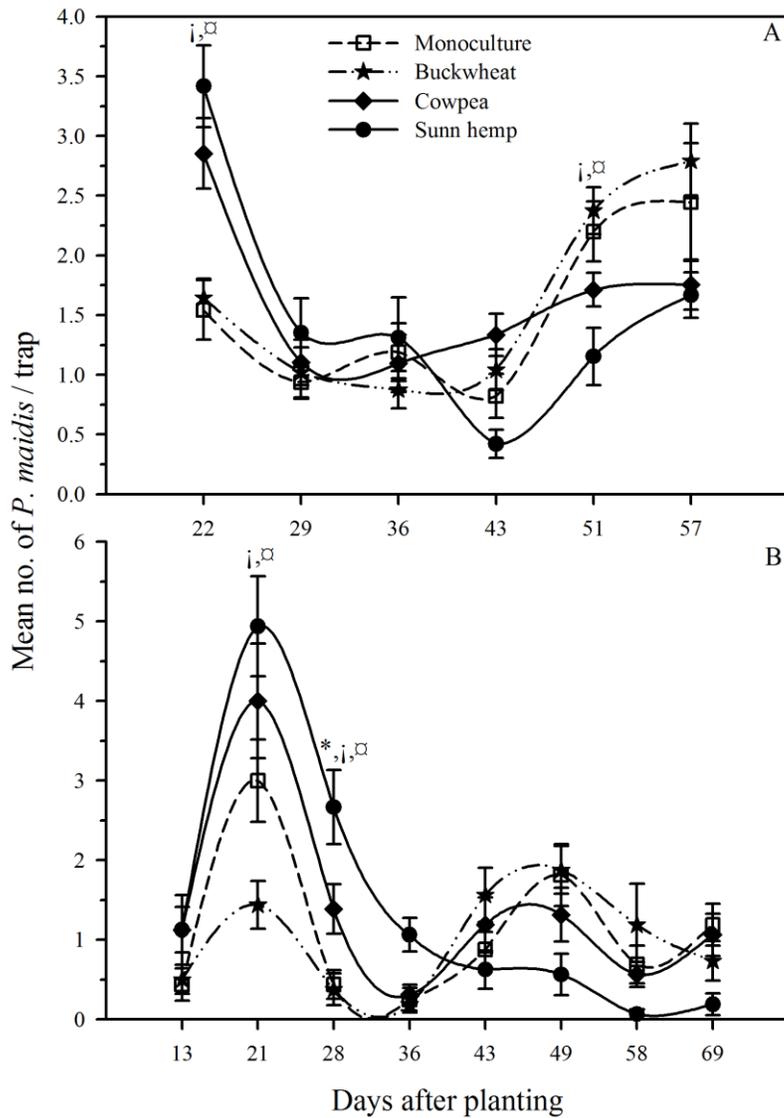
result of increased within-field activity. Higher colonization rates of *P. maidis* were found in the cowpea habitat, with large numbers of hoppers moving from the cowpea onto the corn, resulting in complete loss of corn plants before they reached the R stage. Observations showed that cowpea provided suitable shelter for *P. maidis*, and their movement from cowpea to corn increased *P. maidis* densities on corn plants. Similar results were obtained from the 2010 experiment, where the density of colonized *P. maidis* was higher in cowpea treatments on several sampling dates.

### **Incidence of hopperburn and MMV**

Management of persistently transmitted plant virus diseases using habitat management technique is not easy, owing to persistence of virus in the insect vector for their lifetime (Hogenhout et al. 2008). Persistent viruses have long incubation periods and require prolonged feeding by the vector to transmit successfully (Hogenhout et al. 2008). Therefore, vector tenure time in plants and vector movement are the key components that determine spread of the associated virus in the field. Power (1987) showed that increasing plant community diversity by intercropping maize with beans or weeds lowered corn leafhopper, *Dalbulus maidis* populations and decreased the incidence of corn stunt disease. Further analysis indicated leafhopper movement and emigration rates were higher in the polyculture plot that possibly contributed to lower leafhopper abundance and decrease in disease incidence. Similarly, population densities of oat bird cherry aphid, *Rhopalosiphum maidis* (L.) was significantly lower in genetically diversified oat field (*Avena sativa*) resulting reduced incidence of aphid - transmitted persistent virus, *Barley yellow dwarf virus*. Disease reduction in the diverse population of oat was mainly attributed to significantly higher movement of aphids and lower plant tenure time (Power

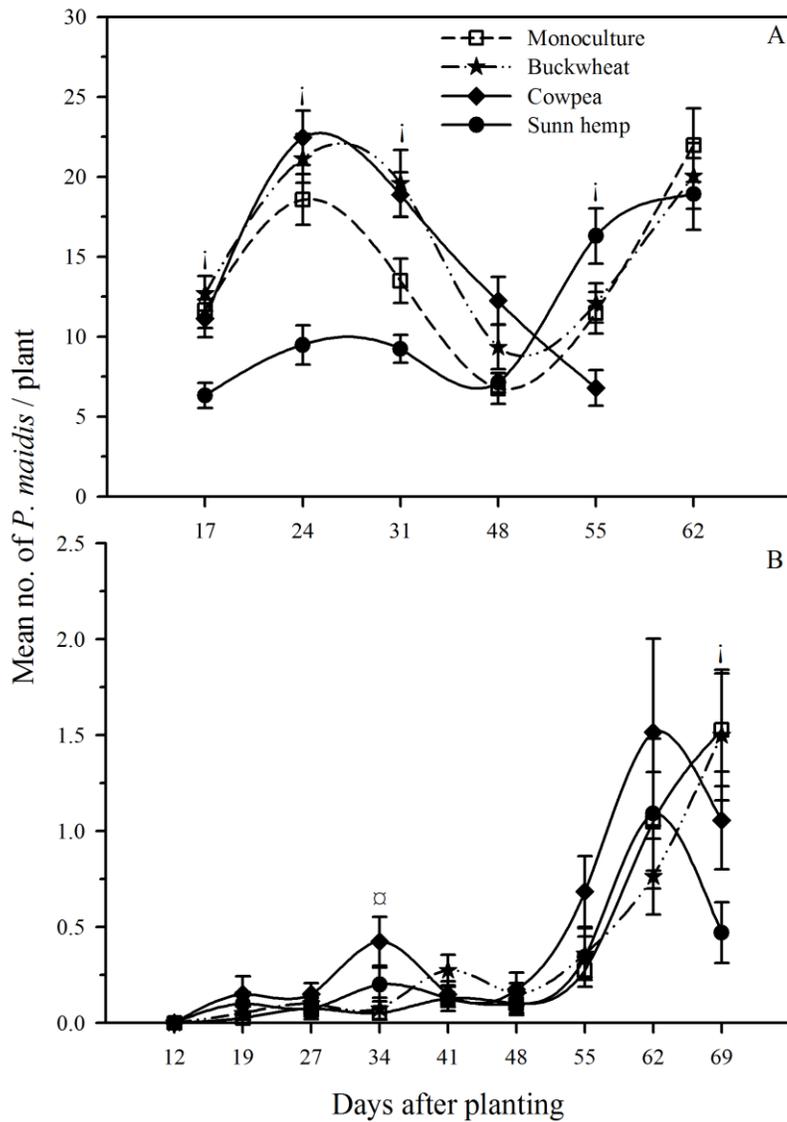
1991). Similar to these previously published results, the current study showed increased within-field movement of *P. maidis*, reduced numbers of colonized *P. maidis* on the corn plants during the V2 – V8 stages, resulting in reduced incidence of hopperburn symptomatic plants during the V2 – V8 stages and MMV symptomatic plants at the reproductive stages of the corn plant.

Results showed some evidence that a tall-statured sunn hemp intercrop may be helpful in altering *P. maidis* within-field movement behavior, resulting in lower population densities and reduced incidence of hopperburn and MMV symptomatic plants. Conversely, cowpea did not contribute to suppressing *P. maidis* densities and their damage and MMV symptoms in corn plants, but exacerbated the problem. The results from this study showed use of a suitable intercrop may be useful for management of insect vectors of persistent viruses, which are usually considered unmanageable by cultural modifications to cropping systems. Further similar studies will be made using sunn hemp as an intercrop in a higher intercropping ratio in larger field plots to mimic conditions similar to a large-scale agriculture landscape. These studies will examine the spatial-temporal distribution pattern of colonizing *P. maidis* as influenced by sunn hemp intercropping and their association with severity of hopperburn and MMV symptoms in the cornfield (see chapter IV). In addition, within-field activity *P. maidis* as influenced by sunn hemp intercrop and their effect on incidence MMV in cornfield will be studied in detail (see Chapter V).

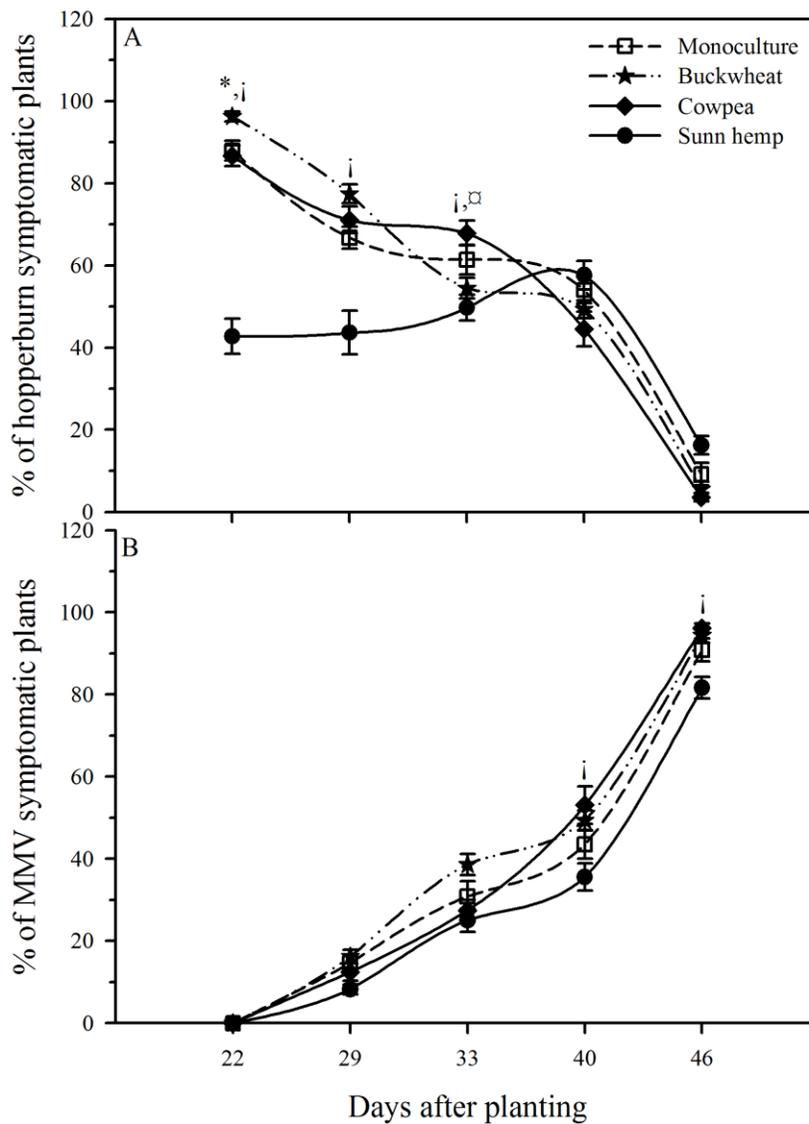


**Figure 3.1:** Mean number macropterous planthoppers, *Peregrinus maidis* ( $\pm$  Standard Error, SE) per sticky trap in different treatment habitats in (A) 2009 and (B) 2010.

Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures. \* Indicates diculture is significantly higher than monoculture, j indicates sunn hemp is significantly different from buckwheat and cowpea, i indicates buckwheat is significantly lower than cowpea.



**Figure 3.2:** Mean number macropterous planthoppers, *Peregrinus maidis* ( $\pm$  SE) per corn plant in different treatment habitats in (A) 2009 and (B) 2010. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures. j Indicates sunn hemp is significantly different from buckwheat and cowpea and a indicates buckwheat is significantly higher than cowpea.



**Figure 3.3:** Percentage of plants showing (A) hopperburn and (B) MMV symptoms ( $\pm$  SE) in different treatment habitats in the year 2009. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures. \* Indicates diculture is significantly lower than monoculture, j indicates sunn hemp is significantly lower than buckwheat and cowpea, i indicates buckwheat is significantly lower than cowpea.

## CHAPTER III

### **Optimizing intercropping density for corn earworm, *Helicoverpa zea* (Boddie) management: enhancing predators and parasitoids in a corn - cover crop intercropping system**

#### **Abstract**

Field experiments were conducted in Hawaii to examine effects of agricultural diversification through intercropping corn with flowering cover crops on (i) parasitism of corn earworm, *Helicoverpa zea* (Boddie) eggs by *Trichogramma* spp., (ii) abundance of the predator (*Orius* spp.) as influenced by prey [*H. zea* eggs and thrips (primarily, *Frankliniella occidentalis*)], and (iii) corn growth and ear yield. Sweet corn, *Zea mays* L. was grown with three flowering cover crops, buckwheat, *Fagopyrum esculentum* (Moench), cowpea, *Vigna unguiculata*, and sunn hemp, *Crotalaria juncea* L. at 2:1 and 4:1 (corn: intercrop) intercropping ratio in 2009 and 2010, respectively. Results showed that cowpea and sunn hemp provided a source of *Trichogramma* spp., resulting in higher parasitism of *H. zea* eggs on corn in the year 2010. Abundance of *Orius* spp. was significantly higher in the buckwheat treatment compared to the monoculture at similar levels of prey availability in the year 2009. Intercropping at a 4:1 corn: intercrop ratio minimized effects of plant competition, and resulted in similar plant growth and sweet corn yield as compared to the monoculture. Enhancement of natural enemies at the 4:1 intercropping ratio, without impacting on plant growth and sweet corn yield, supports the use of this technology in the large-scale corn farming without altering grower's practice to manage *H. zea* and thrips on corn.

## Introduction

Corn earworm, *Helicoverpa zea* (Boddie.) continues to be an important pest of numerous vegetable and field crops through out the United States (Capinera 2000, Cook and Weinzierl 2004). In many states, particularly in Hawaii, where corn, *Zea mays* L. is grown year round, *H. zea* has become a key perennial pest of sweet corn (Brewbaker 2003). High infestations of *H. zea* can develop over a short period as highly mobile adults lay their eggs on newly emerging corn silks (Nishida and Napometh 1974). After hatching, larvae move down to the ear tip through the silk channel and soon begin feeding on young developing kernels (Archer and Bynum 1997). Once *H. zea* larvae become established in the corn ear, they are protected against most pest management tactics including insecticides (Flood and Rabaey 2007, Burkness et al. 2009). In addition, *H. zea* larvae along with thrips, *Frankliniella occidentalis* provides access to the ear rot fungus, *Fusarium verticillioides* (Sacch.) through the feeding tunnels causing a serious contamination problem (Sobek and Munkvold 1999, Parsons and Munkvold 2010).

In the past, corn breeders in Hawaii have achieved a high degree of *H. zea* control by developing varieties with tight husks, which restricts *H. zea* larvae from moving down into the ear (Kim and Brewbaker 1975). However, even a single larva making into the ear can cause substantial damage. Additionally, husk morphology also influences intra-ear thrips populations and determines susceptibility to *Fusarium* ear rot disease (Warfield and Davis 1996, Farrar and Davis 1991). However, tolerance to *Fusarium* ear rot has been found to be variable and few, if any commercial varieties have shown adequate levels of resistance (Munkvold et al. 1997, Munkvold 2003, Clements et al. 2004). Recently developed transgenic *Bt* corn is effective against *H. zea* and can

reduce insecticide use (Burkness et al. 2001, 2002). EPA enforcement of growing *Bt* corn with at least 20% of area of non – *Bt* crop as the law for insect resistant management (EPA website), have not been popular among the corn growers in Hawaii. Thus few alternatives to the above practices that reduce insecticide dependence and provide food security are available to corn growers to effectively manage *H. zea* in corn.

Augmentative release of laboratory reared *Trichogramma* spp. is one approach to managing *Helicoverpa* populations by enhancing the rate of parasitism (King and Coleman 1989, Smith 1996). This strategy has been used effectively for suppressing European corn borer, *Ostrinia nubilalis* Pang through inoculative release of *T. ostriniae* Pang and Chen in US sweet corn fields (Hoffman et al. 2002, Wright et al. 2001, Wright et al. 2002). Another approach to *Helicoverpa* management is employing trap crops (e.g. marigold, *Bt*-corn, sorghum) which are preferred for oviposition and act as a sink for ovipositing moths (Srinivasan et al. 1981, Javaid et al. 2005, van den 2006, Tillman 2006). Incorporating floral resources is a conservation method that can enhance natural enemy populations and biological control of pests in agricultural systems (Landis et al. 2000, Zehnder et al. 2007). The goal is to create a suitable ecological infrastructure within the agricultural system to provide resources such as food for adult natural enemies, alternative prey and hosts, and shelter from adverse conditions (Gurr and Wratten, 1999, Baggen et al. 1999, Jonsson et al. 2008, Gurr et al. 2012). Therefore, the conservation biological control method may hold additive benefits over other alternatives for its potentiality to enhance multiple biological control agents (e.g. parasitoids, and predators) targeting multiple pests.

In several instances, higher numbers of insect natural enemies (e.g. parasitoids, and predators) were found in floral diversity through cover crops than monoculture that resulted in improved pest suppression in crops (Nicholls et al. 2000, English-Loeb et al. 2003, Tillman et al. 2004, Tillman and Mullinix 2004). Thus, increasing floral diversity with cover crops may enhance parasitoids including *Trichogramma* spp. and other predators (such as anthocorids, chrysopids) devouring *H. zea* eggs and thrips on corn. However, selecting flowering cover crops for a given pest and a crop scenario depends on the phenology, attractiveness and accessibility of the flowers to natural enemies (Patt 1997). For instance, early blooming cover crops can benefit natural enemies from their flowers and shelter they provide, and move to the cash crop at the time of pest outbreak. This can be achieved by adjusting planting dates of cover crops with corn to synchronize flowering as well as pest outbreak.

In this study, flowering cover crops which are not preferred *H. zea* oviposition sites, but may provide shelter and food (prey, pollen and nectar) for natural enemies, were investigated for their potential to help manage *H. zea* in corn. Preliminary investigations of arthropods occurring on sunn hemp showed their flowers and buds provide oviposition sites for *Lampides boeticus* (Linnaeus) (Lepidoptera: Lycaenidae). On an average, 40% of *L. boeticus* eggs on sunn hemp (*Crotolaria juncea* L., Leguminosae) flowers were found to be parasitized by *Trichogramma* spp. at different sites in Hawaii (personnel observation). Additionally, it has been reported that Lepidoptera oviposit on cowpea (*Vigna unguiculata*, Leguminosae) flowers or pods (Jackai 1981, 1986), which could potentially provide a reservoir egg hosts for *Trichogramma* spp. Thus, growing sunn hemp and cowpea may create a natural insectary

for egg parasitoids. Buckwheat [*Fagopyrum esculentum* (Moench), Polygonaceae], attracts pollinators and other predators such as syrphids, chrysopids, anthocorids and parasitoids (Platt et. al. 1999, Baggen et. al. 1999, Nicholls et al. 2000, English-Loeb et al. 2003). Hence, three flowering cover crops, buckwheat, cowpea, sunn hemp, were chosen as intercrops for creating diverse cropping systems. From here onwards, “diculture” indicates a diverse habitat achieved through intercropping a cover crop with corn, and “monoculture” indicates a simpler habitat with corn only.

The objective of this chapter is to examine the effect of flowering cover crops on parasitism of *H. zea* by *Trichogramma* spp. and the abundance of a generalist predator, *Orius* spp. as influenced by prey (*H. zea* eggs and thrips) availability in corn - cover crop intercropping systems. This chapter examines the following hypotheses (i) parasitism of *H. zea* eggs by *Trichogramma* spp. will be higher in dicultures than a monoculture (iv) densities of predators, *Orius* spp. as influenced by prey availability, will be higher in dicultures than a monoculture (v) greater corn growth and higher yield will be achieved in dicultures than a monoculture.

## **Methods**

Two field experiments were conducted to examine the effects of cover crops on parasitism of *H. zea* eggs and predator - prey relationships in corn - cover crop intercropping systems. Experiments were conducted at a parent seed-corn farm, Pioneer Hi-Bred International Inc. at Waialua and the University of Hawaii Manoa’s

experimentation station, Waimanalo Research Station at Waimanalo on Oahu, Hawaii in summer 2009 and spring 2010, respectively.

### **Experimental layout**

The experiment was set up in a randomized complete block design with each treatment replicated four times. Treatment plots were 13.7 m x 13.7 m, separated by 9 m in a block and each block was separated by 12.2 m. Treatments within blocks included three intercropped plots and a monoculture. Two and four rows of corn were grown in between the rows of cover crops in 2009 and 2010, respectively. In 2010, the number of rows of corn between two cover crop rows was increased in order to minimize competition between the cover crop and corn. A total of 12 rows of corn were grown per plot between 7 and 4 rows of cover crops in 2009 and 2010, respectively. The monoculture plot had 19 rows of corn. Time of corn planting was adjusted in order to synchronize flowering of the pre-planted and post-planted cover crops with the corn silking stage.

### **Crop planting**

Sunn hemp (variety Tropic Sun, Source: Crop Care Hawaii LLC) and cowpea (variety Iron and Clay, Source: Peaceful Valley Farm Supply, CA) seeds were sown approximately a month before corn planting at the rate of 40 g / row and 50 g / row, respectively. After seeding, plots were treated with a pre-emergence herbicide, Pendimethalin (Prowl H 20, BASF, NC) to check weeds during cover crop growth. A temperate corn variety Passion (Seminis Vegetable Seeds, CA), susceptible to *H. zea* was seeded on 26 May and 4 Feb in 2009 and 2010 experiments, respectively. Wheeler

(Precision Garden Seeder, Earthway Products Inc.) and Jab planters were used to seed corn in the 2009 and 2010 experiments, respectively. At least two seeds per a hole were planted at ~15 cm intra-row and ~75cm inter-row spacing. Buckwheat (Source: Peaceful Valley Farm Supply, CA) seeds were broadcasted a week after corn planting in a strip enclosed by two drip tubes placed 12 inches apart at the rate of 200 g / strip. In the 2009 experiment, a selective herbicide to broad leaf weeds, Mesotrione (Callisto, Syngenta, NC) was applied to rows of corn at the V2 - V3 stage (vegetative stage with initiation of 2 - 3 leaves), followed by hand weeding at the V8 stage to suppress weeds. In the 2010 experiment, a spray of pre-emergence herbicide, Atrazine (AAtrex 4L, Syngenta, NC) was applied immediately after corn seeding to suppress weeds until the final harvest. Corn plants were thinned out to one per hole at the V8 stage and fertilized following standard dosage and application schedules of each field station. Areas in between the field plots were cultivated to keep them weed free in both the years.

### **Ear sampling**

Sampling of corn ears was initiated at the point when approximately 40% of corn ears were producing silks. On each sampling date, 10 corn ears were randomly sampled and brought to the laboratory to isolate *H. zea* eggs from the corn silks. Each *H. zea* egg found on the silks was capsulated in a gel cap and observed for emergence of either larvae or parasitoid. Observations of number of eggs, and emergence of larvae or parasitoid were recorded. Additionally, each corn ear was beaten 30 - 40 times by hand onto a clean surface to dislodge thrips and *Orius* spp. The numbers of thrips and *Orius* spp. dislodged from each corn ear were counted. Corn ears were sampled at

approximately at five day intervals from July 14 (49 DAP) to July 24 (59 DAP), and March 29 (52 DAP) to April 7 (62 DAP) in 2009 and 2010, respectively.

To confirm if adults of *L. boeticus* use sunn hemp and cowpea flowers as an oviposition site and attract *Trichogramma* spp., sunn hemp and cowpea flowers were monitored prior to corn silking stage in the 2010 experiment. This was done by random sampling of 10 sunn hemp and cowpea inflorescences three times at 27, 36 and 43 days after corn planting and brought to the lab to isolate eggs of *L. boeticus*. Each *L. boeticus* egg was capsulated in a gel cap and observed for emergence of either larvae or parasitoids.

### **Corn growth and ear yield**

Corn plant height and canopy density were quantified to assess corn growth in the treatment plots. Plant height was measured from ten randomly selected corn plants in each treatment plot. Canopy density measurement was taken at five randomly sampled locations in each plot, using a Spherical densiometer (Forest Densiometer, Forestry Suppliers Inc.). The unfilled densiometer grids with corn foliage were counted by placing a densiometer directly below corn canopy at the ground level. The percentage of canopy density was determined by multiplying unfilled grid by 1.04 and subtracting the number from 100% as instructed in the instrument manual. Both the parameters were measured repeatedly at the same sampled plant or location within each plot, from June 19 (24 DAP) to July 13 (48 DAP) and March 8 (32 DAP) to April 12 (67 DAP) in 2009 and 2010, respectively.

Corn ears were harvested for fresh sweet corn yield at the grain filling stage as exhibited by recently dried silks on the ear. At harvesting stage, two rows were randomly selected and all corn ears were harvested. Total number of ears and corn ear mass for each plot were recorded. The mean sweet corn yield from each treatment plot was estimated assuming similar plant densities in the intercropped- and the monoculture treatments in tones / hectare.

### **Data Analyses**

Data from arthropod counts, percentage parasitism of *H. zea* eggs, and sweet corn yield (t / ha) were analyzed by using a mixed model analysis of variance (PROC MIXED, SAS Institute). In all cases, the model was constructed to examine the main effect of treatment by date, with block designated as a random factor. Data from plant measurements were analyzed using repeated-measures analysis of variance to examine between and within treatment effect with plant or location as a repeated subject (PROC MIXED, SAS Institute). Within each model, the following pre-planned orthogonal contrasts were conducted: dicultures (corn intercropped with buckwheat, corn intercropped with cowpea and corn intercropped with sunn hemp) vs. monoculture control, corn intercropped with sunn hemp vs. corn intercropped with buckwheat and corn intercropped with cowpea, and corn intercropped with buckwheat vs. corn intercropped with cowpea.

The proportion of *H. zea* eggs, which emerged into either larvae or parasitoids in each treatment, was compared with the monoculture using Fisher exact tests (PROC FREQ, SAS Institute). In analyzing the relationship between predator and prey, the

abundance of *Orius* spp. was correlated with the numbers of *H. zea* eggs, thrips and total of *H. zea* eggs and thrips (PROC CORR, SAS Institute). This analysis was made assuming each *H. zea* egg and each thrips constituted a prey item, and *Orius* spp. is the only dominant predator in the insect assemblage associated with the corn ears. In most cases, abundance of predator may depend on prey availability and there relationship may vary between the treatments. In such cases, an analysis considering prey availability as covariate could explain the actual effects of treatments on *Orius* spp. abundance. Effect of treatments on abundance of *Orius* spp. was examined using mixed model analysis of covariance (PROC MIXED, SAS institute) with their most highly correlated variable as a covariate. The data for each year were fitted to the three models to test the hypotheses whether the slopes are zero, unequal or parallel. A zero slope indicates treatments do not have significant effect on abundance of *Orius* spp. and not influenced by prey availability. Unequal slopes indicate treatments have a significant effect on abundance of *Orius* spp. as influenced by prey availability. Parallel slopes indicate that the treatments may not have significant effect on abundance of *Orius* spp. as influenced by prey availability. In such case, if the regression lines do not overlap, the significance of distance between two parallel regression lines is tested to examine any differences between treatments at a given prey density. The following regression models were fitted to the data in order to compare change in *Orius* densities with respect to prey availability in the treatment plots:

1.  $Y_{ij} = \alpha_i + \beta_i X_{ij} + b_j + e_{ij}$

2.  $Y_{ij} = \alpha_4 + (\alpha_i - \alpha_4) + \beta_4 X_{ij} + (\beta_i - \beta_4) X_{ij} + b_j + e_{ij}$

$$3. Y_{ij} = \alpha_i + \beta X_{ij} + b_j + e_{ij}$$

Where,  $i = 1, 2, 3, 4$  (treatments)

$j = 1, 2, 3, 4$  (blocks)

$Y$  = dependent variable (abundance of *Orius* spp.)

$X$  = independent variable (levels of *H. zea* eggs ( $H_z$ ) + thrips, treatment x  $H_z$  + thrips)

$\alpha_i$  = the intercept of  $i^{\text{th}}$  treatment model

$\beta_i$  = the slope of  $i^{\text{th}}$  treatment model

$\beta$  = the common slope of the treatment models

$b_j$  = the effect of  $j^{\text{th}}$  block

$e_{ij}$  = the experimental error

*Model 1:* The type 3  $F$ -statistics corresponding to ‘treatment x  $H_z$  + thrips’ tests the hypothesis (Slopes equal to zero),  $H_0: \beta_1 = \beta_2 = \beta_3 = \beta_4 = 0$ , i. e. the slopes are most likely equal to zero versus  $H_a$  (not  $H_0$ ). The type 3  $F$ -statistics corresponding to ‘treatment’ tests the hypothesis  $H_0: \alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = 0$  versus  $H_a$ : (not  $H_0$ ) at ‘ $H_z$  + thrips’ = 0.

*Model 2:* The type 3  $F$ -statistics corresponding to ‘treatment x  $H_z$  + thrips’ tests the hypothesis (Unequal slopes),  $H_0: \beta_1 - \beta_4 = \beta_2 - \beta_4 = \beta_3 - \beta_4 = 0$ , i. e. slopes are most likely unequal versus  $H_a$  (not  $H_0$ ). The type 3  $F$ -statistics corresponding to ‘treatment’

tests the hypothesis  $H_0: \alpha_1 - \alpha_4 = \alpha_2 - \alpha_4 = \alpha_3 - \alpha_4 = 0$  versus  $H_a: (\text{not } H_0)$  at ‘ $H_z + \text{thrips}$ ’ = 0.

*Model 3:* The type 3  $F$ -statistics corresponding to ‘ $H_z + \text{thrips}$ ’ tests the hypothesis for a common slope model (Parallel slope),  $H_0$ : common slope = 0 versus  $H_a$ : common slopes are significantly different from zero. The type 3  $F$ -statistics corresponding to ‘treatment’ tests the hypothesis that the distance between two regression lines are not significantly different.

All the statistical analysis procedures involving mixed models in SAS were followed as described by Littell et al. (2006).

## Results

### Parasitism of *H. zea* eggs

The mean number of *H. zea* eggs on silks per ear was lower ( $1.42 \pm 0.13$ , mean  $\pm$  SE) in the 2009 experiment compared to ( $3.13 \pm 0.15$ ) in the 2010 experiment. In 2009, parasitism of *H. zea* eggs by *Trichogramma* spp. was negligible, observed on only two occasions, once in sunn hemp treatment plot (SH) and monoculture control. In contrast, the average percentage parasitism of *H. zea* eggs was higher ( $27.18 \pm 0.15$ ) in the 2010 experiment. The overall percentage parasitism was not significantly different among the treatment habitats ( $F_{3,9} = 2.10$ ,  $P = 0.17$ ), but was significantly higher in cowpea treatment plots (CP) compared to buckwheat treatment plots (BW) on two sampling dates (55DAP:  $F_{1,9} = 7.85$ ,  $P = 0.02$ ; 59 DAP:  $F_{1,9} = 8.74$ ,  $P = 0.02$ ) (Figure 4.1). During the

silking stage, percentage parasitism of *H. zea* eggs on silks in the monoculture treatment generally remained low during the first two sampling dates, but eventually increased on the final sampling date. Considering the increase in parasitism at the final date as a rare event, chi-square analysis was conducted excluding the data set from the final sampling date. In this analysis, proportion of *H. zea* eggs which hatched into larvae or *Trichogramma* spp. were significantly different between the treatment habitats ( $\chi^2 = 20.60$ ,  $df = 3$ ,  $P < 0.01$ ). Further, Fisher exact tests showed that proportion of eggs, which yielded into *Trichogramma* spp. was significantly higher in CP and SH compared to the monoculture control (CP:  $df = 1$ ,  $P = 0.01$ ; SH:  $df = 1$ ,  $P = 0.01$ ) (Figure 4.2). Further observations made on sunn hemp and cowpea flowers prior to corn silking stage showed abundance of *L. boeticus* eggs were significantly higher on sunn hemp than cowpea whereas parasitism of *L. boeticus* eggs were significantly higher in cowpea than sunn hemp flowers (Figure: 4.3). *Trichogramma* spp. yielded from samples of *L. boeticus* eggs and *H. zea* eggs were identified as *T. pretiosum*, and *Trichogrammatoidea* spp. (less than 5% of samples) by R. Stouthamer using the internally transcribed spacer 2 (ITS2) gene (as described in Stouthamer et al. 1999).

### **Predator - prey densities**

Average numbers of thrips on silks per ear were higher ( $9.13 \pm 0.59$ ) in the 2009 compared to ( $6.07 \pm 0.19$ ) in the 2010 experiment. In 2009, number of thrips was significantly lower in dicultures compared to the monoculture ( $F_{1,6} = 19.09$ ,  $P < 0.01$ ) and SH compared to BW ( $F_{1,6} = 8.94$ ,  $P = 0.02$ ). In 2010, thrips numbers were significantly higher in SH compared to BW and CP ( $F_{1,9} = 5.62$ ,  $P = 0.04$ ) (Figure 4.4A). Average numbers of *Orius* spp. were higher ( $4.63 \pm 0.20$ ) in the 2009 compared to ( $0.26$

$\pm 0.02$ ) in the 2010 experiment. The *Orius* numbers were not statistically significant in the 2009 ( $F_{2,6} = 1.85$ ,  $P = 0.2362$ ), were significantly higher in BW compared to CP ( $F_{1,9} = 5.58$ ,  $P = 0.0425$ ) (Figure 4.4B). Composite samples of *Orius* spp. individuals collected from the Waialua site were identified as *O. persequens* (87.5%) and *O. tristicolor* (12.5%), whereas all individuals from a composite sample collected from the Waimanalo site were identified as *O. persequens* (identified by Dr. D. R. Horton, ARS, USDA, Wapato, WA).

### **Predator - prey relationship**

Pearsons correlation coefficients ( $r$ ) between ‘*Orius*’ and ‘*H<sub>Z</sub>*’, ‘thrips’ and ‘*H<sub>Z</sub>* + thrips’ are shown in Table 1.1. The correlation between ‘*Orius*’ and ‘*H<sub>Z</sub>* + thrips’ was higher than either ‘*H<sub>Z</sub>*’ or ‘thrips’ alone in both experiments. This result showed abundance of *Orius* spp. is dependent on the prey ‘*H<sub>Z</sub>* + thrips’ availability. Hence, the number of ‘*H<sub>Z</sub>* + thrips’ was considered as a covariate when analyzing the effect of treatments on abundance of *Orius* spp. on silks. The data for each year were fitted to the three models (described in Data Analyses in Methods section) to test the hypotheses whether the slopes are zero, unequal or parallel.

*Year 2009:* Fitting model (1) to the data, the  $F$ -statistics corresponding to ‘treatment x *H<sub>Z</sub>* + thrips’ ( $F_{3,258} = 7.79$ ,  $P < 0.01$ ) indicated that the slopes are most likely not all equal to zero. The  $F$ -statistics corresponding to ‘treatment’ ( $F_{3,62.4} = 39.63$ ,  $P < 0.01$ ) indicated that the intercepts are most likely, but not all equal to zero, at ‘*H<sub>Z</sub>* + thrips’ = 0. Fitting model (2) to the data, the  $F$ -statistics corresponding to ‘treatment x *H<sub>Z</sub>* + thrips’ ( $F_{2,258} = 1.80$ ,  $P = 0.17$ ) indicated there is no sufficient evidence that the

treatments have unequal slopes, hence a common slope model should be adequate to describe the relationship between ‘*Orius*’ and ‘*H<sub>Z</sub>* + thrips’. Fitting model (3) to the data, the *F*-statistics corresponding to ‘*H<sub>Z</sub>* + thrips’ ( $F_{1,263} = 19.62, P < 0.01$ ) indicated that the common slope is significantly different from zero. The *F*-statistics corresponding to ‘treatment’ ( $F_{3,41.1} = 54.04, P < 0.01$ ) indicate that the distance between the regression lines are significantly different (Table 1.2, Figure 4.5). The pairwise comparison among the treatment models at mean density of ‘*H<sub>Z</sub>* + thrips’ = 10.56 showed a significant difference in distance between models for monoculture and BW ( $\beta = -1.2412, t_{252} = -2.80, P = 0.01$ ). This result indicated abundance of *Orius* spp. was significantly higher in the buckwheat-intercropped treatment compared to the corn monoculture at the mean density of prey.

*Year 2010:* Fitting model (1) to the data, the *F*-statistics corresponding to ‘treatment x *H<sub>Z</sub>* + thrips’ ( $F_{4,454} = 2.56, P = 0.04$ ) indicated that the slopes are most likely not all equal to zero. The *F*-statistics corresponding to ‘treatment’ ( $F_{4,105} = 2.21, P = 0.07$ ) indicated that the intercepts are most likely, but not all equal to zero, at ‘*H<sub>Z</sub>* + thrips’ = 0. Fitting the model (2) to the data, the *F*-statistics corresponding to ‘treatment x *H<sub>Z</sub>* + thrips’ ( $F_{3,454} = 0.43, P = 0.73$ ) indicated there is no sufficient evidence that the treatment has unequal slopes, hence common slope may be adequate describe the relation between the ‘*Orius*’ and ‘*H<sub>Z</sub>* + thrips’. Fitting model (3) to the data, the *F*-statistics corresponding to ‘*H<sub>Z</sub>* + thrips’ ( $F_{1,456} = 8.98, P < 0.01$ ) indicated that the common slope is significantly different from zero, The *F*-statistics corresponding to ‘treatment’ ( $F_{3,455} = 2.47, P = 0.06$ ) indicates the distance between the regression lines are not significantly different (Table 1.3). However, pairwise comparisons among the treatment models at

mean density of ‘*H<sub>Z</sub>* + thrips’ = 9.20 showed significant difference in distance between models for BW and CP ( $\beta = 0.1477$ ,  $t_{455} = 2.12$ ,  $P = 0.03$ ), and SH and CP ( $\beta = -0.1512$ ,  $t_{455} = -2.14$ ,  $P = 0.03$ ). This result indicated that abundance of *Orius* spp. was significantly higher in the buckwheat- and sunn hemp-intercropped treatments compared to the cowpea-intercropped treatment at the mean density of prey.

### **Corn growth and yield**

In the 2009 experiment, although rate of increase in canopy density during the development of corn plant was similar among the treatments (treatment x time:  $F_{9,292} = 1.13$ ,  $P = 0.34$ ), increased rates of canopy density development were observed in monoculture and BW compared to CP and SH (Figure 4.6A). The rate of increase in corn plant height was significantly different among the treatments (treatment x time:  $F_{9,606} = 5.80$ ,  $P < 0.01$ ). The plant height was significantly lower in dicultures compared to the monoculture from 34 – 48 DAP (34 DAP:  $F_{1,9} = 10.03$ ,  $P = 0.01$ ; 41 DAP:  $F_{1,6} = 6.81$ ,  $P = 0.02$ ; 48 DAP:  $F_{1,6} = 14.71$ ,  $P < 0.01$ ) (Figure 4.6B).

In the 2010 experiment, rate of increase of canopy density during the development of corn plants was significantly different among the treatments (treatment x time:  $F_{3,140} = 2.97$ ,  $P = 0.03$ ), however no significance difference was detected on each measured date ( $F_{3,9} = 0.46$ ,  $P = 0.72$ ) (Figure 4.7A). The rate of increase in plant height during the development of corn plants was significantly different among the treatments (treatment x time:  $F_{9,612} = 5.92$ ,  $P < 0.01$ ). The plant height was significantly lower in BW compared to CP from 32 – 49 DAP (32 DAP:  $F_{1,9} = 10.00$ ,  $P = 0.01$ ; 41 DAP:  $F_{1,9} = 5.93$ ,  $P = 0.04$ ; 49 DAP:  $F_{1,9} = 8.92$ ,  $P = 0.02$ ). Additionally, the plant height was

significantly higher in SH compared to BW and CP from 49 – 67 DAP (49 DAP:  $F_{1,9} = 10.02$ ,  $P = 0.01$ ; 67 DAP:  $F_{1,6} = 11.25$ ,  $P = 0.01$ ) (Figure 4.7B).

In the year 2009, corn plants in CP did not attain reproductive stage, owing to plant mortality caused by *Peregrinus maidis* feeding damage and *Maize mosaic virus*, MMV. On the other hand, corn ears in SH did not attain marketable size, due to competition posed by tall sunn hemp plants. The sweet corn yields from monoculture and BW was substantially low and not recorded for statistical analysis. In the year 2010, higher marketable sweet corn yield was recorded, however no significant difference ( $F_{3,9} = 0.79$ ,  $P = 0.53$ ) was observed on ear yield among the treatment plots (Figure 4.8).

## Discussion

The results of the field experiments showed that the two experimental sites had different pest densities and variable associations with natural enemies. The experimental site at Waialua (2009) had high population density of thrips, and low abundance of *H. zea* eggs. At this site, the abundance *Orius* spp. as influenced by prey (thrips and *H. zea* eggs) availability was significantly higher in the buckwheat treatment compared to the corn monoculture, whereas parasitism of *H. zea* eggs by *Trichogramma* spp. was negligible. In contrast, the experimental site at Waimanalo (2010) had low population density of thrips and high abundance of *H. zea* eggs. At this site, the abundance of *Orius* spp. as influenced by prey availability was not significant between the intercropped treatment and monoculture, whereas parasitism of *H. zea* eggs was significantly higher in cowpea and sunn hemp intercropped treatments compared to the monoculture.

### **Parasitism of *H. zea* eggs**

Trap crops grown with the main crop have been used as an attractant for *Helicoverpa* spp. oviposition, and have been successfully used to control these insects in tomato (Srinivasan et al. 1981) and soybean (Javaid et. al. 2005). In these studies, trap crops such as African marigold and *Bt*-corn were used as a sink for *Helicoverpa* eggs. Grain sorghum, *Sorghum bicolor* (L.) Moench efficiently trapped *H. zea* eggs laid by emerging adults from adjacent cornfields in a mixed sorghum - cotton field; parasitism of *H. zea* eggs by *T. pretiosum* and number of predatory insects including *O. insidiosus*, were significantly higher in cotton plots with sorghum traps than the cotton plots only (Tillman and Mullinix 2004). This study indicated that trap plants are not only a sink for *H. zea* eggs but may also provide a nursery of natural enemies. Similarly, the current study showed that cover crops intercropped with corn enhanced biological control agents, *Trichogramma* spp. and *Orius* spp., resulting in increased parasitism of *H. zea* eggs and predation of thrips and *H. zea* eggs, compared to the corn monoculture.

In several studies, buckwheat was used to provide floral resources that led to increased parasitism and improved pest suppression. Adding floral diversity to vineyards by planting buckwheat and sunflower, *Helianthus annuus* Linnaeus, increased abundance of the parasitoid *Anagrus epos* Girault (Hymenoptera: Myrmaridae) which caused increased mortality of grape leafhopper, *Erythroneura elegantula* Osborn eggs, compared to monoculture plots (Nicholls et al. 2000). A similar study by English-Loeb et al. (2003) showed that parasitism of *Erythroneura* leafhopper eggs by *Anagrus* parasitoids was higher in commercial vineyards when grown with buckwheat compared to clover (*Trifolium repens* L.) and mowed sod (*Dactylis glomerata* L.), indicating parasitism by

*Anagrus* is enhanced by providing floral resources in vineyards. Brendt et al. (2006) showed that adding buckwheat in vineyard plots increased parasitism of leafroller, *Epiphyas postvittana* (Lepidoptera: Tortricidae) larvae by *Dolichogenidea tasmanica* (Hymenoptera: Braconidae) compared with vine plots without buckwheat. These studies concluded that floral resources (nectar and pollen) of buckwheat flowers enhanced survival, longevity and fecundity of parasitoids and their potential to contribute to pest suppression in the agriculture systems. In the current study, buckwheat-intercropped treatment did not increase parasitism of *H. zea* eggs by *Trichogramma* spp. on corn plants, but parasitism was significantly greater in sunn hemp- and cowpea-intercropped treatments compared to the monoculture. Although parasitism rates by *Trichogramma* spp. as influenced by flowering resources has not been studied previously, greenhouse and cage experiments showed increased survival, longevity and fecundity of *Trichogramma* spp. in presence of flowering plants (Begum et al. 2006, Bassinger – Witting et al. 2008). In the current study, the greater parasitism of *H zea* eggs in cowpea and sunn hemp was mainly attributable to their flowers, which provided oviposition sites for *L. boeticus* (*L. boeticus* eggs were parasitized by *Trichogramma* spp.), and partially to their floral resources (pollens and nectar).

Parasitoid exploitation of alternative hosts in non-crop vegetation has been shown to be an effective means of providing a natural enemy reservoir. A study using marking and tracking techniques showed that the parasitic wasp *Diadegma semiclausum* (Helen) moved from buckwheat to broccoli, causing greater parasitism of *Plutella xylostella* larvae in broccoli planted with buckwheat strips compared with broccoli alone (Lavandero et al. 2005). In this study, *Trichogramma* spp. that persisted in cowpea and

sun hemp flowers seem to have dispersed to corn rows at the silking stage resulting higher parasitism of *H. zea* eggs. Abundance of *L. boeticus* eggs was significantly higher on sun hemp flowers, whereas parasitism of *L. boeticus* eggs was significantly higher in cowpea. As a result, parasitism of *H. zea* eggs on corn silks was higher in cowpea compared to sun hemp-intercropped treatments. On the other hand, buckwheat did not provide an oviposition site for *L. boeticus*, and thus resulted in lower parasitism of *H. zea* eggs on corn silks in the buckwheat-intercropped treatment. These results showed that cowpea and sun hemp provided shelter (oviposition site for *L. boeticus*) for *Trichogramma* spp. that increased their abundance, resulting greater parasitism of *H. zea* eggs on corn.

### **Predator - prey relationships**

Field mortality of *H. zea* eggs has typically been attributable to hemipteran predators, such as *O. tristicolor* (White), *O. insidiosus* (Say), and *Geocoris punctipes* (Say), as well as *Coleomegilla maculata* (DeGeer) (Nuessly and Sterling 1994, Pfannenstiel and Yeorgan 2002). In a diverse cropping system, cover crops provide suitable habitats to predators that may have dispersed to crops to contribute greater predation of pest population. For example, the predators of heliothines that are found in cover crops dispersed to cotton plants, resulting in reduced *H. virescens* (F.) and *H. zea* infestation levels in a cover crop – cotton cropping system (Tillman et al. 2004). Similarly, Bickerton and Hamilton (2012) showed seasonal predation of *O. nubilalis* eggs by *O. insidiosus* (Say) was significantly higher in bell pepper intercropped with flowering plants (Dill, *Anethum graveolens* L., Coriander, *Coriandrum sativum* L. or buckwheat) compared with non-intercropped plots. However, the current study did not show

significant increases in *Orius* spp. density in corn intercropped with cover crops compared to the monoculture. This may be the result of a density dependent relationship between *Orius* spp. and their prey populations in the treatment plots.

Density dependence relationship been demonstrated in other predator - prey systems. For example, Sansone and Smith (2001) showed a rapid increase in *Orius* spp. numbers at the time of cotton blooming, which coincided with increase in abundance of *H. armigera* and *H. virescens* eggs on flowers. Further, the authors examined predator gut contents using ELISA, and found a higher frequency of predators to be positive for moth egg antigen at the cotton blooming stage, indicating a density dependent predator - prey relationship. Similarly, the relationship between predators [*O. insidiosus* (Say) and *O. pumilio* (Champion)] and prey (*F. bispinosa*) were also found to be density dependent, with a large initial prey population followed by a rapid decline, as the predator population increased on the flowers of *Daucus carota* L. and *Ammi majus* L. (Shirk et al., 2012). Similarly, in the current study, the predator - prey relationship was found to be of moderate strength, indicating moderate density dependence at the corn silking stage.

The current study examined the effect of intercropped treatments on abundance of *Orius* spp. as influenced by prey availability. The results showed that the abundance of *Orius* spp. was significantly greater in the buckwheat treatment compared to the monoculture at the mean density of prey. This result indicated predator - prey relationships was stronger in the buckwheat treatment as compared to the monoculture. Increased survival, longevity and fecundity of *O. majusculus* (Reuter) has been demonstrated in alyssum plant (*Lobularia maritima* L.) with prey, eggs of *Ephestia kuehniella* than without prey, suggesting a companion plant needs to provide both flower

resources (nectar and pollens) and prey items to enhance omnivorous predators effectively (Pumarino and Alomar 2012). In the current study, stronger predator - prey relationship in the buckwheat-intercropped treatment indicated buckwheat might have provided better quality floral resources and habitats for other preys, such as thrips, aphids and whiteflies.

### **Corn growth and ear yield**

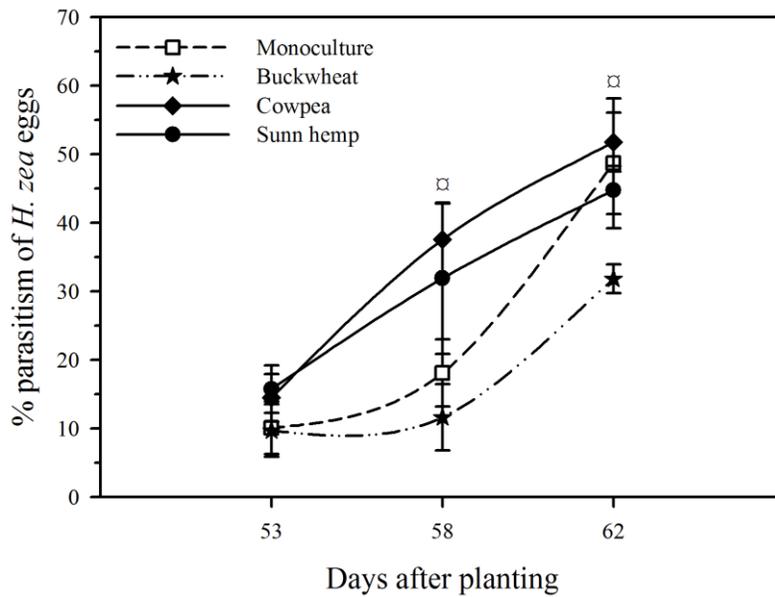
In the 2009 experiment, significant inter-specific competition had caused poor corn growth and no marketable ear yield in corn intercropped with sunn hemp and cowpea treatments. This inter-specific competition was primarily due to lower corn to cover crop ratio (2:1) and vigorous growth of cowpea and sunn hemp, as fueled by long day length during summer. However, higher *P. maidis* feeding damage and MMV infection in the cowpea-intercropped treatments were the primary cause of plants dying before reaching the silking stage (Chapter II). In addition, sunn hemp did not flower and cowpea flowered at the end of the experiment (until 73 days after corn planting) and thus did not provide an oviposition site for *L. boeticus* at the right time to correspond with the target crop. This might be the reason for the rare occurrence of parasitism of *H. zea* eggs by *Trichogramma* spp. in the 2009 experiment.

In the 2010 experiment, despite the sunn hemp being taller than the corn, the higher intercropping ratio (4:1) provided enough space for crops to grow independently, eliminating possible inter-specific competition. Sunn hemp, cowpea and buckwheat flowered before the corn silking and persisted through the reproductive stages. Hence, benefits of a flowering cover crop were observed on parasitism of *H. zea* eggs. Plant

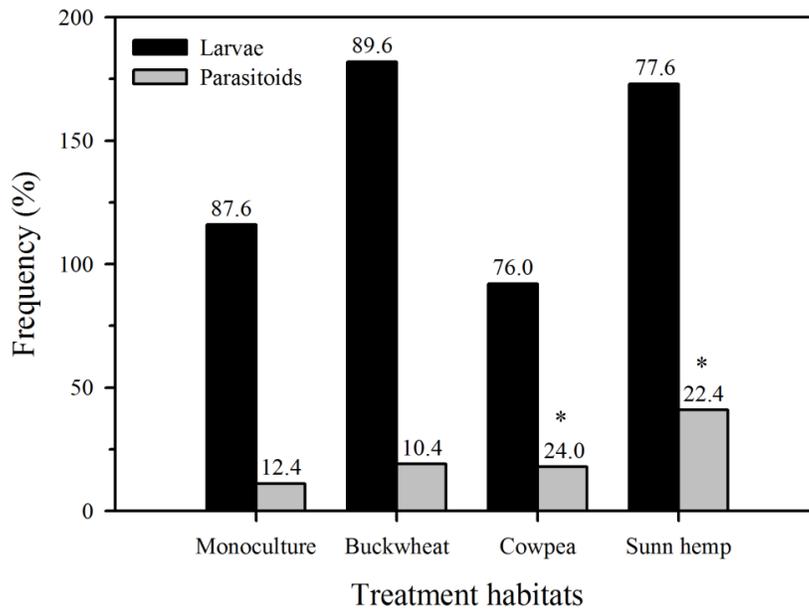
height and canopy density of corn plants were similar among the treatment habitats, producing similar sweet corn yield in treatment plots. Although quantitative measurements of sweet corn quality were not made, higher parasitism of *H. zea* eggs and stronger predator - prey relationships in the cover crop intercropped treatments suggests corn ears with *H. zea* and *Fusarium* rot infestation should be lower in the cover crop intercropped treatments than in the monoculture.

Cover crops used in this study had variable effects on the biological control agents and the pest targeted on corn. Sunn hemp and cowpea increased parasitism of *H. zea* eggs whereas buckwheat increased predation of *H. zea* eggs and thrips in corn - cover crop intercropping systems. Therefore, in areas with high *H. zea* densities, either cowpea or sunn hemp intercropping is recommended to suppress *H. zea* infestation on corn. In addition, intercropping buckwheat can enhance generalist predators, suppressing multiple corn pests such as *H. zea* and thrips, can be useful in the areas with greater *Fusarium* ear rot problems. However, intercropping cowpea is not recommended in the areas with high *P. maidis* densities, to avoid severe feeding damage and MMV spread in corn (Chapter II). The effectiveness of cover crops in reducing pest infestation level at a greater corn to cover crop ratio could be compatible with large-scale corn farming without altering grower's practices. As such, either strips or patches of suitable cover crop can be grown in a corn farm landscape to provide services in enhancing biological control agents of corn pests. This may be helpful in sustaining populations of natural enemy populations at the time of pest outbreak and at the time of insecticidal spray for suppressing other related pest problems.

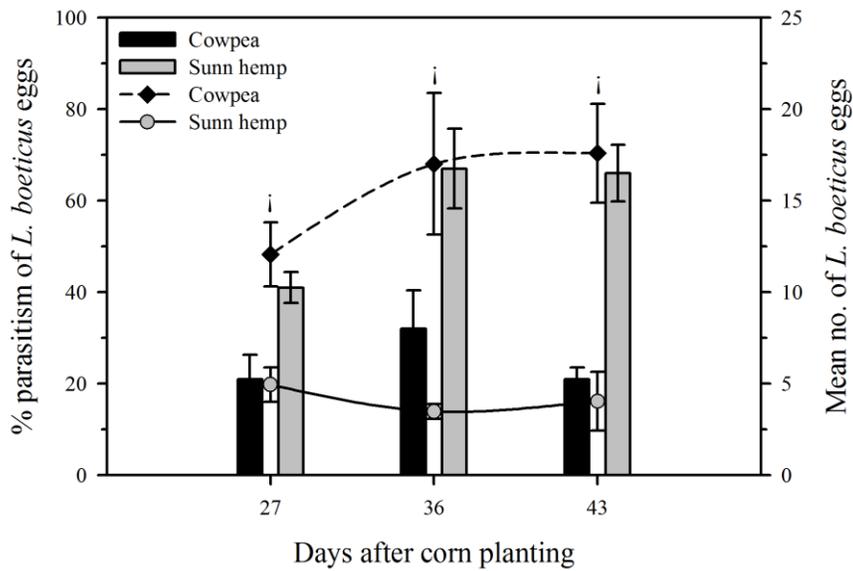
Chapter II and the current chapter showed that sunn hemp provided overall benefits by enhancing parasitism and predation of *H. zea* eggs, and reducing *P. maidis* densities and their associated plant damage on corn plants. Further, similar studies will be made using sunn hemp as an intercrop in a higher intercropping ratio in larger field plots to mimic conditions similar to a larger scale agriculture landscape (Chapters IV and V). In addition, conservation biological control through incorporating floral resources of sunn hemp in corn will be compared with augmentative biological control with release of *T. pretiosum* in corn monoculture to examine best alternative pest management option to suppress *H. zea* infestations on corn (see chapter VI).



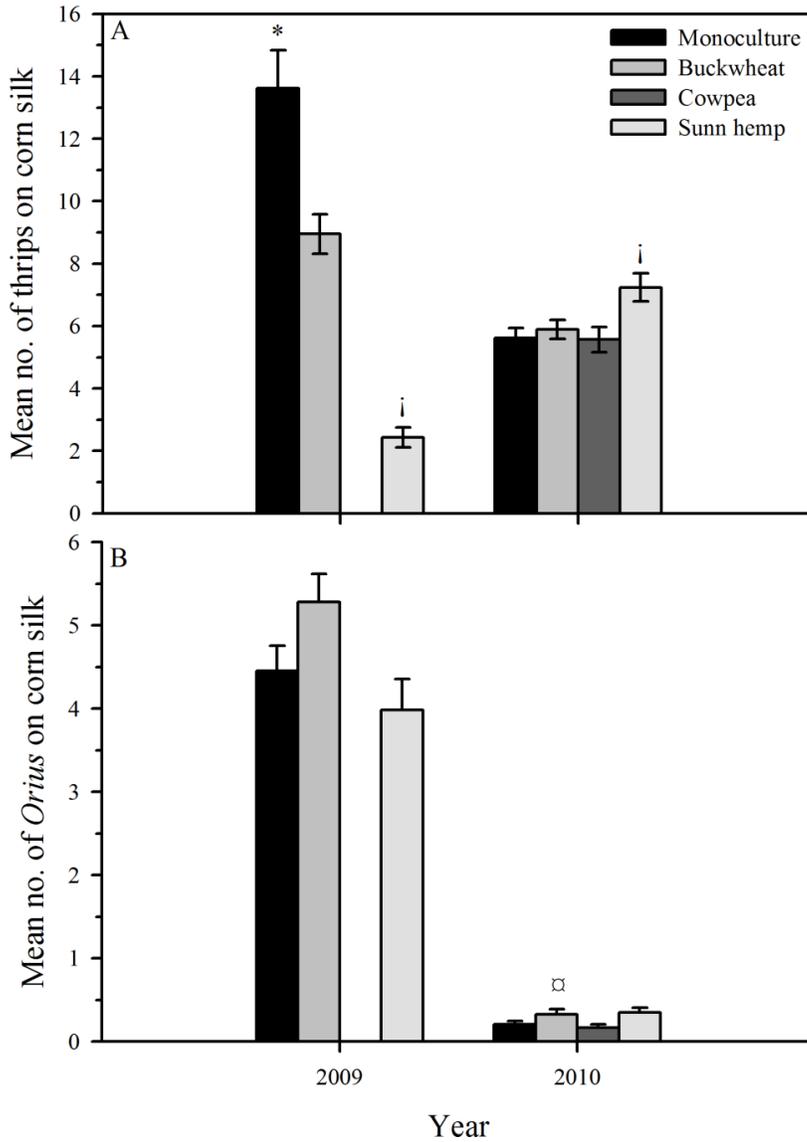
**Figure 4.1:** Percentage parasitism of *Helicoverpa zea* eggs ( $\pm$  SE) by *Trichogramma* spp. in different treatment habitats in the year 2010 at Waimanalo. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures.  $\boxtimes$  Indicates buckwheat is significantly lower than buckwheat cowpea.



**Figure 4.2:** Proportion of *Helicoverpa zea* eggs emerged into larvae or parasitoids in different treatment habitats in the year 2010 at Waimanalo. Larvae and parasitoids indicate *H. zea* larvae and *Trichogramma* spp. emerged from *H. zea* eggs, respectively. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures. \*Indicates cowpea and sunn hemp is significantly higher than monoculture.



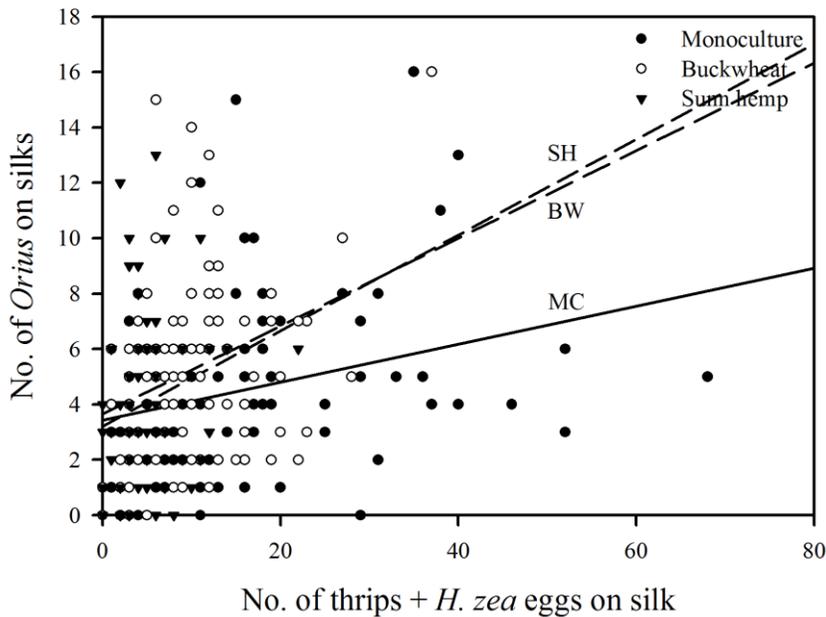
**Figure 4.3:** Mean numbers of *Lampidus boeticus* eggs ( $\pm$  SE) on cowpea (CP) and sunn hemp (SH) inflorescence and percentage parasitism ( $\pm$  SE) of the *L. boeticus* eggs by *Trichogramma* spp. collected from respective inflorescence in the year 2010 at Waimanalo. j Indicates CP is significantly different from SH.



**Figure 4.4:** Mean number of (A) thrips and (B) *Orius* spp. on silk ( $\pm$  SE) per corn ear in different treatment habitats in 2009 (Waialua) and 2010 (Waimanalo). Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures. \*Indicates diculture is significantly lower than monoculture, † indicates sunn hemp is significantly different from buckwheat and cowpea and ‡ indicates buckwheat is significantly higher than cowpea.

**Table 1.1:** Pearson’s correlation coefficient ( $r$ ) between variables, *Orius* spp. (*Orius*) with *Helicoverpa zea* eggs (*Hz*), thrips, and *Hz* + thrips, and their respective  $P$ -values (parentheses) in 2009 (Waiialua) and 2010 (Waimanalo).

<i>Year</i>	<i>Hz / Orius</i>	<i>Thrips / Orius</i>	<i>Hz + thrips / Orius</i>
2009 $r$ ( $P$ )	0.16 (0.01)	0.23 (<0.01)	0.26 (<0.01)
2010 $r$ ( $P$ )	0.13 (<0.01)	0.13 (0.01)	0.16 (<0.01)



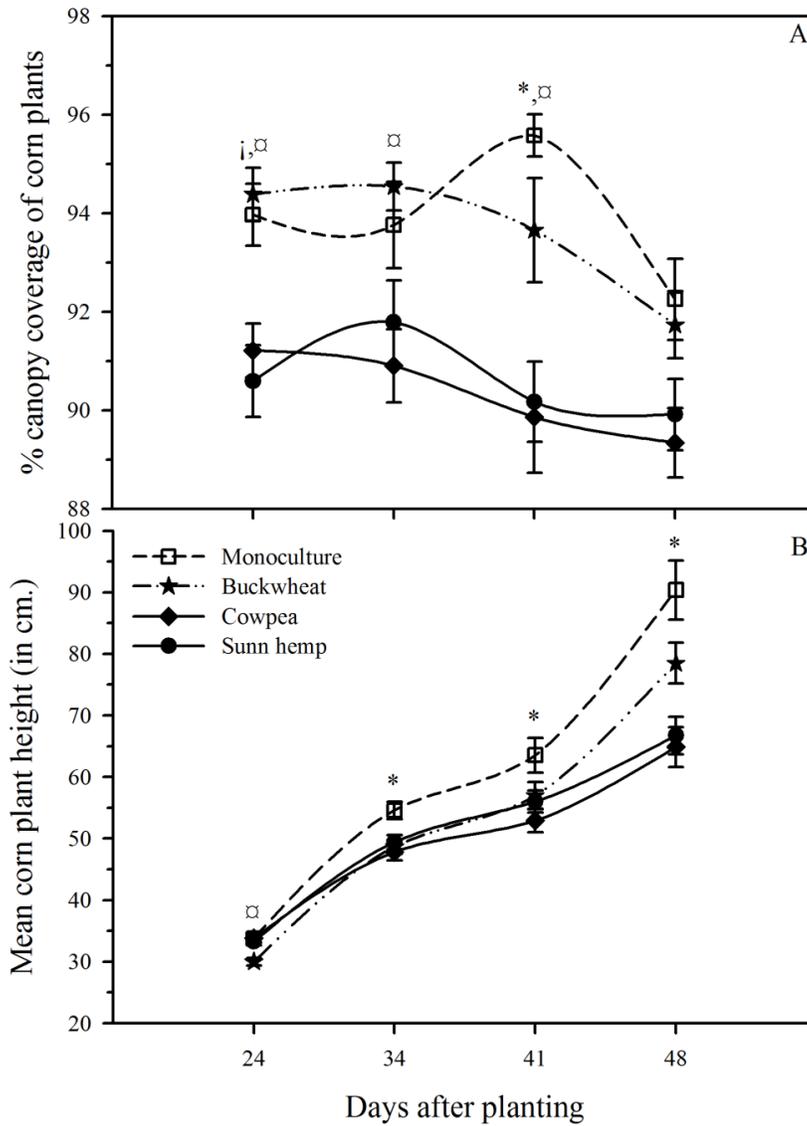
**Figure 4.5:** Relationship between *Orius* spp. at varying levels of *Helicoverpa zea* eggs and thrips (*Hz* + thrips) on corn silks in different treatment habitats in the year 2009 at Waiialua. Monoculture (MC) represents corn mono crop. Buckwheat (BW) and Sunn hemp (SH) are cover crops intercropped with corn, representing dicultures

**Table 1.2:** Linear relationship between predator, ‘*Orius*’ and prey, ‘*Hz* + thrips’ in the year 2009 (Waialua) fitted to three regression models to test hypotheses whether the slopes for treatment models are equal to zero, unequal or parallel. Table shows intercept and slopes for each model with type 3 *F*-statistics and *P*-values for intercept and slopes for each model in the year 2009. MC represents corn monoculture. BW (buckwheat), CP (Cowpea), and SH (sunn hemp) are cover crops intercropped with corn representing dicultures.

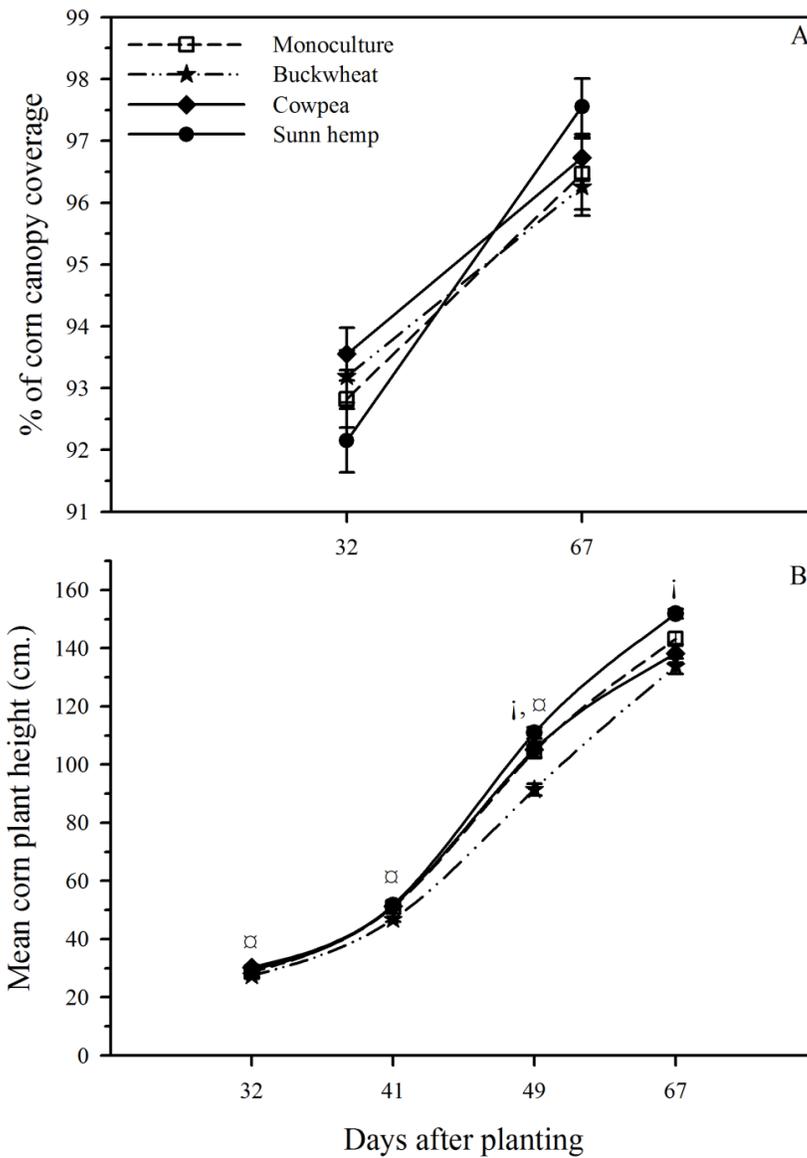
Model	Effect	Intercept			Slopes	
		treatment	<i>Hz</i> + thrips	<i>Hz</i> + thrips x treatment		
1. $Y_{ij} = \alpha_i + \beta_1 X_{ij} + b_j + e_{ij}$	MC	3.43		0.07		
	BW	3.66		0.16		
	SH	3.21		0.17		
<i>F</i> ratio (df)		39.63 (3, 62.4)		7.79 (3, 258)		
<i>P</i> value		<0.01		<0.01		
2. $Y_{ij} = \alpha_3 + (\alpha_i - \alpha_3) + \beta_3 X_{ij} + (\beta_i - \beta_3) X_{ij} + b_j + e_{ij}$	MC	3.43		0		
	BW	3.66		0.09		
	SH	3.21		0.10		
				0.07		
<i>F</i> ratio (df)		39.63 (3, 62.4)		1.80 (2, 258)		
<i>P</i> value		<0.01		0.17		
3. $Y_{ij} = \alpha_i + \beta X_{ij} + b_j + e_{ij}$	MC	3.08				
	BW	4.32				
	SH	3.58				
				0.09		
<i>F</i> ratio (df)		54.04	19.62 (1, 263)			
<i>P</i> value		<0.01				

**Table 1.3:** Linear relationship between predator, ‘*Orius*’ and prey, ‘*Hz* + thrips’ in the year 2010 (Waimanalo) fitted to three regression models to test hypotheses whether the slopes for treatment models are either equal to zero, unequal or parallel. Table shows intercept and slopes for each model with type 3 *F*-statistics and *P*-values for intercept and slopes for each model. MC represents corn monoculture. BW (buckwheat), CP (Cowpea) and SH (sunn hemp) are cover crops intercropped with corn representing dicultures.

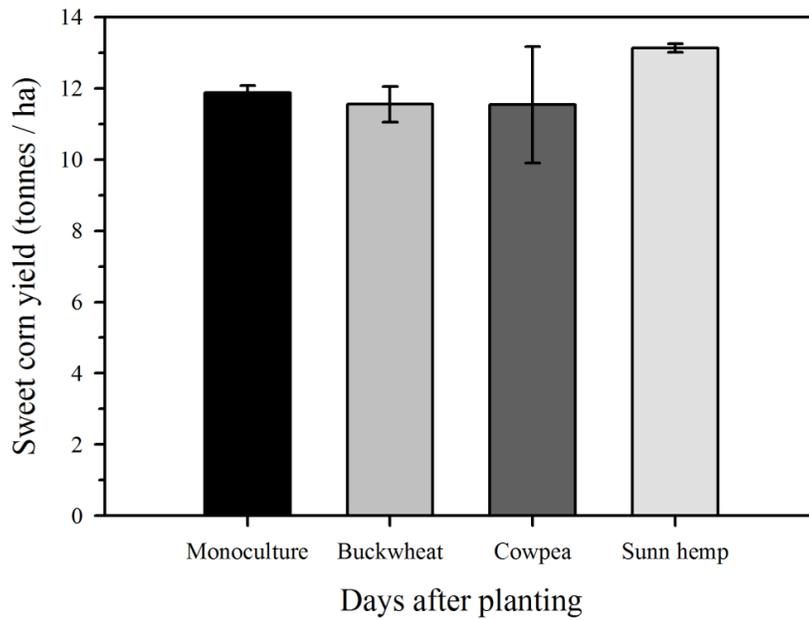
Model	Effect	Intercept			Slopes		
		treatment	<i>Hz</i> + thrips	<i>Hz</i> +thrips x treatment			
1. $Y_{ij} = \alpha_i + \beta_i X_{ij} + b_j + e_{ij}$	MC	0.07		0.02			
	BW	0.13		0.02			
	CP	0.07		0.01			
	SH	0.26		0.01			
<i>F</i> ratio (df)		2.21 (4, 105)		2.56 (4, 454)			
<i>P</i> value		0.07		0.04			
2. $Y_{ij} = \alpha_3 + (\alpha_i - \alpha_3) + \beta_3 X_{ij} + (\beta_i - \beta_3) X_{ij} + b_j + e_{ij}$	MC	0.07		0			
	BW	0.13		0.01			
	CP	0.07		-0.01			
	SH	0.23		-0.01			
				0.02			
<i>F</i> ratio (df)		2.21 (4, 105)	9.70 (1, 452)	0.43 (3, 452)			
<i>P</i> value		0.07	<0.01				
3. $Y_{ij} = \alpha_i + \beta X_{ij} + b_j + e_{ij}$	MC	0					
	BW	0.11					
	CP	-0.03					
	SH	0.12					
				0.1			
<i>F</i> ratio (df)		2.47 (3, 455)	8.98 (1, 456)				
<i>P</i> value		0.06	<0.01				



**Figure 4.6:** (A) Mean percentage of canopy density ( $\pm$  SE) and (B) mean plant height in cm. ( $\pm$  SE) of corn plants in different treatment habitats in the year 2009 at Waialua. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures. \*Indicates diculture is significantly lower than monoculture.



**Figure 4.7:** (A) Mean percentage of canopy coverage ( $\pm$  SE) and (B) mean plant height in cm. ( $\pm$  SE) of corn plant in different treatment habitats in the year 2010 at Waimanalo. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures.  $\ddagger$  Indicates sunn hemp is significantly higher than buckwheat and cowpea,  $\sphericalangle$  indicates buckwheat is significantly lower than cowpea.



**Figure 4.8:** Mean sweet corn yield (t / ha) ( $\pm$  S.E.) in different treatment habitats in the year 2010 at Waimanalo. Monoculture represents corn mono crop. Buckwheat, cowpea and sunn hemp are cover crops, intercropped with corn representing dicultures.

## CHAPTER IV

### **Within-field spatio-temporal distribution pattern of corn planthopper, *Peregrinus maidis* and their association with severity of hopperburn and *Maize mosaic virus* symptoms as influenced by sunn hemp intercropping**

#### **Abstract**

Intercropping is known to alter dispersal behavior and movement in corn planthopper, *Peregrinus maidis* (Hemiptera: Delphacidae) and this may in turn affect patterns and severity of hopperburn and *Maize mosaic virus* (MMV) caused by their feeding. Four field experiments were conducted, each consisting corn intercropped with sunn hemp and corn monoculture treatments. In each experiment, spatially explicit data were obtained by sampling corn plant to quantify number of macropterous *P. maidis* and severity of hopperburn and MMV symptoms at weekly intervals. The spatially explicit count data were used to compare the within-field spatial patterns of *P. maidis* and their association with severity of hopperburn and MMV between corn-intercropped with monoculture treatment plots using **Spatial Analysis for Distance IndicEs** (SADIE) methodology.

Results of field experiments showed distinct within-field spatial patterns of *P. maidis*, producing gaps than patches during peak densities in the corn-intercropped and corn monoculture plots. The spatial pattern showed a temporal trend with the population randomly distributed during early-crop growth, aggregated during mid-crop growth and then random to uniform during later-growth stages in both corn-intercropped and

monoculture plots. At high *P. maidis* densities, the spatial patterns were stronger in the corn-intercropped plot producing larger gaps than patches as compared to the corn monoculture. This result suggested sunn hemp intercropping may have restricted within-field movement of *P. maidis* resulting in higher densities of *P. maidis* developing in the patch areas during mid-crop growth stage. The patterns of *P. maidis* were unassociated with severity of injury and disease in both corn-intercropped and corn monoculture plots. At high *P. maidis* densities, significant spatial patterns in the corn-intercropped plot resulted in lower spread MMV than that observed in the monoculture. These dispersion patterns of *P. maidis* may be helpful in developing a precise sampling plan for *P. maidis* in cornfields. Such sampling plans which can identify of within-field *P. maidis* patches in a cropping system may be valuable for making pest management decisions.

## Introduction

Corn planthopper, *Peregrinus maidis* (Hemiptera: Delphacidae) is a serious economic pest of corn and sorghum in many tropical and sub-tropical areas (Brewbaker 2003, Singh and Seetharama 2008). In corn; *Zea mays* L., crop loss may result from either feeding damage, or transmission of *Maize mosaic virus*, MMV disease (Brewbaker 2003, Backus et al. 2005, Singh and Seetharama 2008). High numbers of planthoppers feeding on young corn plants often result a condition known as “hopperburn” (Takara and Nishida 1983). Corn plants with hopperburn show symptoms of necrosis, reduced plant vigor and stunting, and are often pre-disposed to moisture stress (Backus et al. 2005, Singh and Seetharama 2008). Symptoms of MMV include acute chlorosis with

elongated white streaks over the leaf veins, leaf sheath and ear husks (Ming et al. 1997). Plants may be severely or mildly stunted, bearing small ears with shortened husks (Brewbaker 2003). These effects can cause serious economic losses due to plant death or poor ear development, especially when infection occurs in the early growth stage (Brewbaker 2003, Singh and Seetharama 2008).

The current approach to MMV management is solely dependent upon resistant varieties. All economically important Hawaiian super-sweet, field-corn inbreds and hybrids carry the *Mv* gene for general resistance to these pathogens (Brewbaker 2003). However, the temperate field- and sweet corn varieties rarely show any tolerance to MMV and it is challenging to grow these varieties in Hawaii (Brewbaker 2003). This problem has caused major setbacks for the parent seed-corn industries in Hawaii, which generated 222.56 million US dollars in the year 2009 (NASS 2009). Hawaii serves as an important winter-nursery for the production of parent seed-corn. An alternative to current management strategies is needed, targeting MMV susceptible corn varieties.

Increasing vegetational diversity in cropping systems (eg, intercropping, mixed cropping) often results in reduced pest incidence compared to monocultures (Andow 1991, Altieri 1999). However, effects of intercropping or mixed cropping on population dynamics of *P. maidis* and incidence of MMV have been poorly understood. The previous study (Chapter II) on effect of cover crops on *P. maidis* showed that the sunn hemp intercrop altered within-field activity and colonizing behavior of *P. maidis* on corn plants, resulted in lower densities and reduced incidence of MMV in the intercropped plot compared to the monoculture. The results of Chapter II also provided some evidence of differential bimodal periodicity in their colonization and within-field activity in

intercropping systems, and hence may have bearing on within-field spatio-temporal dynamics. An understanding of dispersion pattern of *P. maidis* is of significance because their prolonged feeding on the susceptible plants permits successful transmission of MMV, a persistent virus (Hogenhout et al. 2008). The dispersion patterns of *P. maidis* in relation to severity of damage inflicted on corn plants is fundamental to developing a precise sampling plan for *P. maidis* in cornfields. A sampling plan could thus be developed to identify within-field *P. maidis* patches in a cropping system and may provide a valuable tool for making pest management decisions.

Within-field abundance and activity of arthropods is typically patchy (Holland et al. 1999, Warner et al. 2000, Ferguson et al. 2002), and generally an aggregated dispersion pattern is common for insects (Pedigo and Rice 2006). Dispersion patterns in insects may be mediated by mating behavior where mating males converge on receptive females to produce an aggregated dispersion pattern (Wickman and Rutowski 1999). Mating behavior in leafhoppers (Hemiptera: Cicadellidae) often leads to the aggregation of disease incidence transmitted in a semi-persistent manner (Hunt et al. 1993, Smith et al. 2000). Habitat management (e.g. intercropping) has been shown to disrupt mating in leafhopper vectors resulting in reduced plant disease incidence (Page et al. 1999). However, typical dispersion patterns of insects (Hemiptera: Cicadellidae, Delphacidae) have been found to be modified through a change in dispersal or migration behavior as influence by adjacent non-crop habitats near target crops (Park et al. 2005, Decante et al. 2009, Bressan et al. 2009). Previous studies (Power 1987, 1988, 1991) and results reported in Chapter II showed that changes in habitat characteristics (e.g. intercropping) increase movement rates of vector insects and ultimately reduce disease incidence of

persistent viruses. Therefore, increased within-field movement in intercropped habitats may alter dispersion pattern of planthoppers, from a typical pattern as observed in the monoculture plot. Further, under conditions of overcrowding and reduced host plant nutritional quality, planthoppers can leave the host plant to seek nutritionally superior hosts (Denno et al.1986, Denno and Roderick 1990), may influence the relationship between patterns crop injury or yield loss and pest distribution. This study examines within-field spatio-temporal distribution patterns of *P. maidis*, as influenced by sunn hemp intercropping, and the concomitant effects on the severity of hopperburn and MMV symptoms. In this study, spatially explicit sampling techniques were applied and spatial data were analyzed using Spatial Analysis for Distance Indices (SADIE) to address the following questions (i) does sunn hemp intercropping with corn alter the spatial pattern of *P. maidis* distribution, (ii) does sunn hemp intercropping alter the spatial correlation of *P. maidis* and severity of hopperburn and MMV symptoms, and (iii) does sunn hemp intercropping lower the density of *P. maidis* and MMV symptoms in the corn crop?

## **Methods**

Within-field spatial pattern of *P. maidis* distribution in corn-intercropped plots were compared with corn monocultures using spatial data from four experiments. The indices explaining degree of spatial heterogeneity of *P. maidis* distribution and their association with severity of hopperburn and MMV symptoms (severity of symptoms) were generated in a temporal sequence for each experiment. Three experiments were conducted at the University of Hawaii's experimentation station, Waimanalo Research

Station at Waimanalo. One experiment was conducted at the Pioneer Hi-Bred International Inc. field station at Mililani Agricultural Park, Mililani. The experimental sites are located on the island of Oahu in Hawaii and characterized by diversified agricultural systems, consisting of fruits, vegetables, ornamentals and seed-corn farming. The experiments were planted at the stations on randomly chosen planting dates during the study period from October 2010 to December 2011.

### **Crop planting**

A total of five strips of sunn hemp, each measuring 30.5 m x 1.8 m were planted in a field plot of 30.5 m x 30.5 m area. Six rows of corn were planted in an area between each pre-established strips of sunn hemp, to make 24 rows of corn plants in a corn-intercropped plot. Continuous 34 rows of corn were planted in an area of 30.5 m x 30.5 m in a corn monoculture plot. A sweet corn variety, Passion (Seminis Vegetables) and a fodder variety, P0125xR (Pioneer HiBred International Inc.), both susceptible to MMV were used at Waimanalo and Mililani sites, respectively. The experiments were planted on October 5, 2010 at Waimanalo (fall 2010), November 23, 2010 at Mililani (spring 2011) followed by April 14, 2011 (summer 2011) and October 11, 2011(fall 2011) at Waimanalo.

Each plot was designated  $x$  and  $y$  axes corresponding with field edges, with all sampled plants within each plot having  $x$  and  $y$  co-ordinates. Here,  $y$  and  $x$  axes represented directions along the crop rows and against the crop rows, respectively. The distance away from  $x = 0$  with respect to inter-row spacing determines the  $x$  ordinate and the distance away from  $y = 0$  with respect to distance between two sampled plants

determines the  $y$  ordinate. Thus, spatial data for *P. maidis* counts and severity of symptoms were generated for each cropping system.

### ***P. maidis* counts on plants**

Observations showed that seedling corn plants were not colonized by the macropterous *P. maidis*, owing to minimal plant biomass being available. Initial colonization of *P. maidis* occurred at around 14 - 17 DAP at the V2 - V3 stages (initiation of 2 to 3 leaves), therefore *P. maidis* counts on the corn plant were taken after 17 DAP (Chapter II). Five corn plants were sampled at an interval of 6.0 m in each row to quantify macropterous *P. maidis*. The sampled plants were either 1.5 m or 3.0 m away from the beginning of row along the  $y$  direction, forming a staggered mosaic-sampling pattern. This implies that if the sampled plants were 1.5, 7.6, 13.7, 19.8 and 25.9 m away from the beginning in a row, the subsequent row had sampled plants at 3.0, 9.1, 15.2, 21.3 and 27.4 m away from the beginning. Corn plants were sampled from all 24 rows between the 5 strips of sunn hemp in the corn-intercropped plot. An identical sampling pattern was followed in the corn monoculture plot by not sampling corn plants where sunn hemp strips were located in the intercropped plot. In the monoculture plots, corn rows at field margins (3-4 rows at beginning and end of the field along the  $x$  direction) and middle (3 rows after each 6 sampled rows along the  $x$  direction) were not sampled in order to produce an identical sampling pattern as the corn-intercropped plot. There were a total of 120 and 120-145 sampled plants in corn-intercropped and monoculture plot, respectively. The sampled plants were flagged and sampled weekly for *P. maidis* counts, from the V2-V3 stage until silking. The macropterous *P. maidis* were counted in the leaf

whorls at the beginning of the crop cycle, then sampling was extended to leaf sheaths, flag leaves and ears at later developmental stages.

### **Severity of hopperburn and MMV symptom evaluation**

Rows of corn plants designated for *P. maidis* counting were used for evaluating severity symptoms in the both treatments. 20 plants were sampled at an interval of 1.5 m in each row. There were a total of 480 and 459-480 sampled plants in each corn-intercropped and monoculture plot, respectively. Each of the plants was scored at scale of 0-3 on the basis of severity of hopperburn and MMV symptoms. Ranking was as follows: 0 represented healthy plants, 1 represented mild chlorosis, with or without hopperburn (necrosis) symptoms, 2 represented acute chlorosis, heavy hopperburn, or initial MMV symptoms or a combination of all, 3 represented the same symptoms described for 2, with MMV symptoms visible (white streak along the leaf veins) or green plants with prominent MMV symptoms. The plants were evaluated as the first occurrence of visual symptoms of MMV and continued until harvest at weekly intervals. In addition, number of plants showing visual symptoms of MMV was counted in each row and presented as percentage MMV symptomatic plants per row for each treatment plot. At least 3 leaf samples of corn plants showing each severity symptom score were collected from each plot. The leaf samples were diagnosed to detect MMV and *Maize chlorotic mottle Virus* (MCMV), separately using a double antibody sandwich enzyme-linked immunosorbent assay (DAS – ELISA) at the end of each experiment. MCMV is another corn virus, transmitted by a thrips species, *Frankliniella williamsi* that was suspected during the experimental period. ELISA reagent kits were specific for MMV and MCMV (Agdia Inc., US).

## Data Analyses

The spatial patterns for *P. maidis* and severity of symptoms in each cropping system were examined using **Spatial Analysis by Distance IndicEs** (SADIE version 1.22, Perry et al. 1999), which has been developed for the spatial analysis of ecological data in the form of spatially referenced counts (Perry 1995). A complete description of this methodology is available in Perry (1995, 1998), Perry et al. (1999) and Perry and Dixon (2002). Rather than using more traditional geostatistical methods such as semivariograms, SADIE was chosen because of its additional ability to describe association among datasets sharing the same locations (Perry et al. 2002).

SADIE produces a number of indices, which are used to test the null hypothesis that the counts within the field are arranged randomly with respect to each other. The distance to regularity,  $D$  is the minimum mean value of the total distance that the individuals in the sample would have to move from unit to unit, so that all units contained identical numbers (Perry 1998). In particular, division of the observed mean value of  $D$  by the mean value  $D$  of several hundred randomized data gives an index of aggregation,  $I_a$  (Perry 1998). The value of  $I_a = 1$  indicates random distribution,  $I_a < 1$  indicates regular or uniform distribution, while  $I_a > 1$  indicates aggregation into clusters (Perry 1998).  $P_a$  is the probability that the observed counts are randomly distributed among the given sampling units and was considered significant when  $< 0.05$  (Perry 1998).

SADIE also quantifies the degree of clustering in the count data. The clustering indices give a measure of the proportion of the data that fit into patch clusters (groups of counts higher than the sample mean,  $v_i$ ) and gap clusters (groups of counts lower than the

sample mean,  $v_j$ ) (Perry et al. 1999). Usually, values of  $v_i$  greater than 1.5 and  $v_j$  less than -1.5 indicate significant larger patches and gaps (Perry et al. 1999). Additionally, the clustering test (one tailed) determines whether the observed data are significantly clustered than the random. The measure of cluster indices were considered significant when the probability associated with this index is less than 0.05, indicating the observed data were more clustered than the random data. The interpolated maps from count data, indicating gaps and patches were produced in a temporal sequence to display the spatial pattern within each field graphically. A geostatistical method, Inverse Distance Weighing (IDW), was used to interpolate values at unsampled locations. This method works well with small data sets (Kravchenko 2003), and interpolates by linear weighed combination of observed values in the neighborhood of each processing cell. The maps were generated using ArcView 9.2 (ESRI 2006) software.

The overall spatial association,  $c$  between the two data sets (*P. maidis* counts and severity of symptoms ratings) was assessed by examining the correlation between clustering indices of two sets of data (Perry and Dixon 2002). A measure of local spatial association,  $\chi_p$  is calculated between the first set of cluster indices and the second set of cluster indices at each  $x, y$  point. If the  $\chi_p$  is negative, there is negative association (dissociation) between two data sets at this  $x, y$  point. If the  $\chi_p$  is positive there is a positive association between the two data sets at this  $x, y$  point. The association test is two tailed and determines whether the clusters of two data sets are associated ( $P < 0.025$ ), unassociated ( $0.025 > P > 0.975$ ), or dissociated ( $P > 0.975$ ) (Conrad, 2006). The QUICK ASSOCIATION ANALYSIS SHELL (Version 1.22) program was used to assess the degree of association between the two variables measured at the same  $x, y$  location within

the field. The measures of local association were mapped to display graphical patterns of association in a temporal sequence. Any change in spatial association pattern between treatments and within treatment in a temporal sequence could thus be visualized.

Analysis of variance (PROC MIXED, SAS Institute Inc., 2003) was used to compare *P. maidis* densities and percentage of MMV symptomatic plants in the two cropping systems. The pooled analysis for *P. maidis* densities was conducted designating “planting season” as a random factor. Additionally, *P. maidis* densities were compared for each experiment by sampling date with row nested with sampled plant “row (plant)” as a random factor. Finally, percentage of plants showing visual symptoms of MMV per row was compared between treatment plots with “row (plant)” as a random factor for fall 2010 and spring 2011 experiments.

## Results

### Within-field spatial pattern

The variables that showed significant within-field spatial patterns of *P. maidis* between treatment plots varied in each experiment (Table 2.1). Results showed that significant *P. maidis* aggregation patterns ( $I_a > 1$ ,  $P_a < 0.05$ ) were more frequent in the corn-intercropped plots than in the corn monoculture plots in fall 2010 (Table 2.1, Figure 5.1A and 5.1B) and spring 2011 experiments (Table 1 and Figure 5.2A and 5.2B). No significant aggregation patterns were observed during the sampling period in both the treatment plots in the summer 2011 experiment (Table 5.1, Figure 5.3A and 5.3B). Significant aggregation patterns were more frequent in the corn monoculture plot than in

the corn-intercropped plot in the fall 2011 experiment (Table 2.1, Figure 5.4A and 5.4B). This significant aggregated spatial pattern corresponding to treatment plots resulted in the development of significant gaps ( $v_j < -1.5$ ,  $P_j < 0.05$ ) and patches ( $v_i > 1.5$ ,  $P_i < 0.05$ ) of *P. maidis* on the respective sampling dates (Table 2.1, and Figure 5.1-5.4).

### **Temporal pattern**

Within-field spatial patterns of *P. maidis* distributions varied by planting season, with significant spatial patterns occurring in the fall plantings compared to moderate and weak patterns in the spring and summer plantings, respectively (Table 2.1). Similarly, spatial patterns of *P. maidis* distributions also varied between each sampling date in all experiments (Table 2.1, Figure 5.5). The distribution of *P. maidis* was found to be random on early sampling dates, and gradually aggregated, forming gaps and patches on subsequent sampling dates, to ultimately develop random or near uniform distribution patterns on later sampling dates (Table 2.1, Figure 5.5). In general, stronger spatial patterns of *P. maidis* distribution, as indicated by values of  $I_a$ ,  $v_j$ , and  $v_i$ , was found in corn-intercropped plots than monocultures at mid-crop developmental stage. (Table 2.1, Figure 5.5-5.6). The spatial pattern of *P. maidis* showing gaps and patches can be visualized in an interpolated map with *P. maidis* counts, overlaid with points showing gaps and patches (Figures 5.1-5.4). The maps showed that the frequency of sampled locations showing gaps were higher than that of patches in both treatment habitats (Figures 5.1-5.4). At high densities of *P. maidis* (as in case in the case of the fall 2010 and 2011 experiments, mean density  $\sim 2.6$  per plant), the weaker patches were formed at the field margin initially, which gradually developed into larger patches at the multiple field margins with larger gaps concentrated at the center of the field on the later sampling

dates (Figures 5.1-5.4). The maps showed no visual difference in distribution of patches and gap between the treatment plots (Figures 5.1-5.4).

### **Within-field spatial association – vectors and severity of symptoms**

Within-field spatial patterns of plants showing severity of hopperburn and MMV symptoms varied between the two treatments (Table 2.2). The aggregation patterns of severity of symptoms were prolonged and occurred earlier in the intercropped plots than in monoculture plots where they were more ephemeral and occurred later in fall 2010 and spring 2011 experiments, (Table 2.2, Figure 5.7-5.8). These aggregated patterns attributable to the treatments applied, produced significant patches and gaps in both treatment habitats on the respective sampling dates (Table 2.2, Figure 5.7-5.8). In general, the patch size of severity of symptoms increased with the development of the corn plants in both treatments (Figures 5.7-5.8). The association between spatial patterns of severity symptoms and *P. maidis* distribution was not consistently correlated and was positively associated on only one occasion in the intercropped plot on 38 DAP in the fall 2010 trial ( $c = 0.195$ ,  $P_c < 0.05$ ). The local spatial association,  $\chi_p$  between the severity of symptoms and *P. maidis* for each experiment can be visualized in the map plotted with the values of local association indices at each common sampled location for *P. maidis* counts and severity of symptoms ratings (Figures 5.9-5.10). The maps showed a generally unassociated relationship between the two variables, with rare locations showing either strongly associated or dissociated relationship (Figures 5.9-5.10).

### ***P. maidis* densities**

*P. maidis* densities were higher at the Waimanalo site ( $2.57 \pm 0.07$ , mean per plant,  $\pm$  SEM) than lower at the Mililani site ( $0.63 \pm 0.03$ ). The densities also varied with planting seasons with highest densities in the fall ( $2.27 \pm 0.09$ ), followed by moderate in the spring ( $0.63 \pm 0.02$ ) and low in the summer ( $0.05 \pm 0.01$ ) plantings. The pooled analysis for all four experiments showed *P. maidis* densities were significantly greater in corn-intercropped plots than monocultures ( $F_{1,6650} = 6.17$ ,  $P = 0.01$ ). However, considering random effect of planting season, the overall treatment effect did not differ significantly for *P. maidis* densities ( $F_{1,2} = 1.09$ ,  $P = 0.41$ ) (data not shown). An individual analysis of each experiment showed that *P. maidis* densities were significantly higher in the corn-intercropped plot than monoculture on three (50%) sampling dates ( $F_{1,119} = 6.9 - 23.0$ ,  $P \leq 0.01$ ) in the fall 2010 experiment (Figure 5.11A). In spring 2011, the densities in the corn-intercropped plot were significantly higher on two (28.5%) sampling dates ( $F_{1,114} = 5.2 - 6.4$ ,  $P \leq 0.02$ ), and lower on one (14.3%) sampling date ( $F_{1,114} = 9.6$ ,  $P < 0.01$ ) than the monoculture (Figure 5.12A). In the summer 2011, the densities were significant lower in the intercropped plot than monoculture on one sampling date (14.3%) ( $F_{1,119} = 7.60$ ,  $P < 0.01$ ) (Figure 5.12B). In fall 2011, the densities in intercropped plot was significantly lower in the intercropped plot on two (33.3%) sampling dates ( $F_{1,115} = 11.0 - 38.5$ ,  $P < 0.01$ ) and was higher on two (33.3%) sampling dates ( $F_{1,118-119} = 4.8 - 10.4$ ,  $P \leq 0.03$ ) than monoculture (Figure 5.11B).

## MMV incidence

The incidence of MMV was found to be higher at Waimanalo than Mililani site and was higher in fall (mean *P. maidis* density ~ 2.3 per plant) than spring (mean *P. maidis* density ~ 0.6) or summer (mean *P. maidis* density ~ 0.1 per plant) planting seasons. The percentage of MMV symptomatic plants per row at silking stage was significantly lower in the intercropped plot ( $71.75\% \pm 3.4$ ) than monoculture ( $82.04\% \pm 0.9$ ) in the fall 2010 ( $F_{1,46} = 8.75$ ,  $P < 0.01$ ) (data not shown). The percentage MMV symptomatic plants was found to be negligible in the spring 2011 and was significantly higher in the intercropped plot ( $0.67\% \pm 0.2$ ) than monoculture ( $0.11\% \pm 0.1$ ) ( $F_{1,46} = 6.22$ ,  $P = 0.02$ ) (data not shown).

All the leaf samples of plants showing visual symptoms of MMV were consistently tested positive for MMV using DAS-ELISA in fall 2010 and spring 2011 experiments. However, the proportion of leaf samples from plants showing differential severity symptoms of MMV were also tested positive for MCMV in the spring 2011. The severity of MCMV was prominent in summer and fall 2011, where all the leaf samples were tested positive for MCMV. Therefore, the severity symptoms rated in spring 2011 and fall 2011 were not used to analyze spatial association test, assuming the ratings of severity of symptoms may represent dual, or MCMV infection alone.

## Discussion

### Within-field spatial pattern

Results of field experiments showed that high densities of *P. maidis* (mean density ~ 2.6 per plant) produced strongly pronounced spatial distribution patterns (as in the case of the fall 2010 and 2011 experiments). On the other hand, medium to low densities of *P. maidis* (density ranged ~ 0.1 – 0.6 per plant) produced moderate to weak spatial distribution patterns (as in case of the spring and summer 2011 experiments). These results are similar to other studies that showed significant spatial pattern at higher densities of insects than at their lower densities (Rahman et al. 2010, Reay-Jones et al. 2010, Reay-Jones 2012). In addition, sunn hemp intercropping favored prolonged *P. maidis* aggregation patterns compared with a shorter period of aggregation in monoculture plots (as in the case of fall 2010 and spring 2011 experiments). This may be due to restriction of within-field movement of *P. maidis*, due to interference caused by a sunn hemp intercrop (Chapter II). A study in vineyards showed that natural vegetation (mixed hedges, woodlands and other vine plants) between vine plots acted as a barrier, which intercepted migrating adults of *Empoasca vitis*, resulting in a clear aggregated pattern adjacent to woodlands along the downwind direction (Decante et al. 2009). In the current study, although evidence of *P. maidis* interception was not observed on the sunn hemp intercrop, a stronger aggregated pattern in the corn-intercropped plots suggested that sunn hemp might have restricted *P. maidis* within-field movement.

Results of fall 2010 experiments showed high *P. maidis* densities with a significant aggregation pattern, which resulted in significantly lower percentages of

plants exhibiting MMV symptoms in the corn-intercropped plot compared to the monoculture. Conversely, low *P. maidis* densities (mean density ~ 0.1 per plant) with weak aggregation patterns in both treatments resulted in significantly higher percentage of plants showing MMV symptoms in the corn-intercropped plot than monoculture in the summer 2011. The significant aggregation in corn-intercropped plot suggests that *P. maidis* adults were confined to a limited area within a field, feeding on less corn plants, which may explain the reduced incidence of MMV that was observed.

The current study indicated that immigration of *P. maidis* occurred from the field margins, and progressed gradually moving to the adjacent plants forming larger patches that permeate into the crop along field margins over time. Warner et al. (2003) found edge effects on immigration of *Psylliodes chrysocephala* (L.), a Chrysomelidae pest that initially infested field edges, invading gradually over time in rape (*Brassica napus* L.) fields, resulting a non-uniform distribution within the crop. Pronounced edge effects were observed in the distribution of stink bugs, *Nezara viridula* L., and *Euschistus servus* (Say) as they colonize new cotton fields (Tillman et al. 2009). Similarly, an aggregated dispersion pattern of the adult weevil, *Listronotus maculicollis* population along the edges of annual blue grass (*Poa annua*) area closest to their overwintering sites, showed progressive movement of these weevils in blue grass areas on golf courses (McGraw and Koppenhofer 2010). Thus, edge effect of immigrating insects into crop fields is common. This behavior results in larger patches along the field margin over time. *P. maidis* within-field dispersion pattern is consistent with such an invasion pattern. The utility value of planting border crops and intercropped rows is thus likely to reduce the spread of MMV in cornfields.

## Temporal pattern

Within-field dispersion patterns of arthropods may vary over time and depend upon availability of resources. For example, dispersion of adult corn rootworm beetles, *Diabrotica* spp. is typically aggregated in the middle of the growing season (at corn silking stage), and random early and late in the crop cycle (Park and Tellefson 2006). Similarly, in the current study the within-field distribution of *P. maidis* showed a temporal trend oscillating from random with initial *P. maidis* colonization at the early-crop growth stage, to aggregated in the mid-crop growth stages, and then random to uniform dispersion at later growth stages. This temporal distribution pattern of *P. maidis* can be explained by their unique ecology, exhibiting wing dimorphism in adults: brachypterous morphs have reduced wings and cannot fly, while macropterous morphs have fully developed wings and can disperse over long distances (Denno and Roderick 1990). In general, planthoppers produce large proportions of macropterous adults under conditions of overcrowding and reduced host plant nutritional quality that can migrate to find superior host plants (Denno et al., 1986, Denno and Roderick 1990). In this context, immigrating macropterous *P. maidis* that land randomly in the cornfield produces an initially random distribution pattern. The initial immigrants reproduce and their population size grows, often leading to overcrowding, resulting aggregated distribution pattern at mid-crop growth stage. Feeding by high numbers of *P. maidis* reduces nutritional quality of corn plants, thereby producing a larger proportion of macropterous forms. Resulting progeny disperse within the field to find nutritionally superior plants, developing random to uniform distribution patterns. Furthermore, the restricted within-field movement of *P. maidis* in intercropped plots produced stronger spatial patterns with

larger gaps than patches in intercropped plots than in monoculture plots during the mid crop growth period.

### **Within-field spatial association – vectors and virus**

Results showed that the aggregation patterns of *P. maidis* related symptoms were stronger in corn-intercropped plots than corn monoculture plots. This is mainly due to the stronger aggregation pattern of *P. maidis* in corn-intercropped plots than monocultures. Further, results of association tests showed a spatially unassociated relationship between *P. maidis* and their related severity symptoms in corn plants in both treatments. A study showed that spatial distribution of stink bugs, *Acrosternum hilare* (Say), *Euschistus servus* (Say) and *Nezara viridula* did not always coincide with the severity of boll injury in cotton fields (Reay-Jones et al. 2010). This result suggests that close association patterns of pest and crop injury are rare, possibly due to pest's abandonment of food patches, owing to their feeding reducing the quality of the resource.

In the current study, the distribution maps of local association indices showed large unassociated area with rare locations showing either associated or dissociated areas. This pattern may reasonably be explained by *P. maidis* feeding on nutritionally poor host plants produce more macropterous morphs, which preferably colonize healthy plants (Denno et al. 1986), producing a disjointed pattern of insect density and severity of symptoms. Further, at high densities of *P. maidis* (as in case in the 2010 fall experiment), association indices were higher in intercropped plots than monoculture, which indicates greater spatial association of *P. maidis* with severity of symptoms in the intercropped plot

compared to corn monoculture. This is likely the result of the restricted movement of *P. maidis* in intercropped plots.

In conclusion, the within-field spatial pattern of *P. maidis* is mediated by their population densities and is most distinct producing gaps than patches at peak densities of the insect in both treatments. The dispersion pattern of *P. maidis* showed temporal trends from initial random, progressing to aggregated during mid-crop growth stages, and then random to uniform dispersion at later growth stages. At high *P. maidis* densities, the spatial patterns were stronger in the corn-intercropped plot producing larger gaps than patches as compared to the corn monoculture. This result suggested sunn hemp intercropping may have restricted within-field movement of *P. maidis* resulting in development of higher densities of *P. maidis* in the patch areas during mid-crop growth stage. The spatial distribution patterns of *P. maidis* were unassociated with patterns of severity of feeding symptoms and MMV infection in both treatments. At high densities, significant aggregation of *P. maidis* in corn-intercropped plots resulted in lower spread of MMV than in monoculture plots.

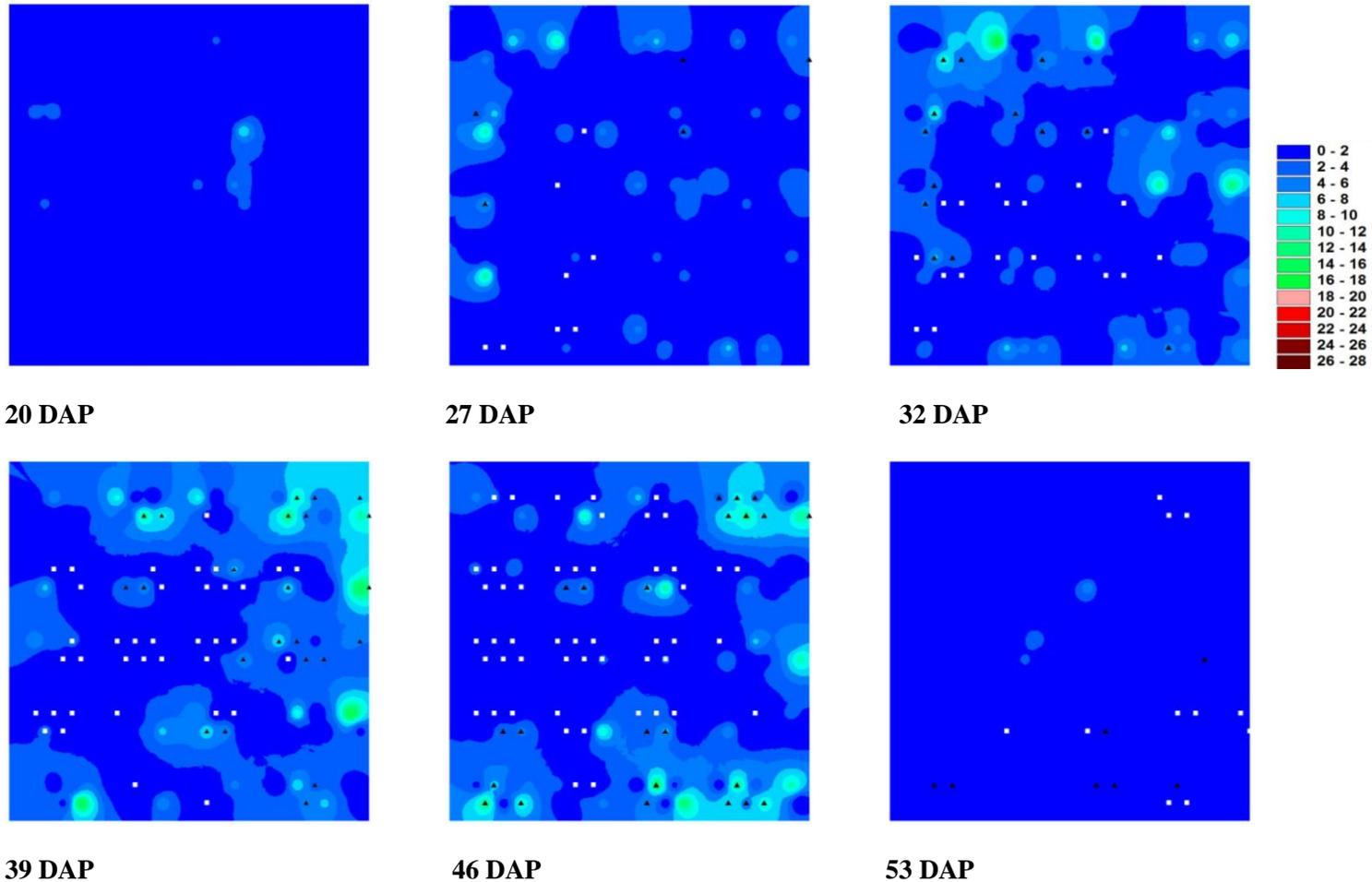
Understanding dispersion patterns of *P. maidis* may be helpful in developing a fixed precision sampling plan for *P. maidis* in cornfields. Such a sampling plan can be used to identify within-field *P. maidis* patches in a cropping system and thus provide a valuable tool for making pest management decisions. The identified *P. maidis* patches could be spot-sprayed with insecticides at peak density, which may reduce frequency of treatments and quantity of insecticide applied. In a corn production system, intercropping corn with sunn hemp, combined with appropriate integrated pest management practices

may provide valuable options for reducing the impacts of insect-borne plant pathogens such as MMV.

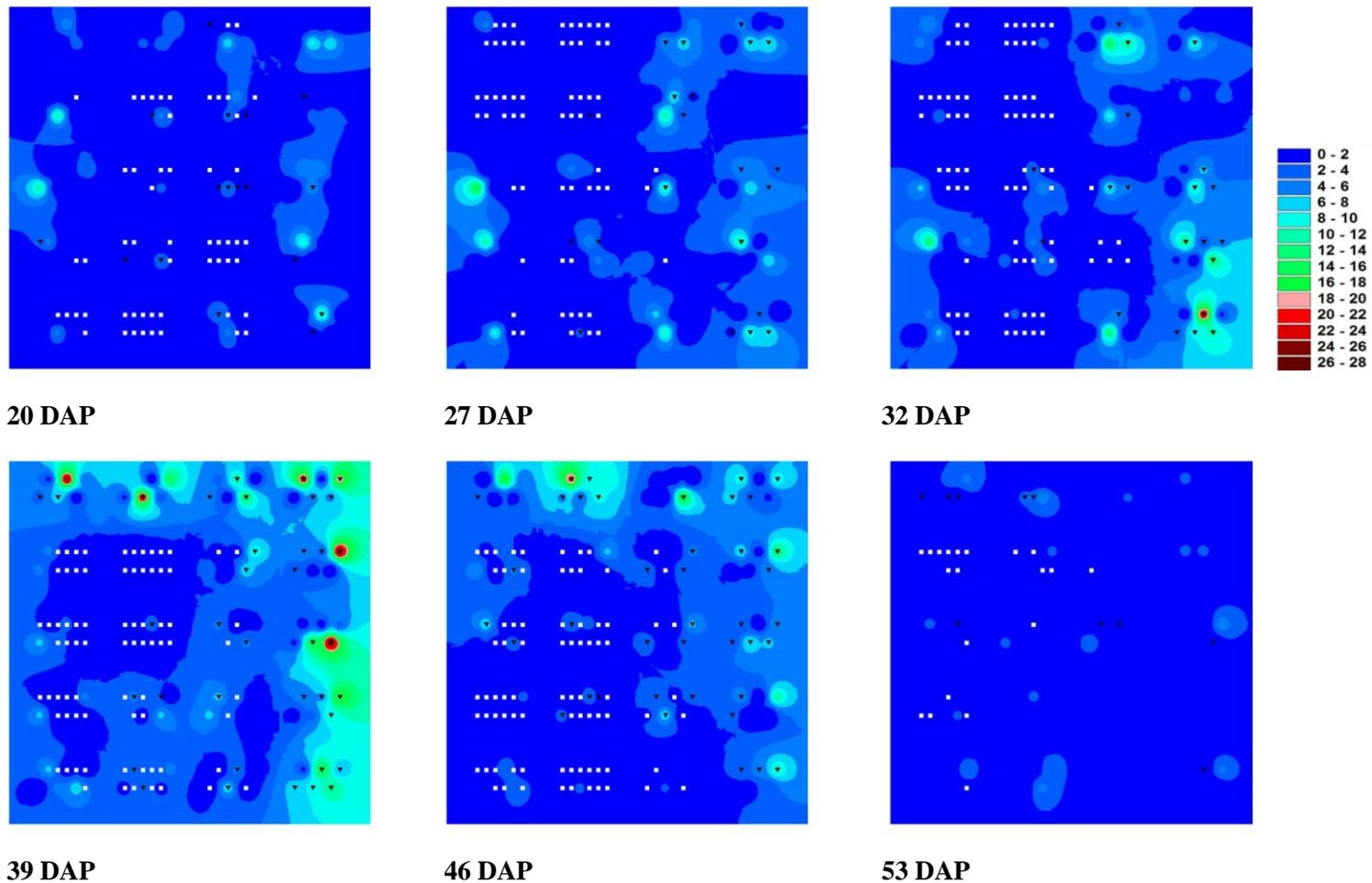
**Table 2.1:** Summary of SADIE analysis results on *Peregrinus maidis* counts in corn monoculture and corn intercropped with sunn hemp (corn + SH), measured at weekly interval during the corn developmental time. The index of aggregation ( $I_a$ ) indicates degree of spatial aggregation of *P. maidis* distribution; the clustering indices,  $\nu_j$  and  $\nu_i$  indicates the distribution forming gaps and patches, respectively.

Site	Planting season	DAP	Treatments					
			Corn monoculture			Corn +SH		
			$I_a$	$\nu_j$	$\nu_i$	$I_a$	$\nu_j$	$\nu_i$
Waimanalo	Fall 2010	20	1.23	-1.26	1.07	1.31	-1.32	1.33
		27	0.95	-0.93	0.85	<b>1.90</b>	<b>-2.04</b>	<b>1.52</b>
		32	1.13	-1.10	1.16	<b>2.12</b>	<b>-2.19</b>	<b>1.75</b>
		39	<b>1.65</b>	<b>-1.49</b>	<b>1.68</b>	<b>2.43</b>	<b>-2.56</b>	<b>2.18</b>
		46	<b>1.62</b>	<b>-1.57</b>	<b>1.66</b>	<b>2.34</b>	<b>-2.48</b>	<b>2.35</b>
		53	0.95	-0.94	0.97	0.98	-0.98	0.86
	Summer 2011	21	0.93	-0.93	0.87	0.90	-0.90	0.87
		28	0.88	-0.88	0.89	0.70	-0.70	0.71
		35	1.07	-1.06	1.10	0.88	-0.88	0.85
		42	0.88	-0.87	0.91	0.89	-0.88	0.96
		49	0.86	-0.88	0.84	-	-	-
		54	0.86	-0.86	0.91	1.05	-1.05	1.09
	Fall 2011	23	<b>1.30</b>	<b>-1.34</b>	1.21	1.16	-1.15	1.14
		29	1.28	<b>-1.38</b>	1.13	1.22	-1.29	1.04
		39	<b>1.72</b>	<b>-1.85</b>	<b>1.37</b>	0.96	-0.93	0.95
		45	<b>1.94</b>	<b>-2.10</b>	<b>1.67</b>	1.19	-1.16	0.95
		52	<b>1.40</b>	<b>-1.45</b>	1.14	1.20	-1.14	1.03
59		<b>1.31</b>	-1.25	1.17	<b>1.98</b>	<b>-2.17</b>	<b>1.69</b>	
Mililani	Spring 2011	25	0.83	-0.84	0.81	0.95	-0.95	0.92
		36	0.89	-0.89	0.93	<b>1.46</b>	<b>-1.43</b>	<b>1.33</b>
		41	1.02	-1.01	0.95	<b>1.58</b>	<b>-1.55</b>	<b>1.41</b>
		48	1.18	-1.13	1.08	0.95	-0.92	1.01
		55	1.08	-1.06	1.28	<b>1.51</b>	<b>-1.49</b>	<b>1.53</b>
		61	1.14	-1.11	1.12	0.98	-0.96	0.93
		70	0.91	-0.96	0.98	0.99	-0.98	0.97

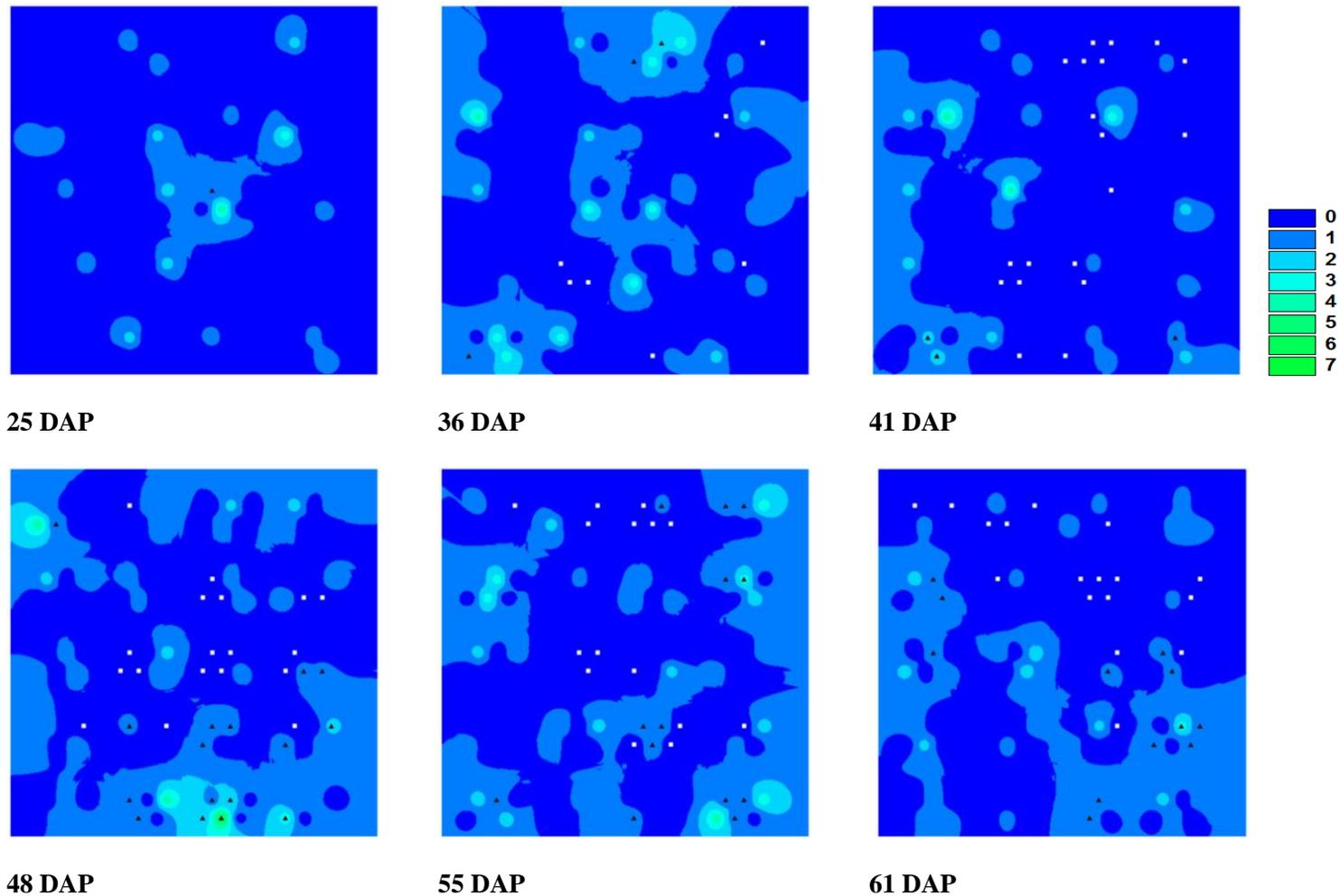
Bold values  $I_a$  suggest ( $P_a < 0.05$ ), that there is significant spatial pattern (i. e. the counts are not arranged randomly). Bold values of  $\nu_j$  suggest ( $P_j < 0.05$ ), that the distribution forms significant gaps. Bold values of  $\nu_i$  suggest ( $P_i < 0.05$ ), that the distribution forms significant patches.



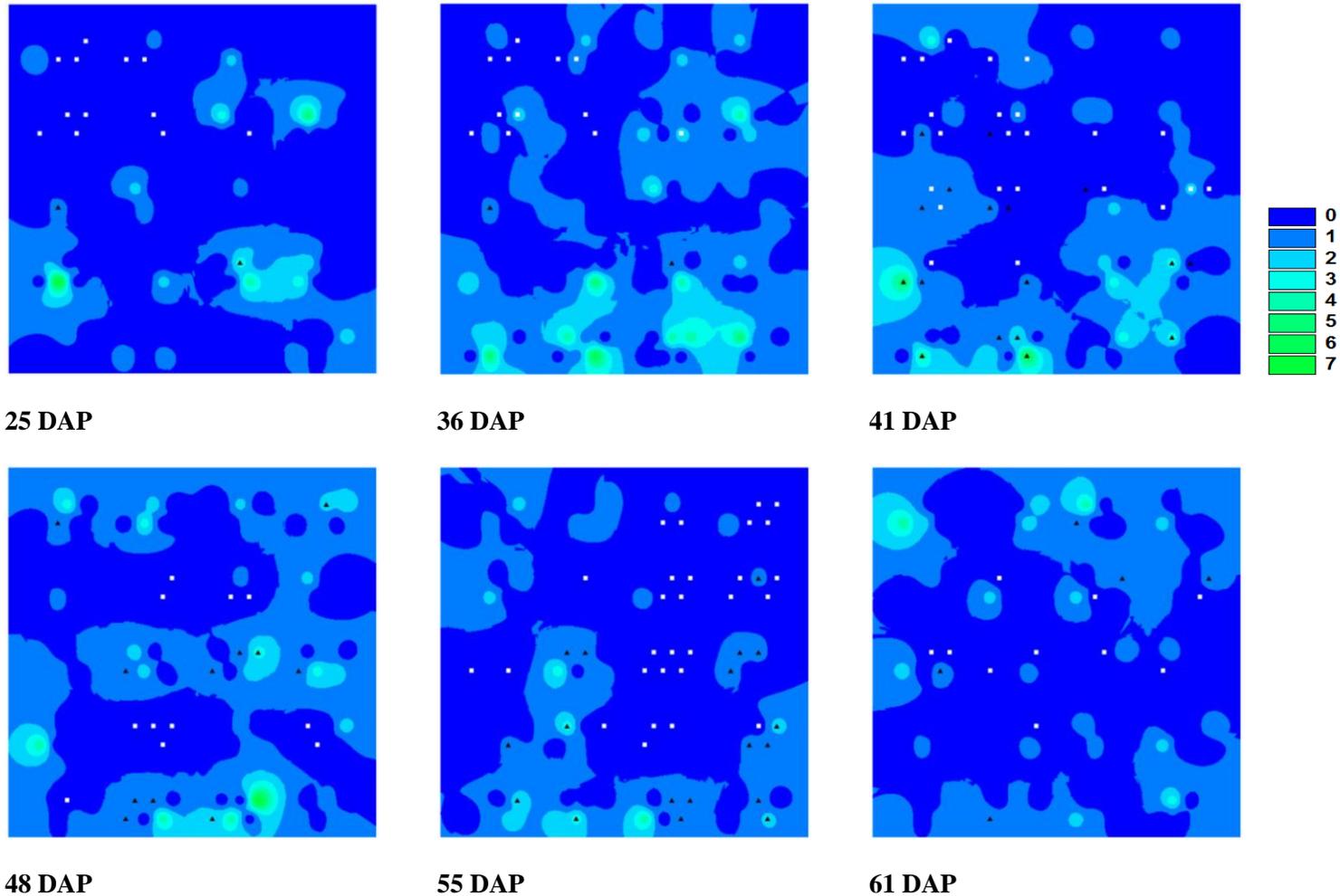
**Figure 5.1(A):** Spatial distribution pattern of corn *Peregrinus maidis* measured at weekly interval during the corn developmental time in corn monoculture in fall 2010 at the Waimanalo Research Station, Waimanalo. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of gaps) and  $v_i > 1.5$  (the presence of patches), respectively. The maps show significant spatial patterns, forming larger gaps than the patches at high densities of *P. maidis* from 32 to 46 DAP. The patches occurred at the field margins, with large gaps at the center of the field.



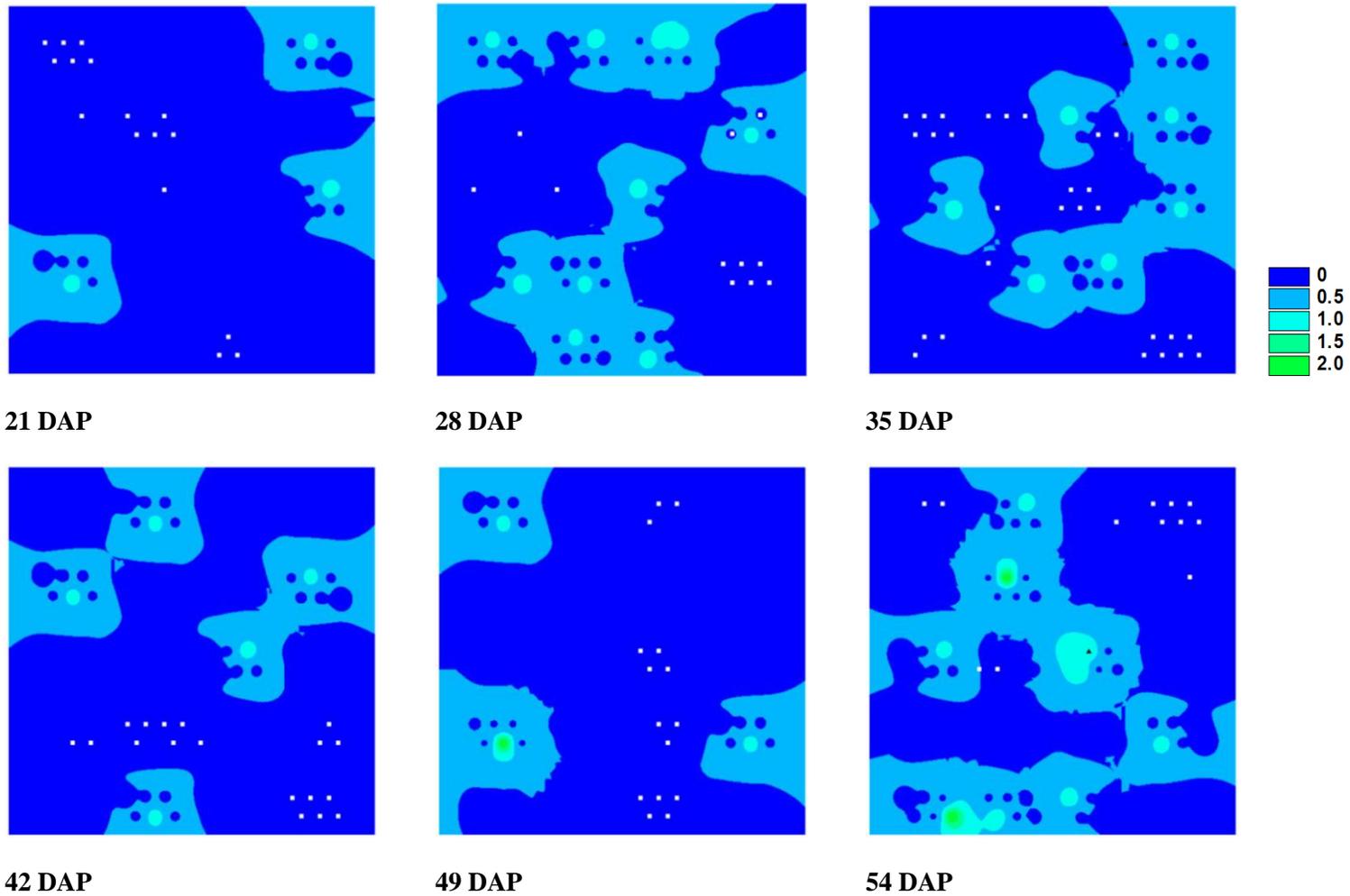
**Figure 5.1(B):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during the corn developmental time in corn intercropped with sunn hemp plot in fall 2010 at the Waimanalo Research Station, Waimanalo. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and indicates  $v_i > 1.5$  (the presence of patches). The maps show significant spatial patterns with larger gaps than patches at high densities of *P. maidis* from 27 to 46 DAP. The patches occurred at the field margins with large gap at the center of the field.



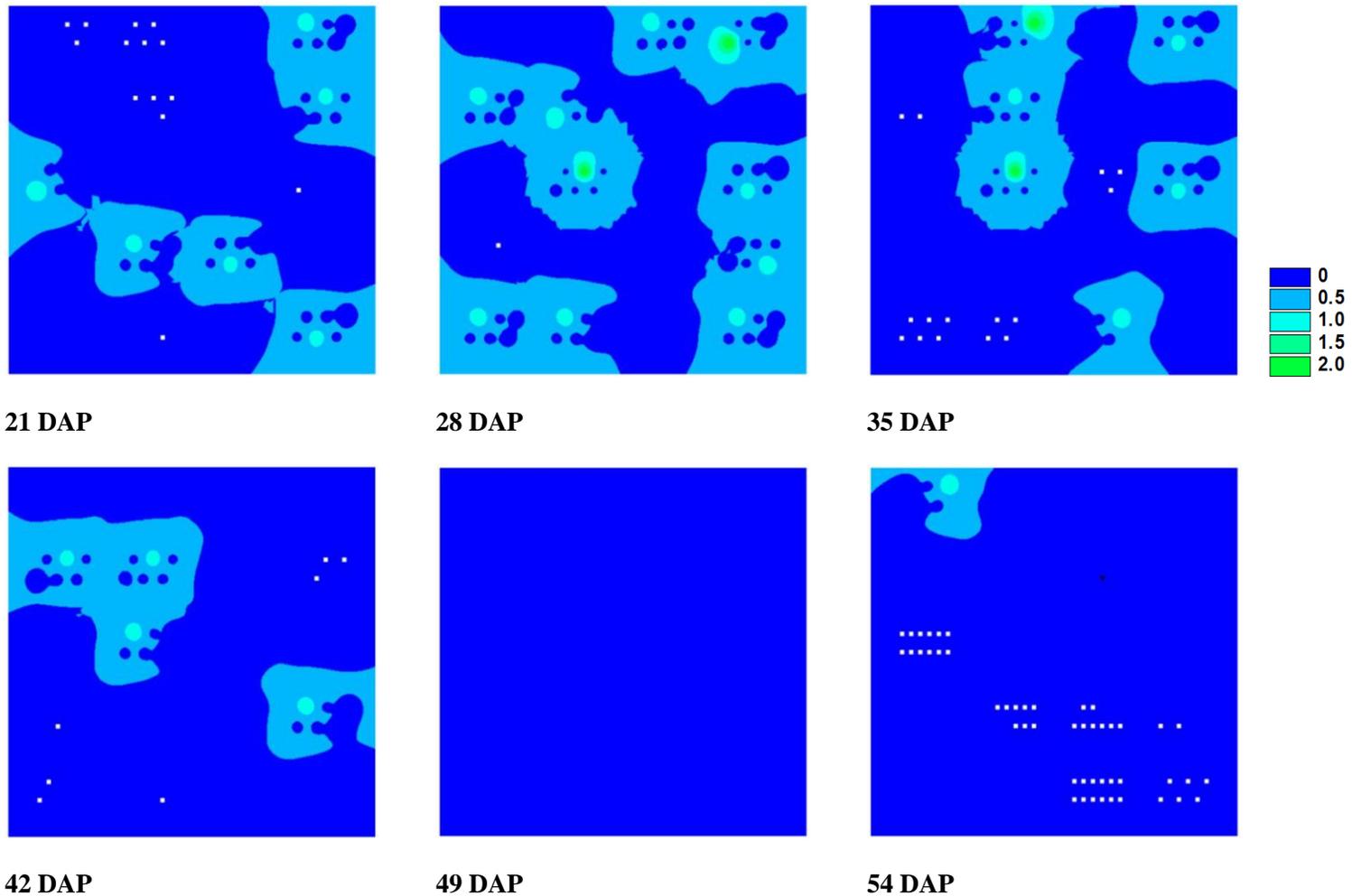
**Figure 5.2(A):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during the corn developmental time in corn monoculture in spring 2011 at the Mililani Agriculture Park, Mililani. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and  $v_i > 1.5$  (the presence of patches), respectively. The maps show moderate spatial patterns with larger gaps than the patches at moderate densities of *P. maidis* from 41 to 61 DAP. The patches occurred at the field margin with large gap at the center of the field.



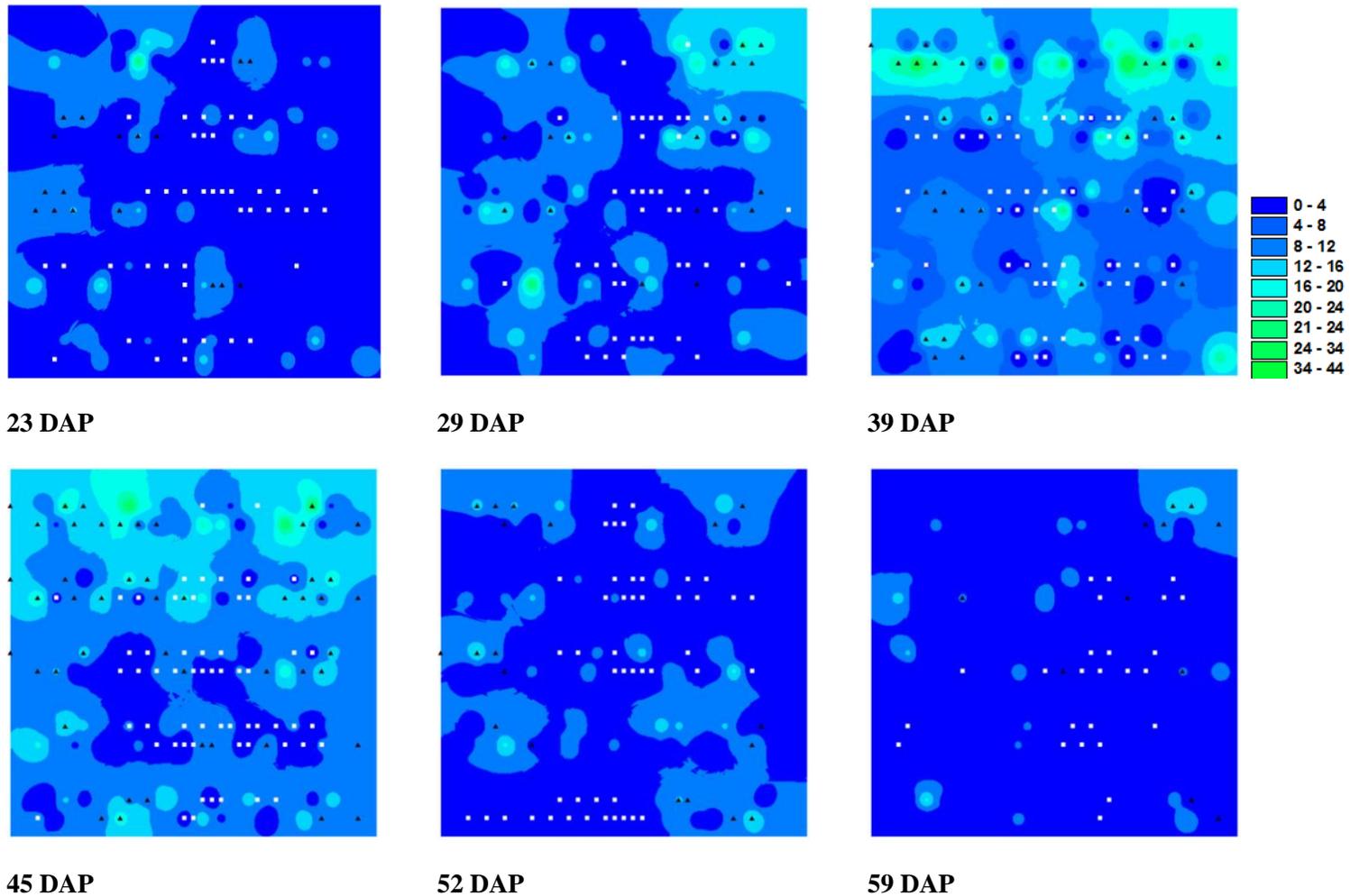
**Figure 5.2(B):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during the corn developmental time in corn intercropped with sunn hemp in spring 2011 at the Mililani Agriculture Park, Mililani. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and  $v_i > 1.5$  (the presence of patches), respectively. The maps show significant spatial patterns with larger gaps than the patches at moderate densities of *P. maidis* from 36 to 55 DAP. The patches occurred at the field margins with large gaps at the center of the field.



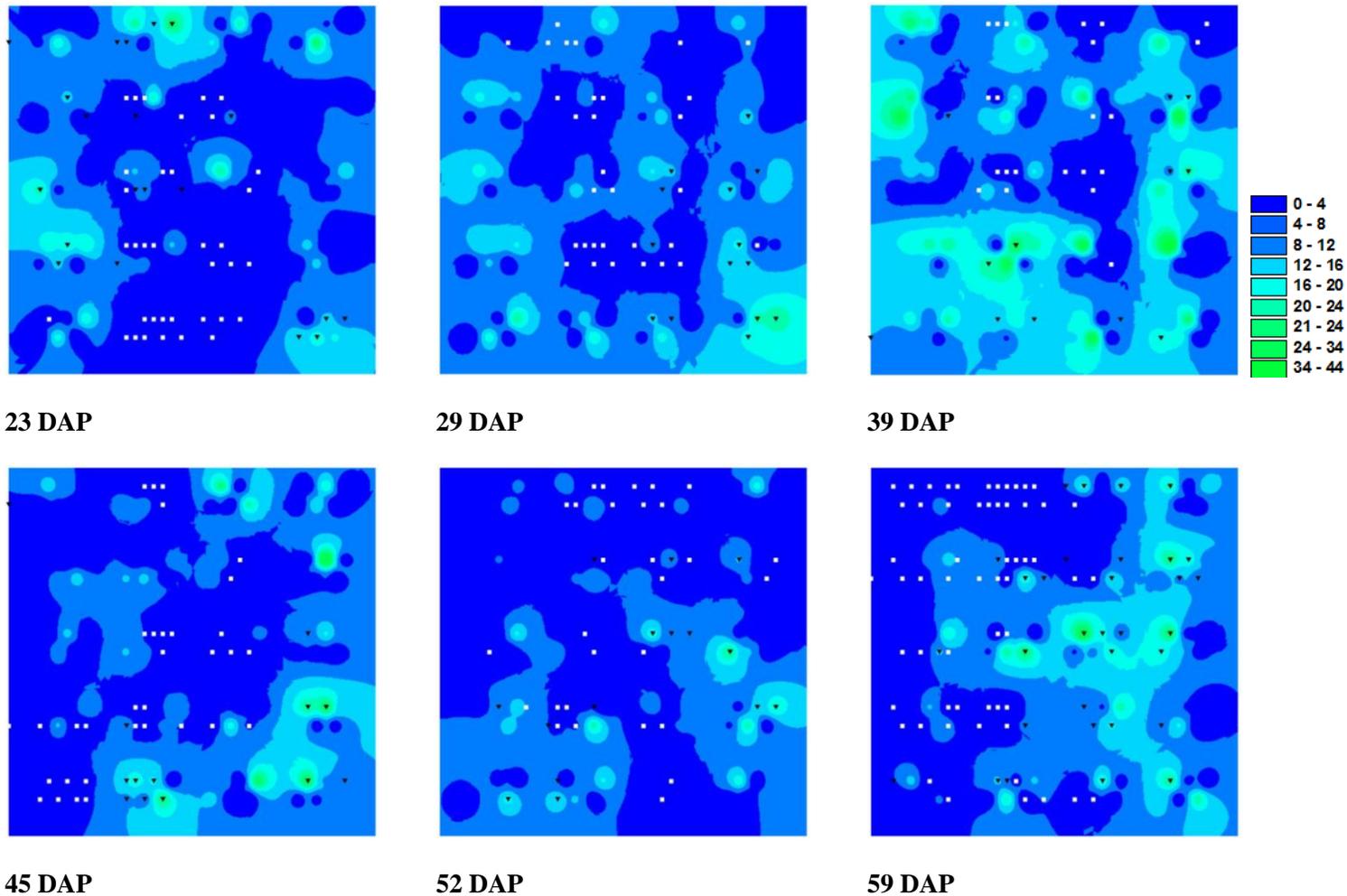
**Figure 5.3(A):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during the corn developmental time in corn monoculture in summer 2011 at the Waimanalo Research Station, Waimanalo. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and  $v_i > 1.5$  (the presence of patches). The maps show uniform to random distribution patterns with large gaps and rare patches at low densities of *P. maidis*.



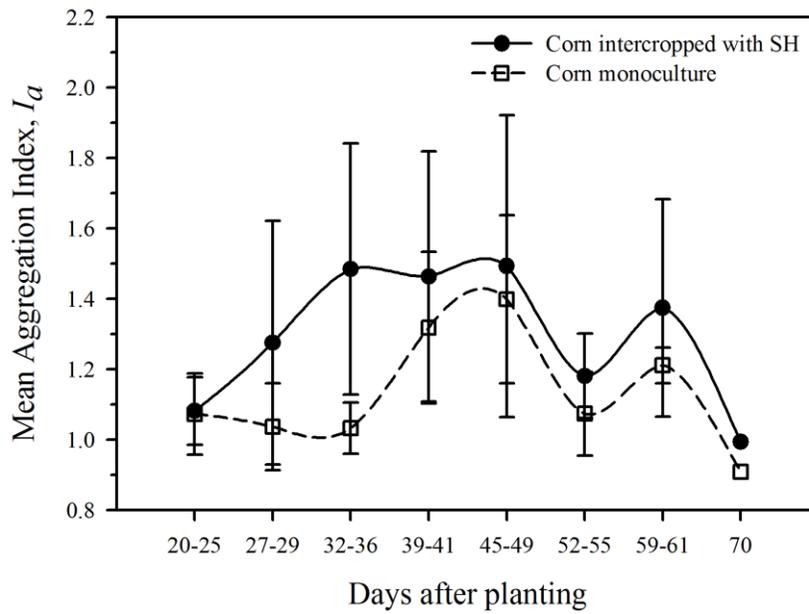
**Figure 5.3(B):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during the corn developmental time in corn intercropped with sunn hemp plots in summer 2011 at the Waimanalo Research Station, Waimanalo. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and  $v_i > 1.5$  (the presence of patches), respectively. The maps show uniform to random distribution patterns with large gaps and rare patches at low densities of *P. maidis*.



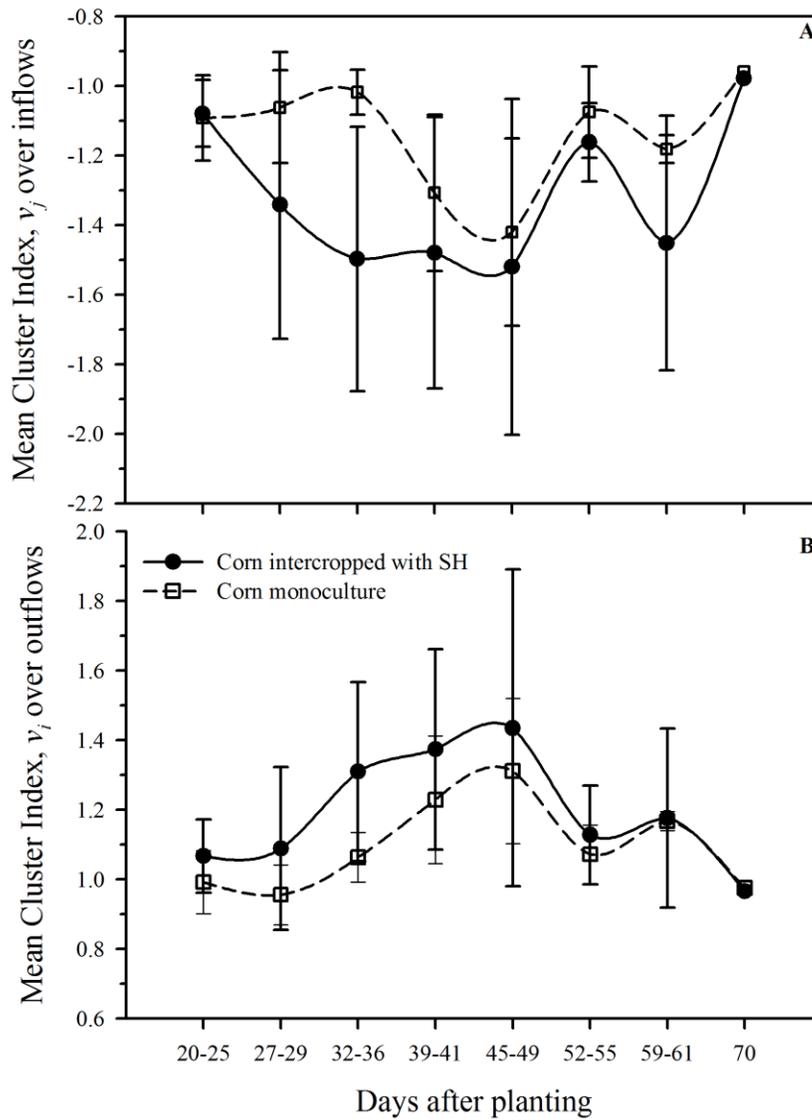
**Figure 5.4(A):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during corn growth in corn monoculture in fall 2011 at the Waimanalo Research Station, Waimanalo. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and  $v_j > 1.5$  (the presence of patches), respectively. The maps show significant spatial patterns with large gaps than patches at high densities of *P. maidis* from 23 to 59 DAP. The patches occurred at the field margins with large gap at the center of the field.



**Figure 5.4(B):** Spatial distribution pattern of *Peregrinus maidis* measured at weekly interval during corn growth in corn intercropped with sunn hemp in fall 2011 at the Waimanalo Research Station, Waimanalo. White squares and dark triangles at the sampled location indicate  $v_j < -1.5$  (the presence of patches) and  $v_j > 1.5$  (the presence of patches), respectively. The maps show significant spatial patterns with larger gaps than patches at high densities of *P. maidis* from 23 to 59 DAP. The patches occurred at the field margins with large gaps at the center of the field.



**Figure 5.5:** Pattern of the index of aggregation,  $I_a$  ( $\pm$  standard error, SE) during the corn developmental time in corn monoculture and corn intercropped with sunn hemp. The values  $I_a$  are the mean of such indices from four experiments at the given range of sampling duration. The figures shows stronger spatial pattern of *Peregrinus maidis* in the intercropped plots than in the corn monocultures on the mid sampling dates.



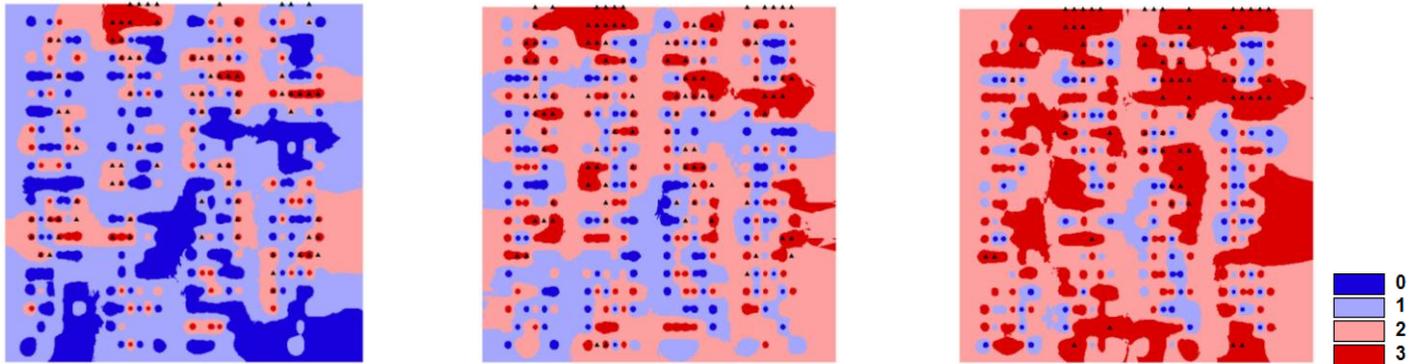
**Figure 5.6:** Pattern of clustering indices (A) forming gaps,  $v_j (\pm SE)$ ; and (B) forming patches,  $v_i (\pm SE)$  during the corn developmental time in corn monoculture and corn intercropped with sunn hemp. The values of each index are the mean of such indices from four experiments at the given range of sampling duration. The figures show stronger spatial distribution patterns of *Peregrinus maidis* in the corn intercropped with sunn hemp plots producing significant gaps and patches than in the corn monocultures.

**Table 2.2:** Summary of SADIE analysis results on severity of plant damage and MMV symptoms (severity of symptoms) scores, and their spatial association with *Peregrinus maidis* counts in corn monoculture and corn intercropped with sunn hemp (corn +SH). The aggregation index  $I_a$  indicates degree of spatial aggregation of severity of symptoms distribution; the clustering indices,  $v_j$  and  $v_i$  indicates the distribution forming gaps and patches, respectively and  $c$ , the association index indicating association between the abundance of *P. maidis* and the severity of symptoms.

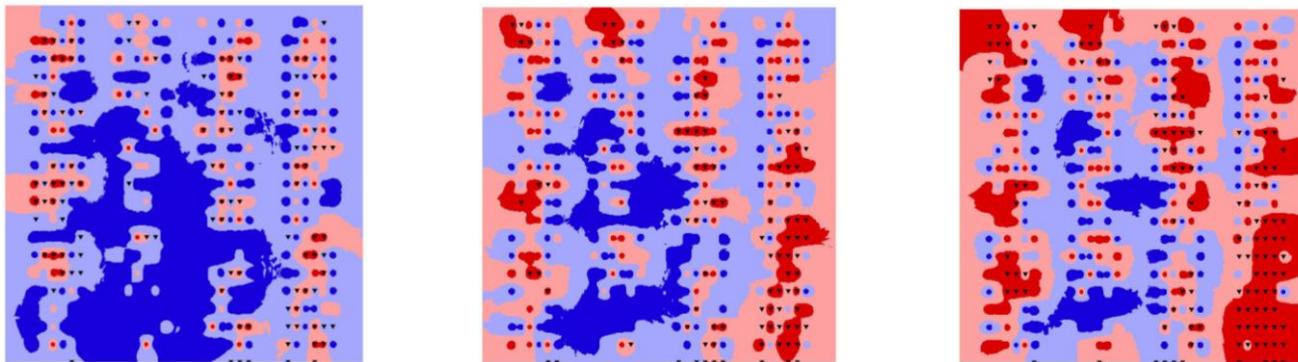
Site	Planting date	DAP	Treatments							
			Corn monoculture				Corn + SH			
			$I_a$	$v_j$	$v_i$	$c$	$I_a$	$v_j$	$v_i$	$c$
Waimanalo	Fall 2010	38	<b>1.94</b>	<b>-1.98</b>	<b>1.86</b>	-0.11	<b>1.65</b>	<b>-1.56</b>	<b>1.67</b>	<b>0.20</b>
		45	<b>1.59</b>	<b>-1.59</b>	<b>1.53</b>	0.05	<b>1.71</b>	<b>-1.60</b>	<b>1.84</b>	0.12
		52	1.12	-1.12	1.11	0.14	<b>1.73</b>	<b>-1.59</b>	<b>1.83</b>	0.41
Mililani	Spring 2011	61	1.19	-1.20	1.15	0.01	<b>1.70</b>	<b>-1.69</b>	<b>1.73</b>	0.03
		70	<b>1.49</b>	<b>-1.51</b>	<b>1.48</b>	-0.11	<b>1.59</b>	<b>-1.60</b>	<b>1.60</b>	0.04
		76	<b>1.41</b>	<b>-1.42</b>	<b>1.35</b>	0.15	1.15	-1.18	1.14	0.05

Bold values  $I_a$  suggest ( $P_a < 0.05$ ), that there is significant spatial pattern (i. e. the severity of symptoms are not arranged randomly). Bold values of  $v_j$  suggest ( $P_j < 0.05$ ), that the distribution forms significant gaps. Bold values of  $v_i$  suggest ( $P_i < 0.05$ ), that the distribution forms significant patches. Bold values of  $c$  suggest ( $P_c < 0.05$ ) that there is positive association between *P. maidis* counts and severity of symptoms

(A) Corn monoculture:



(B) Corn intercropped with SH:



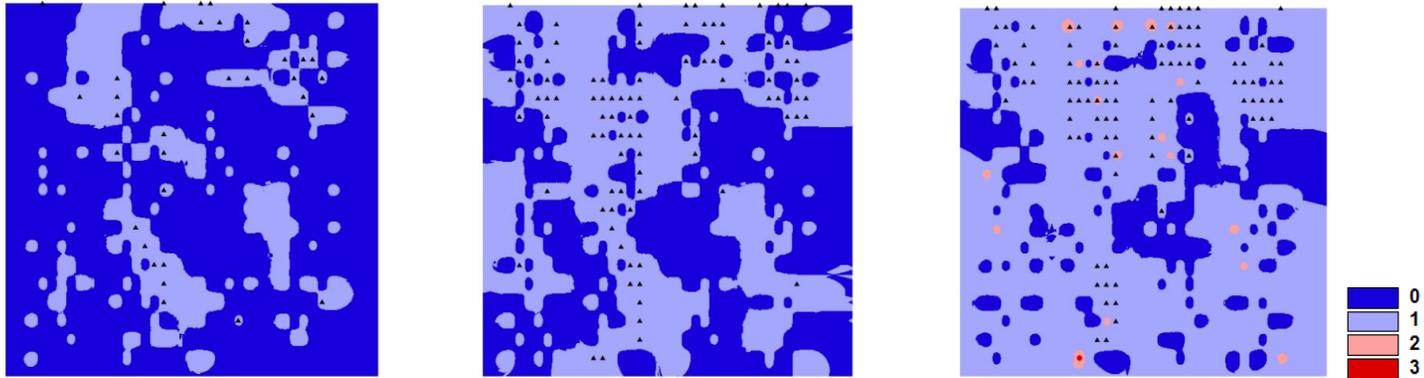
38 DAP

45 DAP

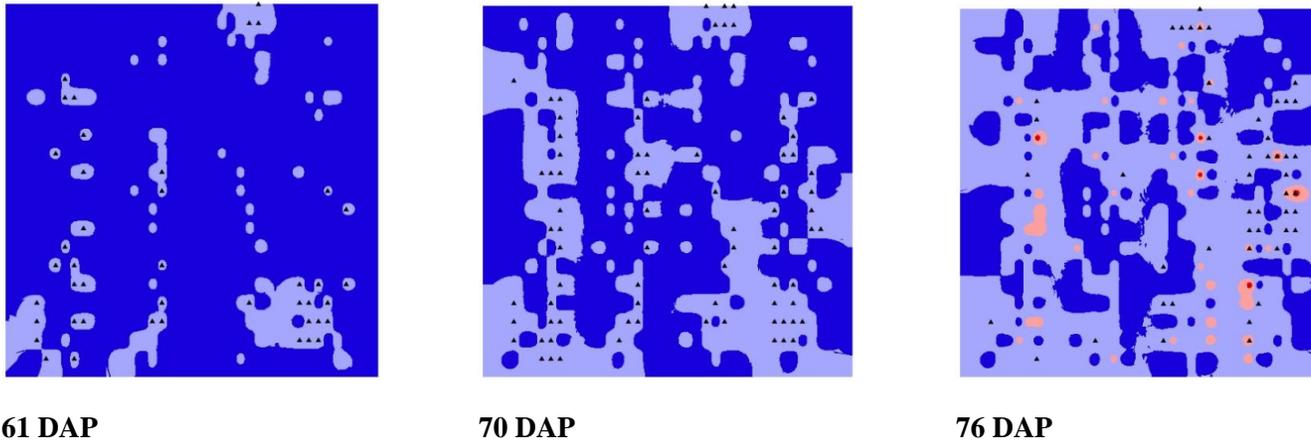
52 DAP

**Figure 5.7:** Spatial distribution pattern of severity of plant damage and MMV symptoms measured at weekly interval during the corn developmental time in (A) corn monoculture and (B) corn intercropped with sunn hemp in fall 2010 at the Waimanalo Research Station, Waimanalo. Dark triangles at the sampled location indicate  $v_i > 1.5$  (the presence of patches). The maps show significant spatial patterns of severity symptoms with patch size increasing with the developmental time. The patch size of plants with MMV symptoms was larger in corn monoculture than intercropped plot.

(A) Corn monoculture:



(B) Corn intercropped Plot:



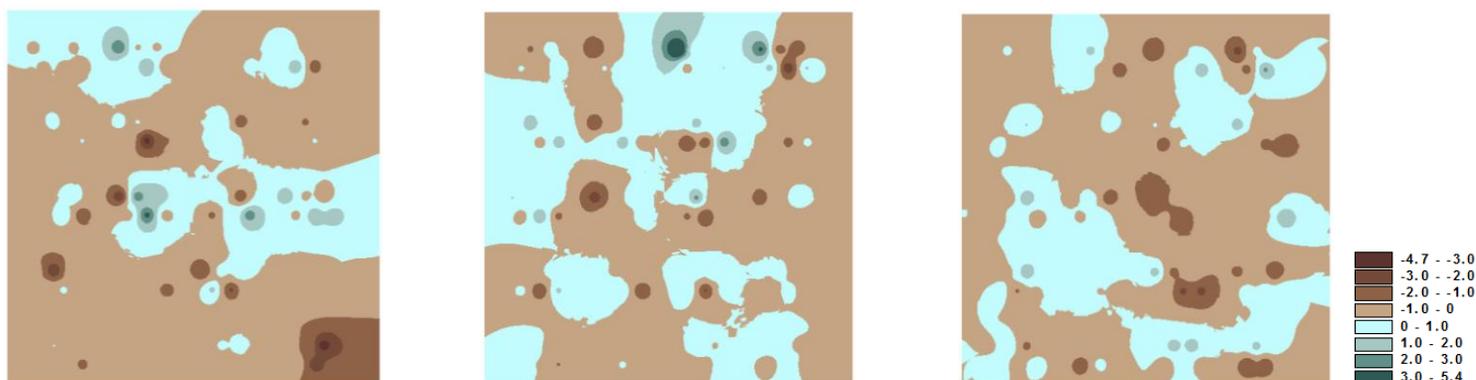
61 DAP

70 DAP

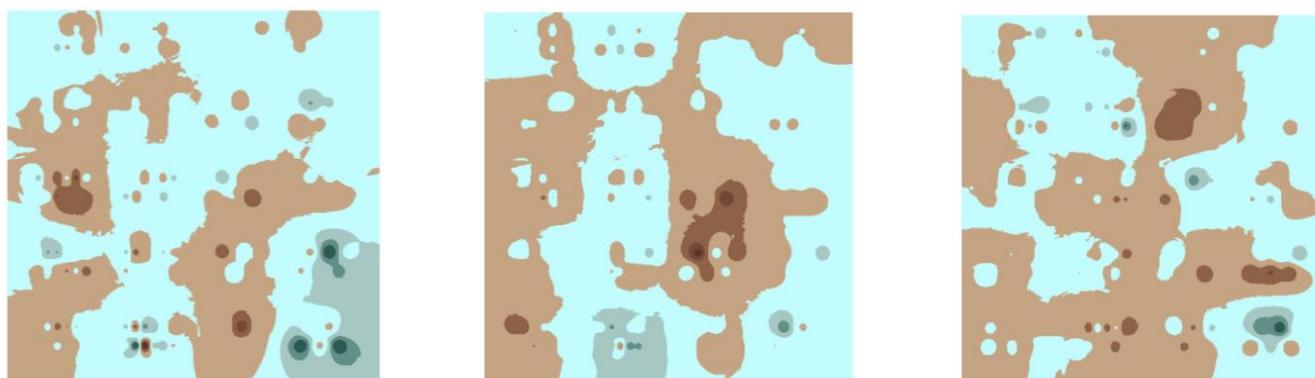
76 DAP

**Figure 5.8:** Spatial distribution pattern of severity of plant damage and MMV symptoms measured at weekly interval during corn growth in (A) corn monoculture and (B) corn intercropped with sunn hemp in spring 2011 at the Mililani Agriculture Park, Mililani. Dark triangles at the sampled location indicate  $v_i > 1.5$  (the presence of patches). The maps show patch size for severity of plant damage increased with the corn developmental time with incidence of MMV.

(A) Corn monoculture:



(B) Corn intercropped plot:



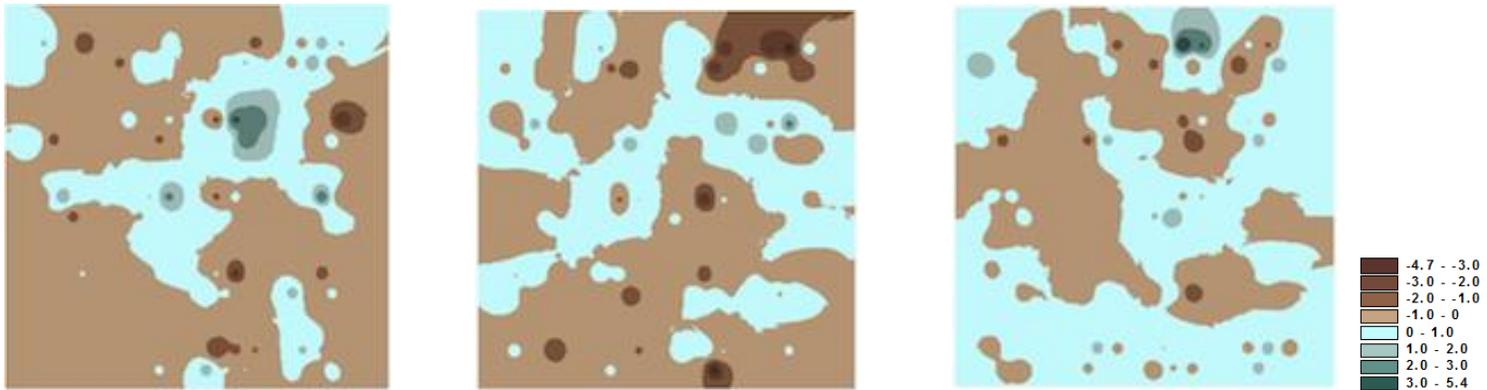
38 DAP

45 DAP

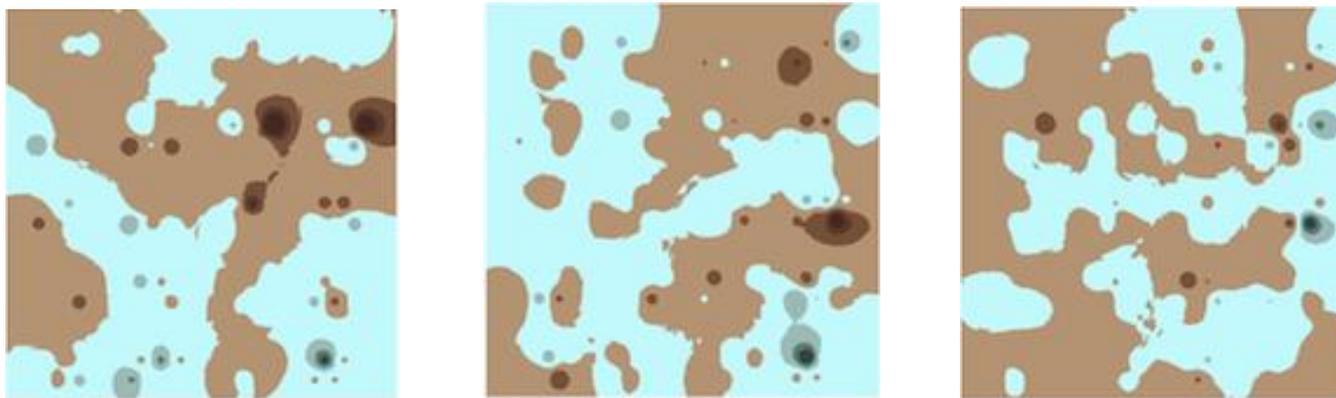
52 DAP

**Figure 5.9:** Local spatial association pattern of *Peregrinus maidis* distribution with the severity of plant damage and MMV symptoms in (A) corn monoculture and (B) corn intercropped with sunn hemp plot measured at weekly interval at the Waimanalo Research station, Waimanalo. The maps show unassociated distribution pattern with a few small dissociated (dark brown) and associated patches (dark green).

(A) Corn monoculture:



(B) Corn intercropped plot:

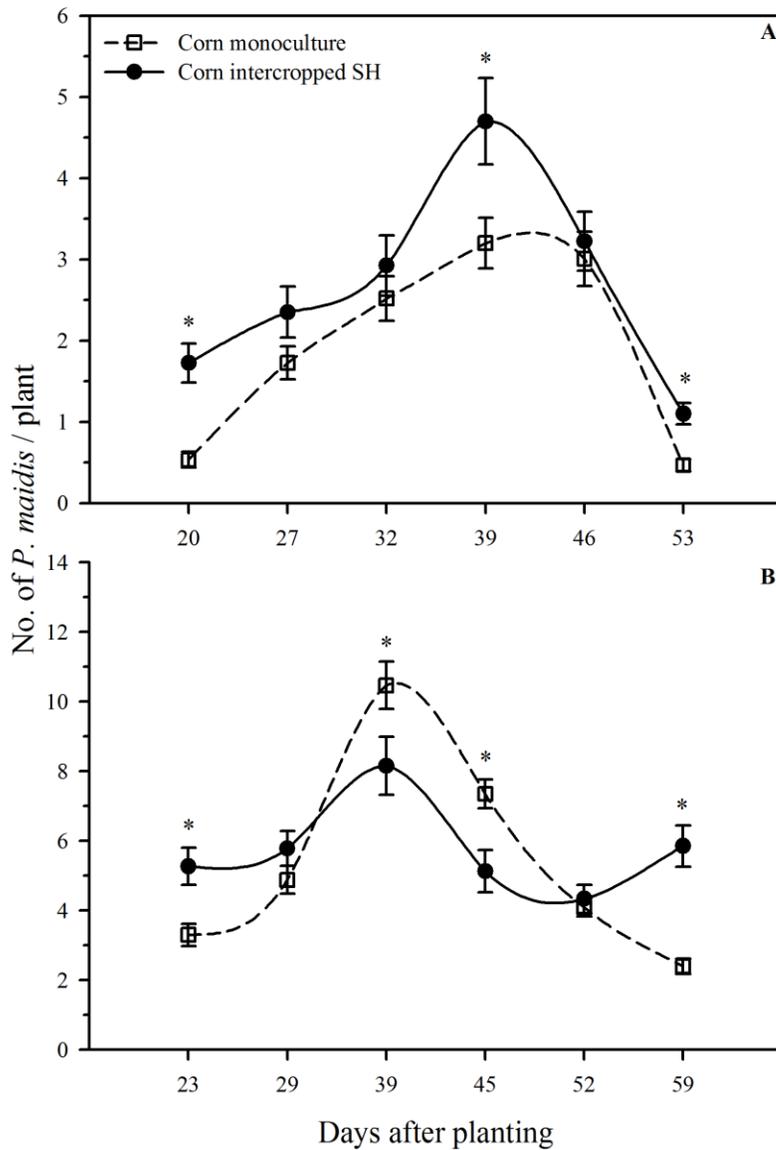


61 DAP

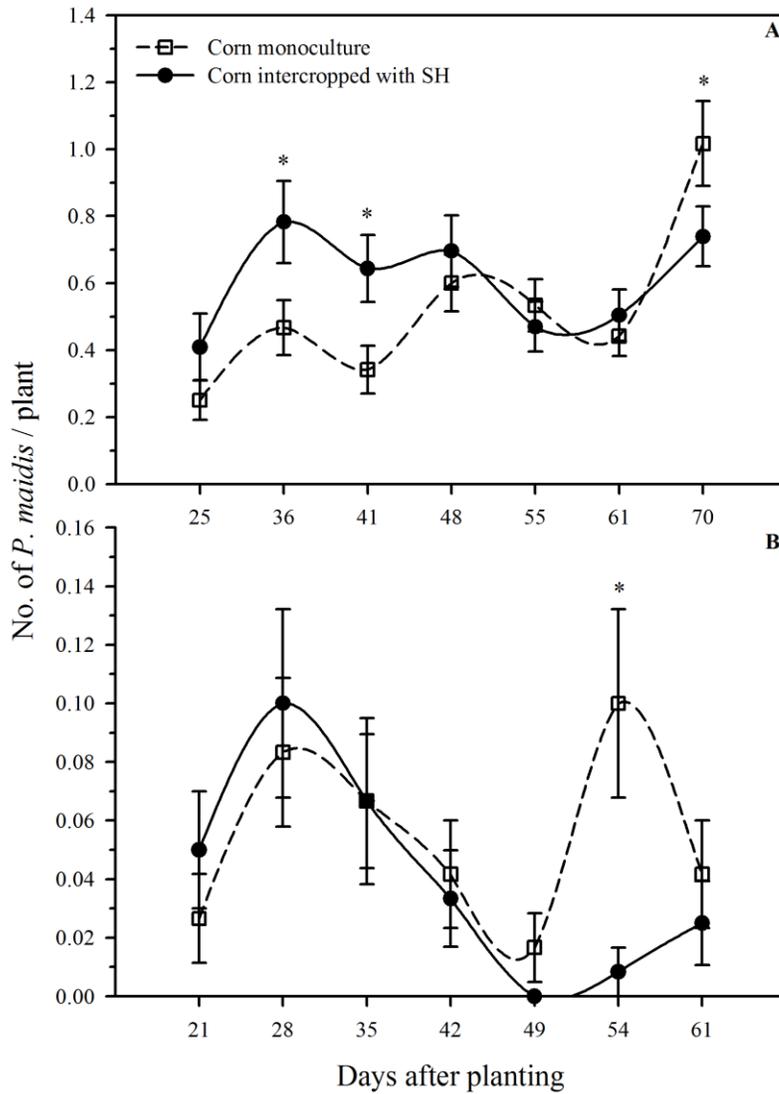
70 DAP

76 DAP

**Figure 5.10:** Local spatial association pattern of *Peregrinus maidis* distribution with the severity of plant damage and MMV symptoms in (A) corn monoculture and (B) corn intercropped with sunn hemp plot measured at weekly interval at the Mililani Agriculture Park, Mililani. The maps show unassociated distribution pattern with a few small dissociated (dark brown) and associated patches (dark green).



**Figure 5.11:** Mean numbers of *Peregrinus maidis* ( $\pm$  SE) on different sampling dates in corn monoculture and corn intercropped sunn hemp plots in (A) fall 2010 and (B) fall 2011 at the Waimanalo Research Station, Waimanalo. \* indicates mean number of *P. maidis* were significantly different in corn monoculture than intercropped plot.



**Figure 5.12:** Mean numbers of *Peregrinus maidis* ( $\pm$  SE) on different sampling dates in corn monoculture and corn intercropped with sunn hemp in (A) spring 2011, and (B) summer 2011 at the Mililani Agriculture Park, Mililani and Waimanalo Research Station, respectively. \* indicates mean number of *P. maidis* were significantly different in corn monoculture than intercropped plot.

## CHAPTER V

### **Incidence of *Maize mosaic virus* and *Maize chlorotic mottle virus* in corn, in relation to within-field activity of their vector insects in corn as influenced by sunn hemp intercropping**

#### **Abstract**

Field experiments were conducted to examine effects of sunn hemp, *Crotalaria juncea* L. intercropping on within field-activity of (i) corn planthoppers, *Peregrinus maidis* Ashmead and corn thrips, *Frankliniella williamsi* Hood, and (ii) incidence of *Maize mosaic virus* (MMV) and *Maize chlorotic mottle virus* (MCMV) symptomatic corn plants in corn – sunn hemp intercropping system. Within-field activity of macropterous *P. maidis* and adults *F. williamsi* was quantified using trap catches as an activity measure. Plants showing MMV symptoms were evaluated in the first two experiments (fall 2010 and spring 2011), while MCMV were evaluated in the later two experiments (summer and fall 2011). Results showed that sunn hemp intercropping significantly increased within-field activity of macropterous *P. maidis* and adults of *F. williamsi* compared to corn monoculture treatments. Increase in within-field activity of *P. maidis* in the intercropped treatment during vegetative stages of corn development coincided with reduced colonization, that might have reduced number of feeding vectors and plant tenure time, resulting lower incidence of MMV as compared to the monoculture, as the events required for persistent transmission of plant virus were disrupted. Increase in within-field activity of *F. williamsi* in the intercropped treatment suggested greater movement of

vectors and contributed to higher incidence of MCMV compared to the monoculture treatment, as is characteristic of semi-persistent transmission of plant virus.

## Introduction

Corn (or maize, *Zea mays*) is a staple crop in many countries and is an important component of US agriculture in general. In addition, production of parent seed-corn is currently the largest agri-based industry in Hawaii (NASS 2009). However, recent pest problems have threatened seed-corn production in Hawaii. This is mainly due to one of the most devastating virus disease of corn, *Maize mosaic virus* (MMV), vectored by the corn planthopper; *Peregrinus maidis* Ashmead (Hemiptera: Delphacidae), which has been problematic in the islands for many years. The viruses of Rhabdoviridae family, such as MMV are propagative in the insect body and transmitted persistently to the susceptible plants (Nault 1989, Ammar and Hogenhout 2008, Hogenhout et al. 2008). The recent epidemics of another virus (*Maize chlorotic mottle virus*, MCMV) vectored by the corn thrips, *Frankliniella williamsi* Hood (Thysanoptera: Thripidae) have created significant additional problems for Hawaii corn growers and the parent seed-corn industry. Epidemic outbreaks of MCMV were first observed on the island of Kauai during 1990s (Jiang et al. 1992). The problem was eradicated for several years by destroying infected cornfields and employing a strict fallow period between cropping seasons. However, the presence of MCMV was suspected during the year 2009 on the island of Oahu (personnel communication with the seed-corn industry). The recent epidemics of this disease were confirmed from cornfields at Pioneer Hi-Bred station at

Kaheka (Kauai, HI) on December 2009 (Cabanas et al. 2013) and at Kahuku sweet corn Farm (Oahu, HI) in 2010, causing severe yield losses (Nelson et al. 2011). Since then the disease has been confirmed on the Oahu, Maui and Kauai islands of Hawaii.

MCMV (Tombusviridae: *Machlomovirus*) is transmitted semi-persistently by several species chrysomelid beetles, including the western corn rootworm, *Diabrotica frugiperla frugiperla* Laconte on the mainland USA (Nault et al. 1978, Jensen 1985). However, none of these chrysomelid beetle species have been reported in Hawaii (Nishida 2002), where *F. williamsi* has been identified as the primary vector of MCMV in Hawaii (Jiang et al. 1992, Cabanas et al. 2013). A recent study on transmission biology of MCMV by *F. williamsi* showed a semi-persistent mode of transmission, similar to transmission by chrysomelid beetles previously examined (Cabanas et al 2013). The symptoms of MCMV include initial longitudinal streaks parallel to leaf vein, streaks may coalesce to create chlorotic mottling, followed by leaf necrosis, stunting and plant death (Nelson et al. 2011). It has been reported that MCMV can reduce corn yield by 10-15% (Castilo and Hobert 1974, Nault et al. 1981). When MCMV co-infects corn plants with other corn viruses from the family Potyviridae, such as *Maize dwarf mosaic virus* (MDMV), *Sugarcane mosaic virus* (SCMV) or *Wheat streak mosaic virus* (WSMV), their synergistic effect cause a more severe disease called Corn Lethal Necrosis (CLN) (Uyemoto et al. 1980, Goldberg and Brakke 1987). It has been reported that CLN disease can reduce crop yield by up to 90% (Niblett and Claffin 1978, Uyemoto et al 1980).

Currently it is evident that both virus diseases (MMV and MCMV) vectored by *P. maidis* and *F. williamsi* is negatively impacting the corn production in Hawaii. The current practice of employing resistant cultivars when available can be ineffectual for the

temperate seed-corn production (Brewbaker 2003) and in areas where mixed infestation of these viruses occurs (Dintinger et al. 2005). As a result Hawaii's seed-corn industries routinely spray pesticides to suppress vector populations. The potentiality of thrips to express resistance to selective insecticides is another concern in managing this vector species (Martin et al. 2003, Yulin et al. 2012). Alternative practices, which are sustainable, environment friendly, cost effective that can simultaneously manage MMV and MCMV, would be of great value to the seed-corn industry.

The barrier or protector plant, which are grown around, or in-between the cash crops acts as a virus sink and effectively reduces incidence of aphid-transmitted non-persistent viruses (Fereres 2000, Hooks and Fereres 2006, Manandhar and Hooks 2011). The diverse cropping habitat increases within-field movement of insect vectors that reduces vector tenure time on the crop plant and results in lower incidence of insect vectored persistent viruses (Power 1987, 1988 and 1991). The previous result of this dissertation work showed, among cover crop used (buckwheat, cowpea and sunn hemp), a tall statured sunn hemp intercropping reduces *P. maidis* within-field activity that minimizes vector colonization on the corn plant and results in reduced incidence of hopperburn and MMV symptomatic plants (Chapter II). Furthermore, no studies involving cover cropping, intercropping or mixed cropping in reducing thrips transmitted persistent viruses (genus: *Tospovirus*) or semipersistent viruses (genus: *Tombusvirus*) in crop plants exist. This study aimed to examine effect of sunn hemp intercropping on within-field activity of *P. maidis* and *F. williamsi*, and incidence of MMV and MCMV symptomatic corn plants, respectively. Unlike previous study of this dissertation work, this study is conducted in a large field plot with high corn to sunn hemp intercropping

ratio (6:1) to mimic large-scale agricultural landscape that can fit into the current cropping practices. This chapter specifically examines the following hypotheses: (i) activity of *P. maidis* and *F. williamsi* will be higher in corn intercropped with sunn hemp (corn-intercropped) plots than corn monoculture plots, and (ii) incidence of and MMV and MCMV will be lower in corn-intercropped plots than monoculture plots.

## **Methods**

Field experiments were conducted to study the effect of sunn hemp intercropping in within-field activity of *P. maidis* and *F. williamsi* in cornfields. Within-field activity of *P. maidis* and *F. williamsi* in intercropped treatments were compared with monoculture controls using sticky trap count data, with trap captures as the measure of insect activity. Three experiments were conducted at the University of Hawaii's experimentation station, Waimanalo Research Station at Waimanalo. One experiment was conducted at the Pioneer Hi-Bred International Inc. field station at Mililani Agricultural Park, Mililani. The experimental sites were located on the island of Oahu in Hawaii and characterized by diversified agricultural systems, including fruits, vegetables, ornamentals and seed-corn farming. The experiments were planted at the stations in various planting seasons during the study period from October 2010 to December 2011.

### **Crop planting**

A total of five strips of sunn hemp, each measuring 30.5 m x 1.8 m were planted in a field plot of 30.5 m x 30.5 m area approximately a month before corn planting. Six

rows of corn were planted in rows between two pre-established strips of sunn hemp, to make 24 rows of corn plants in corn-intercropped plots. Continuous 34 rows of corn were planted in an area of 30.5 m x 30.5 m in corn monoculture plots. A sweet corn variety, Passion (Seminis Vegetables) and a fodder corn variety, P0125xR (Pioneer Hi-bred International Int.), both susceptible to MMV were used at Waimanalo and Mililani sites, respectively. The experiments were planted on October 5 (fall 2010) at Waimanalo, November 23, 2010 (spring 2011) at Mililani followed by April 14 (summer 2011) and October 11 (fall 2011) at Waimanalo.

### ***P. maidis* and *F. williamsi* counts on traps**

Macropterous *P. maidis* and adult *F. williamsi* were monitored using yellow sticky traps (Seabright Laboratories, CA). Each yellow sticky trap was reverse folded to expose its 10.2 cm x 35.6 cm (~360 cm<sup>2</sup>) area coated with adhesive. A 1.2 m long PVC pipe elbowed with 25 cm PVC pipe at the top was pounded in the soil to form a support for hanging the trap. A total of eight traps were hung in each plot, adjusting the height at each sampling to maintain traps at a level equal to the top of the corn canopy. In monoculture plots, traps were arranged systematically by placing two traps in a corn intra-row space, separated ~15 m apart. Each such set of two traps was placed in a corn intra-row space between 4<sup>th</sup> and 5<sup>th</sup>, 12<sup>th</sup> and 13<sup>th</sup>, 20<sup>th</sup> and 21<sup>st</sup>, and 28<sup>th</sup> and 29<sup>th</sup> rows of corn plants from the beginning row. In corn-intercropped plots, traps were placed in a zig-zag pattern, with four traps placed in a corn - sunn hemp inter-row space, followed by another four traps in a corn - corn intra-rows space.

Macroptrous *P. maidis* trapped in the sticky traps were counted in the field at weekly intervals from the V2 - V3 (early vegetative, initiation of 2-3 leaves) stages of corn plant development until final harvest. Each week, counted *P. maidis* were removed from the traps using forceps in order to avoid inclusion in the next week's count. The sticky traps were replaced every two weeks and brought to the lab for counting adult *F. williamsi*. Adults of *F. williamsi* were counted from the central four squares (25 cm<sup>2</sup>) of each side of the trap using a binocular microscope. The mean count from both sides of the trap was reported as a unit *P. maidis* and *F. williamsi* count. The thrips species identified from the sunn hemp flowers are primarily, *F. occidentalis* (Pergande) and *Thrips tabaci*. The corn thrips, *F. williamsi*, were identified using their characteristic feature of three pairs of ocellii on the forehead and the dark antennal tip, while counting on the sticky traps.

### **MMV and MCMV evaluation**

MMV was evaluated on the basis of typical visual symptoms of white streaking on leaf lamina, leaf sheaths and husk covers at the reproductive (R) stages of corn plants in fall 2010 and spring 2011 experiments. MCMV was evaluated on the on the basis of typical symptoms of chlorotic mottling of leaf at the VT (vegetative tassel) and R stages of corn plants in summer and fall 2011 experiments. The number of plants showing visual symptoms of each virus were quantified by evaluating at least 24 rows of corn plants in each treatment plot and presented as percentage per row. In the fall 2010 and spring 2011 experiments, four leaf samples of corn plants showing differential severity symptoms of hopperburn and MMV symptoms (scored 0 - 3) (see chapter IV) were collected from each plot. Similarly, at least three leaf samples were collected showing

differential symptoms of MCMV (scored 0 - 3) were collected from each plot in the summer and fall 2011 experiments. Here, severity of MCMV ratings: 0 indicated no symptoms, 1 indicated chlorosis, 2 indicated longitudinal streaks parallel to veins, and 3 indicated chlorotic mottling. The leaf samples were diagnosed using a double antibody sandwich enzyme-linked immunosorbent assay (DAS – ELISA) to detect of MMV and MCMV. ELISA reagent kits for MMV and MCMV (Agdia Inc., US) were used for diagnosing MMV and MCMV, respectively.

### **Data Analyses**

Effects of treatments on within-field activity of *P. maidis* and *F. williamsi* were analyzed for each experiment by sampling dates using a mixed model analysis of variance (PROC MIXED, SAS Institute). Further, a pooled analysis was conducted on the combined data to examine treatment effects on general within-field activity pattern of *P. maidis* and *F. williamsi* during the corn developmental time. The effect of treatments on incidence of MMV on the basis of visual symptoms was examined for fall 2010 and spring 2011 experiments using a mixed model analysis of variance (PROC MIXED, SAS Institute). Same procedure was used to examine incidence MCMV by sampling dates for the summer and fall 2011 experiments. All the statistical analysis procedures involving mixed models in SAS were followed as described by Littell et al. (2006).

## Results

### ***P. maidis* counts on traps**

Overall mean number of *P. maidis* counts, irrespective of treatments were higher in the fall ( $21.15 \pm 1.0$ , mean  $\pm$  SE), followed by spring ( $0.91 \pm 0.2$ ) and summer ( $0.61 \pm 0.1$ ) experiments. In the fall 2010 experiment, *P. maidis* trap counts were significantly higher in the corn-intercropped- compared to the corn monoculture plot at 24 DAP ( $F_{1,14} = 5.56$ ,  $P = 0.03$ ) and lower in the corn-intercropped- compared to the monoculture plot from 53 – 66 DAP ( $F_{1,13-14} = 7.25 - 17.62$ ,  $P \leq 0.02$ ) (Figure 6.1A). Similarly, in the fall 2011 experiment, the trap counts were significantly higher in the corn-intercropped- compared to the monoculture plot from 21 - 28 DAP ( $F_{1,18} = 11.04 - 18.67$ ,  $P < 0.01$ ) (Figure 6.2B). The trap counts were significantly higher in the corn-intercropped- compared to the monoculture plot at one later sampled date in spring 2011 (41 DAP:  $F_{1,13} = 6.97$ ,  $P = 0.02$ ) (Figure 6.1B) and in summer 2011 (56 DAP:  $F_{1,18} = 6.0$ ,  $P = 0.02$ ) (Figure 2A) experiments. Combining all the experimental data, the trap counts were significantly higher in corn-intercropped- compared to monoculture plots from 24 - 28 DAP ( $F_{1,69} = 7.43$ ,  $P = 0.01$ ) (Figure 6.3).

### ***F. williamsi* counts on traps**

Overall mean *F. williamsi* counts, irrespective of treatment plots were higher in the summer ( $13.16 \pm 1.45$ ), followed by fall ( $8.78 \pm 1.03$ ) and spring ( $0.95 \pm 0.20$ ) experiments. Results of analysis for each experiment by sampling dates showed significantly greater trap counts in the corn-intercropped- compared to the corn monoculture plot on almost all sampled dates ( $P < 0.05$ ) (Figures: 6.4A, 6.4B, 6.5A and

6.5B). Combining all the experimental data, trap counts were significantly greater in corn-intercropped- compared to monoculture plots at first three range of sampled dates (24 - 34 DAP:  $F_{1,69} = 5.91$ ,  $P = 0.02$ ; 39 - 48 DAP:  $F_{1,70} = 11.93$ ,  $P < 0.01$ ; 53 - 65 DAP:  $F_{1,69} = 23.57$ ,  $P < 0.01$ ) (Figure 6.6).

### **MMV and MCMV evaluation**

MMV occurred only in the first two experiments (fall 2010 and spring 2011) and was higher in the fall than spring planting season (Figure 6.7). The percentage of plants showing MMV symptoms per row at the R stage was significantly lower in the corn-intercropped- ( $71.75 \pm 3.4$ ) compared to the monoculture plot ( $82.04 \pm 0.9$ ) in the fall 2010 ( $F_{1,46} = 8.75$ ,  $P < 0.01$ ), while the percentage of plants showing MMV symptoms was significantly higher in the corn-intercropped- ( $0.67 \pm 0.2$ ) compared to the monoculture plot ( $0.11 \pm 0.1$ ) ( $F_{1,46} = 6.22$ ,  $P = 0.02$ ) in the spring 2011 (Figure 6.7).

In the first two planting seasons (fall 2010 and spring 2011), field evaluation on MCMV on the basis their symptoms were not planned, as MMV was anticipated to be the primary virus. Low levels of MCMV detection on analyzing leaf samples by DAS - ELISA in the spring 2011 experiment prompted field evaluation of this virus in subsequent experiments. Visible symptoms of MCMV on the corn plants were clear in the summer and fall 2011 experiments. The percentage of plants showing visual symptoms was higher in fall than summer experiments. In the summer 2011 experiment, the percentage of plant showing visual symptoms of MCMV was significantly higher in the corn-intercropped- compared to the corn monoculture plot at VT and R stages of crop growth (VT stage:  $F_{1,45} = 93.62$ ,  $P < 0.01$ ; R stage:  $F_{1,45} = 60.54$ ,  $P < 0.01$ ) (Figure 6.8A).

While, the percentage was significantly higher in the corn-intercropped- compared to the monoculture plot at the R stage of the corn growth (R stage:  $F_{1,51} = 39.66$ ,  $P < 0.01$ ) in the fall 2011 experiment (Figure 6.8B).

All the leaf samples of plants showing symptoms of MMV consistently tested positive for MMV using DAS – ELISA in fall 2010 and spring 2011. However, the proportion of leaf samples from plants showing differential severity symptoms of MMV were also tested positive for MCMV in the spring 2011. In subsequent experiments (summer 2011 and fall 2011), all the leaf samples showing differential symptoms of MCMV (scored 0-3) tested consistently positive for MCMV, but negative for MMV (Table 3.1). The results showed that even leaf samples of apparently healthy plants (with disease rating 0) tested positive for MCMV.

## **Discussion**

### **Within-field activity of *P. maidis***

In the current study, the combined analysis of *P. maidis* trap counts showed increased within-field activity of *P. maidis* during V2 – V8 and R stages, suggesting bimodal periodicity. This study showed significant increase in within-field activity of *P. maidis* in the corn intercropped with sunn hemp treatment compared to the corn monoculture during the V2 – V8 stages. This result is consistently similar in each experiment conducted in three different seasons. The results of previous work of this dissertation also support the current result (Chapter II). The results of current study and Chapter II suggest immigrating *P. maidis*, which land on sunn hemp, need to find their

host plants through subsequent short distant flights resulting increased activity in the corn-intercropped- compared to the monoculture treatment. The increased *P. maidis* activity in the intercropped treatment indicated reduced settling and colonization rate on corn plants during the V2 – V8 stages (Chapter II). The reduced *P. maidis* colonization on the corn plants is attributable to reduce plant tenure time and less *P. maidis* feeding on the corn plants during the V2 – V8 stages in the intercropped treatment compared to the corn monoculture.

### **MMV incidence**

The results of current study showed that the percentage of plants showing MMV symptoms was significantly lower in the corn intercropped with sunn hemp treatment compared to the corn monoculture. Lower incidence of MMV in the corn-intercropped treatment is likely mainly due to reduced *P. maidis* tenure time, reduced numbers of vectors feeding on the corn plant, disrupting the characteristic course of events required by the persistent mode of transmission. In particular, for transmission of MMV to corn plants, *P. maidis* requires an acquisition access period of two weeks on the MMV infected plants followed by an inoculation access period of one week on the healthy plant (Ammar and Hogenhout 2008). Therefore, a successful transmission of MMV to corn plants requires prolonged feeding by viruliferous *P. maidis*. The current study showed intercropping corn with sunn hemp reduced prolonged feeding by *P. maidis* (as indicated by reduced colonization rate) that resulted in reduced incidence of MMV in corn-intercropped treatments. In general, the current study, results reported in chapter II and references therein (Power 1987, 1988 and 1991), support the hypothesis that plant diversity increases within-field movement of vectors, which reduces numbers of feeding

vectors and their plant tenure time, and the result is reduced incidence of plant disease caused by the persistent viruses. Power (1992) showed that leafhoppers, *Dalbulus maidis* were less abundant and they spread *Maize rayado fino virus* (a persistently transmitted virus) less rapidly in dense stands of corn plants than in sparse stands. This reduced rate of spread was attributed to changes in the insects' dispersal behavior in dense-, compared with less-dense corn plots: if distances between corn plants were less, the insects were more likely to move among plants. In the current study, the sunn hemp barriers may have reduced the movement of the *P. maidis* by increasing the distance between corn plants in alternate strips of intercropped rows (increased distance), thus concentrating the populations on the plants within the corn-rows, where the plants were close to each other.

#### **Within-field activity of *F. williamsi***

The overall results showed corn intercropped with sunn hemp increased within-field activity of *F. williamsi* throughout the corn developmental time compared to corn monocultures. This result was consistent for each experiment conducted during three seasons. Chapter I and references therein identified yellow flowers of companion plants as a trap for mixed species of thrips, thereby potentially reducing their densities in the primary crop. Effect of cover crop intercropping treatments on densities of thrips, primarily *F. occidentalis* on the corn silks have showed mixed results compared to monoculture treatments, probably due to variable effects of different cover crops that were used in the corn - cover crop intercropping systems (Chapter III). It is evident from the current study that sunn hemp flowers host multiple species of thrips (primarily, *F. occidentalis* and *T. tabaci*) and might not have provided attractive hosts for *F. williamsi* from corn plants resulting higher *F. williamsi* activity in the intercropped plots. These

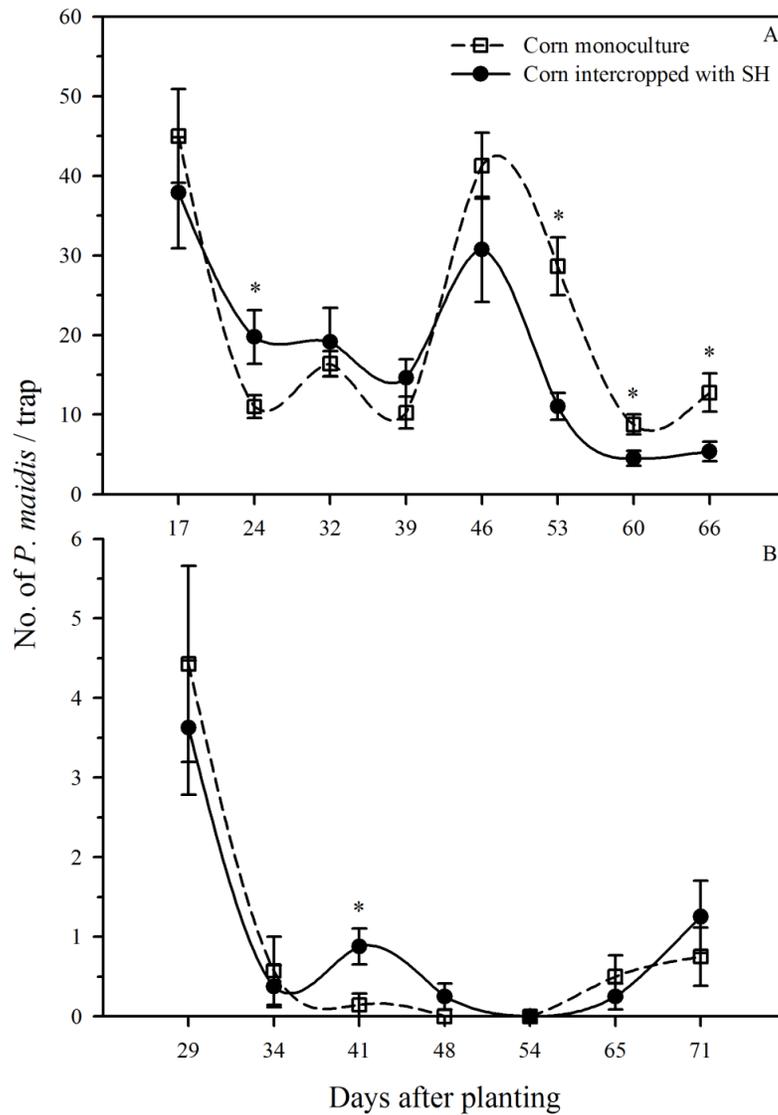
results indicated if the primary crop and intercrop do not host common thrips species, an increase in within-field thrips activity may be predicted.

### **MCMV incidence**

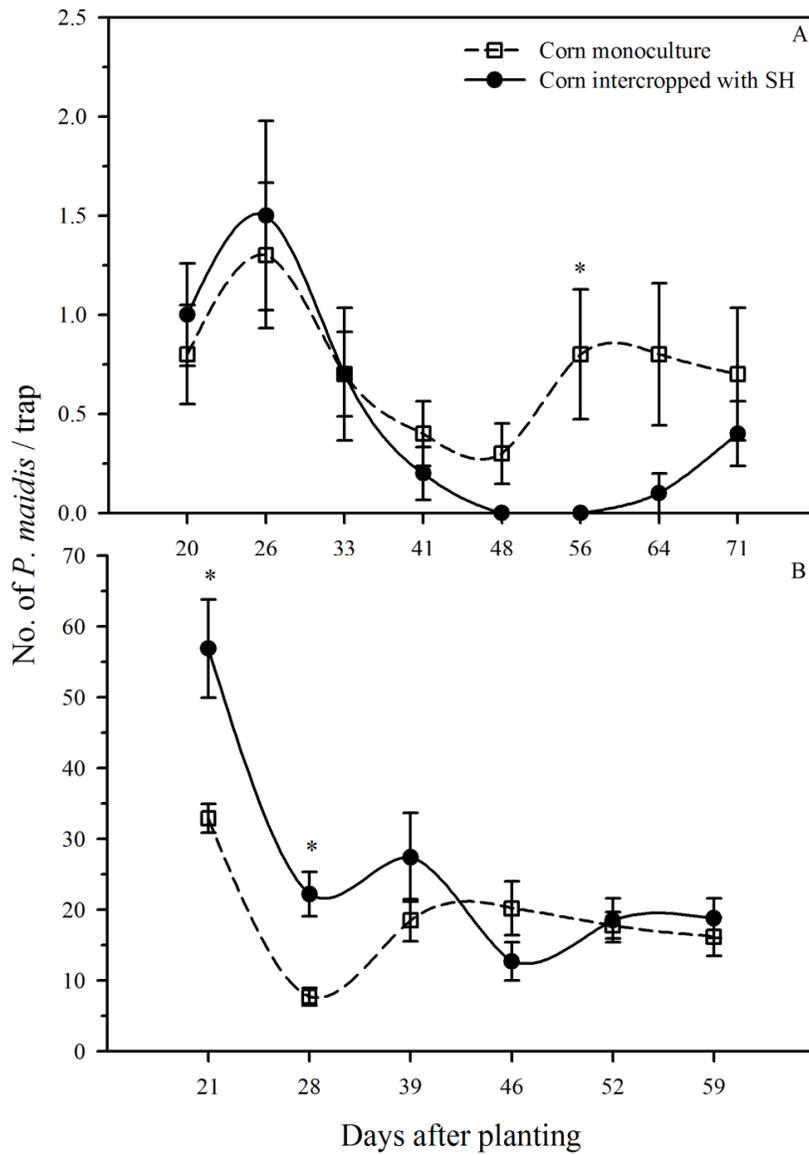
In the current study, the incidence MCMV was higher in the corn-intercropped treatment compared to the corn monoculture. The higher incidence of MCMV in the corn-intercropped treatment is likely mainly due to greater within-field activity of *F. williamsi* and their characteristics semi-persistent mode of transmission. The semi-persistent transmission of plant viruses is characterized by short acquisition access period (minutes to hours), no latent period, a short inoculation access period (minutes to hours) and more importantly, longer retention time (hours to days) (Nault 1997, Ng and Falk 2006). A recent study on MCMV transmission by *F. williamsi* showed shorter acquisition access period (48 hours) and inoculation access period (48 hours) with no evidence of latent period, and longer retention period (6 days). Thus, the insect becomes viruliferous immediately after acquiring the virus and able to transmit with short period feeding on the plant. Additionally, the longer retention time facilitates the viruliferous insect to transmit the virus to several plants as it moves among them and feed. This mode of MCMV transmission by *F. williamsi* likely resulted in rapid transmission rates and infection throughout the entire field. Therefore, greater within-field activity of *F. williamsi* may have facilitated rapid spread MCMV in the corn-intercropped plot compared to the corn monoculture.

In conclusion, intercropping corn with sunn hemp increased the within-field activity of *P. maidis* compared with monoculture cropping, which suggested reduced

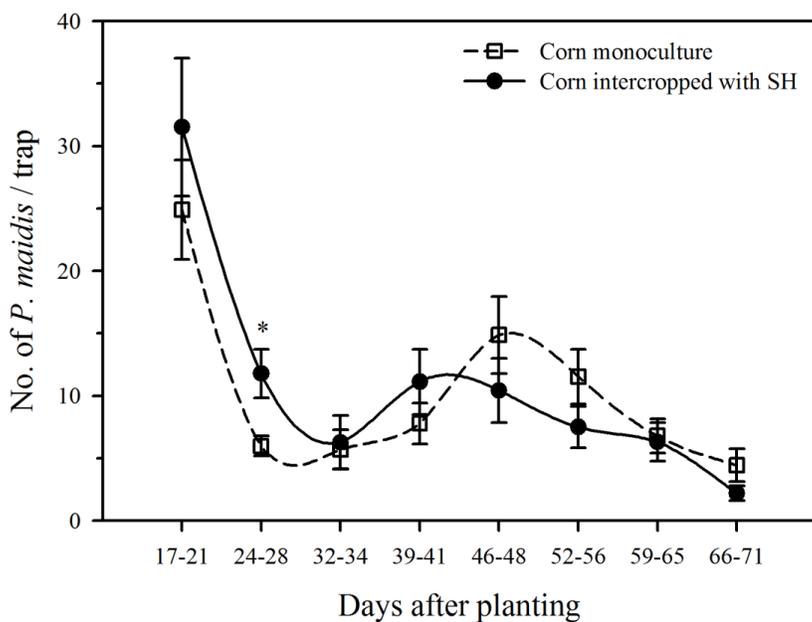
colonization rates (reduction in number of feeding vectors and their plant tenure time) in the corn plants, thereby reducing spread of MMV. This study validated results from the previous study (Chapter II) by conducting large field plot experiments, with higher corn to sunn hemp intercropping ratio. This system of higher corn to sunn hemp intercropping ratio identified for MMV management may fit into current cropping practices and may have significant importance to commercial corn growers and seed-corn industries. On the other hand, sunn hemp increased within-field activity of *F. williamsi* and appears to have resulted in greater movement and rapid spread of MCMV in the intercropped plots. On the basis of these results, use sunn hemp as an intercrop in corn plantings should be avoided in the areas with high epidemics of MCMV.



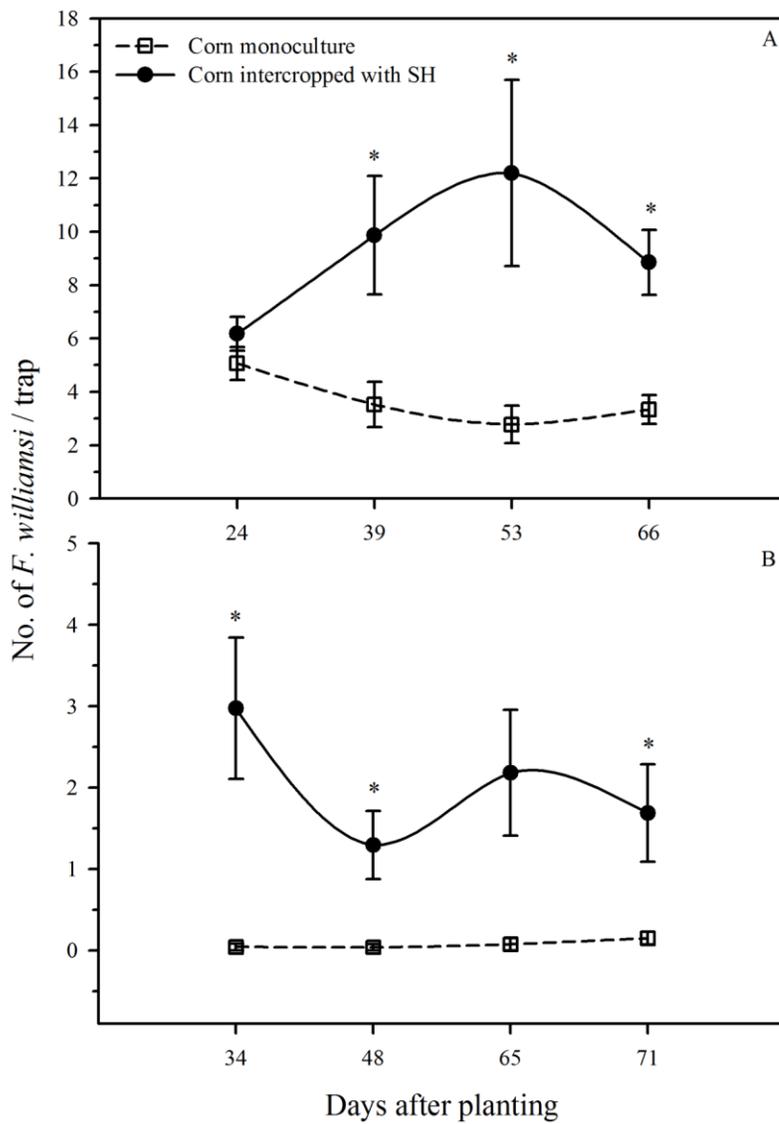
**Figure 6.1:** Mean number corn planthopper, *Peregrinus maidis* ( $\pm$  Standard Error, SE) per sticky trap in corn monoculture and corn intercropped with sunn hemp plot in (A) fall 2010 and (B) spring 2011. \* Indicates corn intercropped plot is significantly different from monoculture.



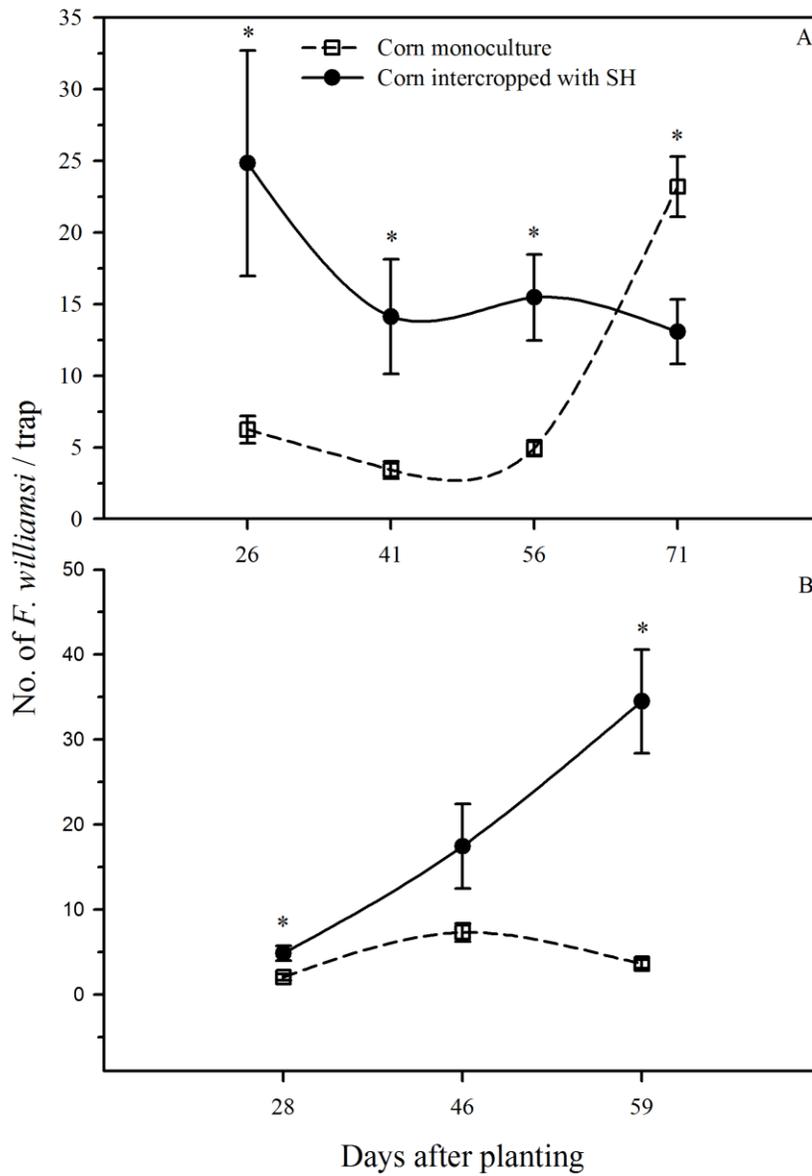
**Figure 6.2:** Mean number corn planthopper, *Peregrinus maidis* ( $\pm$  SE) per sticky trap in corn monoculture and corn intercropped with sunn hemp plot in (A) summer 2011 and (B) fall 2011. \* Indicates corn intercropped plot is significantly different from monoculture.



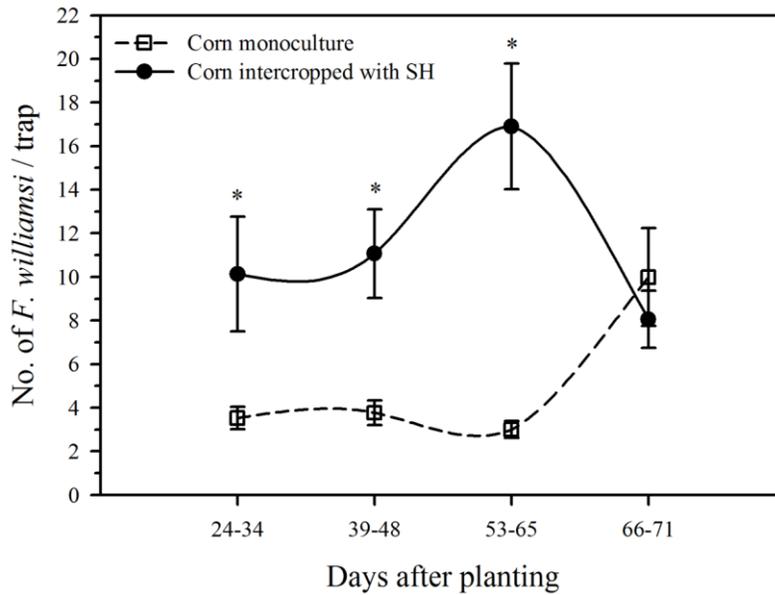
**Figure 6.3:** Activity of corn planthopper, *Peregrinus maidis* as indicated by sticky trap catches during the corn developmental time in corn monoculture and corn intercropped with sunn hemp plot. Each data point showed mean number of *P. maidis* ( $\pm$  SE) from all four experiments (fall 2010, and spring, summer and fall 2011) between a period of days as indicated on the x – axis. \* Indicates corn intercropped with SH plot is significantly higher than monoculture.



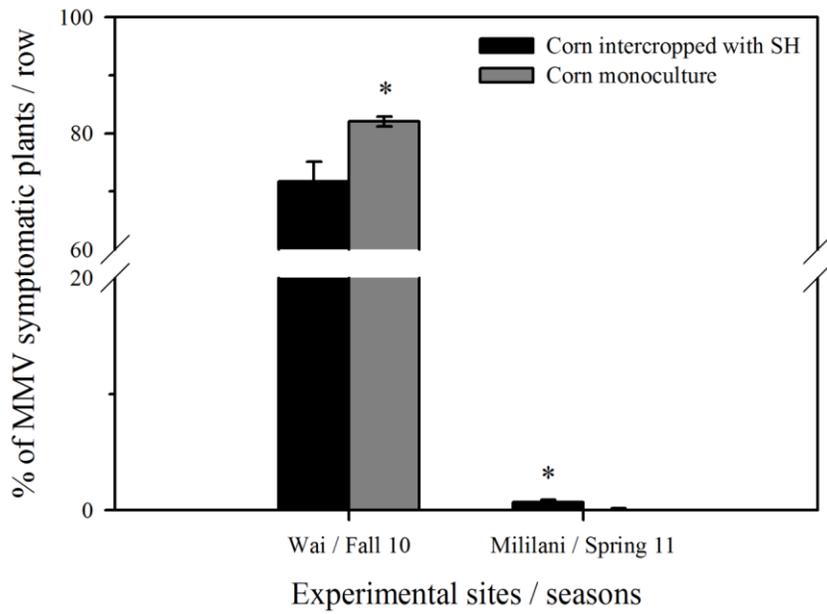
**Figure 6.4:** Mean number of corn thrips, *Frankliniella williamsi* ( $\pm$  SE) per sticky trap in corn monoculture and corn intercropped with sunn hemp plot in (A) fall 2010 and (B) spring 2011. \* Indicates corn intercropped with sunn hemp is significantly higher than monoculture.



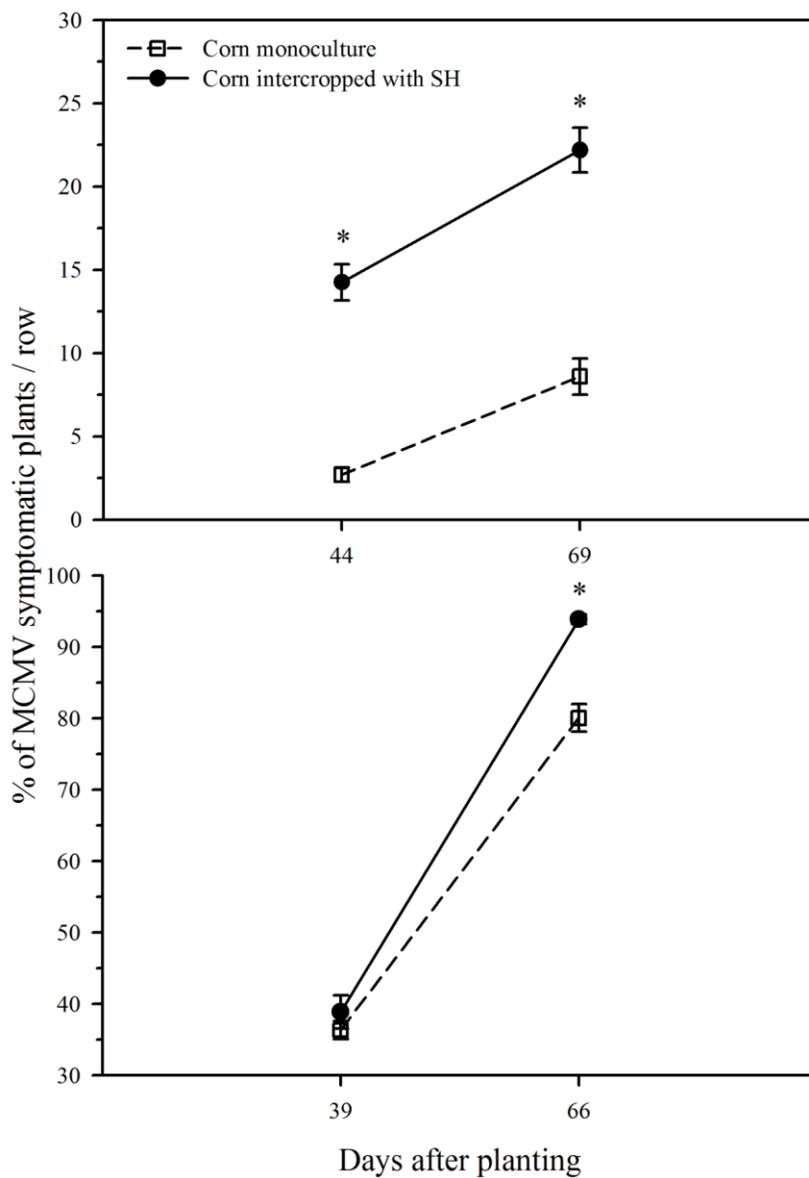
**Figure 6.5:** Mean number of corn thrips, *Frankliniella williamsi* ( $\pm$  SE) per sticky trap in corn monoculture and corn intercropped with sunn hemp plot in (A) summer 2011 and (B) fall 2011. \* Indicates corn intercropped with sunn hemp is significantly higher than monoculture.



**Figure 6.6:** Activity of corn thrips, *Frankliniella williamsi* as indicated by sticky trap catches during the corn developmental time in corn monoculture and corn intercropped with sunn hemp plot. Each data point showed mean number of thrips ( $\pm$  SE) from all four experiments (fall 2010, and spring, summer and fall 2011) between a period of days as indicated on the x – axis. \* Indicates corn intercropped with sunn hemp is significantly higher than monoculture.



**Figure 6.7:** Percentage plants showing *Maize mosaic virus* (MMV) symptoms ( $\pm$  SE) in corn monoculture and corn intercropped with sunn hemp at the reproductive stage, evaluated in experiments at Waimanalo (fall 2010) and Mililani (spring 2011). \* Indicates corn intercropped with sunn hemp is significantly different than corn monoculture.



**Figure 6.8:** Percentage plants showing *Maize chlorotic mottle virus* (MCMV) symptoms ( $\pm$  SE) in corn monoculture and corn intercropped with sunn hemp at vegetative tassel and reproductive stage, evaluated in experiments at the Waimanalo site in summer and fall 2011. \* Indicates corn monoculture is significantly lower than corn intercropped with sunn hemp.

**Table 3.1:** Proportion of corn leaf samples rated on the basis for visual symptoms *Maize mosaic virus* (MMV) (fall 2010 and spring 2011) and *Maize chlorotic mottle virus* (MCMV) (summer and fall 2011) tested positive for either MMV or MCMV or both by using DAS-ELISA.

Treatments	Score	Fall 2010 (Waimanalo)		Spring 2011 (Mililani)		Summer 2011 (Waimanalo)		Fall 2011 (Waimanalo)	
		MMV <sup>+</sup>	MCMV <sup>+</sup>	MMV <sup>+</sup>	MCMV <sup>+</sup>	MMV <sup>+</sup>	MCMV <sup>+</sup>	MMV <sup>+</sup>	MCMV <sup>+</sup>
Corn monoculture	0	0/4	-	0/4	4/4	0/3	3/3	0/4	4/4
	1	0/4	-	0/4	3/4	0/3	3/3	0/4	4/4
	2	0/4	-	0/4	4/4	0/3	3/3	0/4	4/4
	3	4/4	-	2/4	1/4	0/3	3/3	0/4	4/4
Corn + SH	0	0/4	-	0/4	4/4	0/3	3/3	0/4	4/4
	1	4/4	-	0/4	2/4	0/3	3/3	0/4	4/4
	2	4/4	-	0/4	0/4	0/3	3/3	0/4	4/4
	3	4/4	-	4/4	3/4	0/3	3/3	0/4	4/4

## CHAPTER VI

### **Enhancing parasitism and predation of corn earworm *Helicoverpa zea* (Boddie) eggs through habitat management and inundative release of *Trichogramma pretiosum* Riley in corn cropping systems**

#### **Abstract**

Habitat management, a component of conservation biological control, can provide resources such as food for adult natural enemies, alternative prey and hosts, and shelter from adverse conditions, with resulting enhanced activity of natural enemies and pest suppression in agriculture systems. Alternatively, augmentative biological control comprises field releases of laboratory reared natural enemies at a critical stage of the crop pest population growth cycle, and can be an effective and economically viable method to suppress a pest below its economic threshold.

Field experiments comprising three treatments were conducted: corn intercropped with sunn hemp (habitat management technique, conservation biocontrol), corn monoculture with release of the parasitoid *Trichogramma pretiosum* Riley (inundative biocontrol method), and corn monoculture alone as a control. This study aimed to compare effectiveness of (i) conservation biological control and inundative biological control (biocontrol-enhanced treatments) with corn monoculture in terms of improving parasitism of *Helicoverpa zea* (Boddie) eggs, and (ii) conservation biological control with corn monoculture on predation of *H. zea* eggs and thrips [primarily, *Frankliniella*

*occidentalis* (Pergande)] (iii) biocontrol-enhanced treatments on corn ear yield and quality, in corn cropping systems.

Results showed parasitism of *H. zea* eggs on corn silks was greater in biocontrol-enhanced treatments than monoculture controls, and was greater in *T. pretiosum* released plots than intercropped plots. In addition, abundance of *Orius* spp. was greater in intercropped plots than monoculture controls at similar densities of prey. As a result, corn ear yield was greater in biocontrol-enhanced plots than monoculture controls and was in turn greater in parasite release- than intercropped plots. These results suggest that inundative release of *T. pretiosum* is a more efficient and economically viable management tool for *H. zea* control in corn than habitat management techniques with sunn hemp as an intercrop.

## **Introduction**

Habitat management, a component of conservation biological control, is an ecologically based approach aimed at favoring natural enemies and enhancing biological control in agricultural systems (Landis et al. 2000, Zehnder et al. 2007). The goal of habitat management is to create a suitable ecological infrastructure within the agricultural landscape to provide resources such as food for adult natural enemies (e.g. nectar sources for parasitoid wasps), alternative prey and hosts, and shelter from adverse conditions that result in enhanced activity of natural enemies and pest suppression (Jonsson et al. 2008, Gurr et al. 2012). However, attempting to enhance conservation biological control through increasing cropping diversity may become less effective with the increase in pest

diversity and intraguild predation among natural enemies; thus modifying habitats has indeed been found to have neutral and negative impacts on the target pest insects under some circumstances (Staub et al. 2008, Winkler 2010). Therefore, carefully planned management of diversity of crop plants is important to optimize activity of natural enemies and reduce pest density (Landis et al. 2000).

Alternatively, augmentative biological control involves inoculative or inundative release of laboratory reared natural enemies at a critical stage in the crop and pest phenology (Stinner 1977, van Lanteren and Bueno 2003, Collier and van Steenwyk 2004). In some cases, the inundative biological control method can be an effective and economically viable method to suppress a pest below its economic threshold limits (Stinner 1977, Smith 1996, Collier and van Steenwyk 2004). *Trichogramma* spp. is the most popular biological control agent used in augmentative programs against lepidopterous pests (King and Coleman 1989; Smith 1989). Augmentative release of *Trichogramma* spp. had only been successfully used in controlling borer pests of corn such as European corn borer, *Ostrinia nubilalis* and Corn earworm, *Helicoverpa zea* in USA (Smith 1996).

Many studies have compared the efficacy of conservation biological control and augmentative biological control separately with monoculture farming. Studies directly comparing efficacy of conservation biological control and augmentative biological control are limited. Therefore, this study aimed to compare effectiveness of conservation biological control with augmentative biological control, and simultaneously comparing their pooled effect with a monoculture control on parasitism of *H. zea* eggs. In addition, the influence of conservation biological control on predator density was compared with

the monoculture control, quantifying *Orius* spp. (Hemiptera: Anthocoridae) response to the treatments and density of *H. zea* eggs and thrips (primarily, *Frankliniella occidentalis*) in corn cropping systems.

On the basis of results of related works reported earlier in this dissertation, the corn - sunn hemp intercropping system was chosen as a method of conservation biocontrol control to examine the effect on pest suppression. Pest suppression and plant damage reduction is achieved by enhanced parasitism of *H. zea* eggs by *T. pretiosum*, stronger predator (*Orius persequens* and *O. tristicolor*) and prey (*H. zea* eggs and thrips) relationships (Chapter III), or by reduced incidence of *Maize mosaic virus* transmitted by corn planthopper, *Peregrinus maidis* Ashmead (Chapter II), that resulted in higher corn ear yield (Chapter III). The parasitoid *T. Pretiosum* Riley reared from parasitized eggs of *Lampidus boeticus* (Lepidoptera: Lycaenidae) on sunn hemp flowers and buds was chosen for the inundative releases in corn monoculture crops to mass-rear a common parasitoid responsible for *H. zea* egg mortality. From here onwards corn plots receiving the conservation biological control treatment is designated as “corn-intercropped plots”, the inundative biological control treatment with release of *T. pretiosum* in corn monoculture plot is designated as “released plots”. The plots with the two biological control treatments is designated as “biocontrol-enhanced plots” for single degree of freedom contrast comparisons with corn monoculture plots (“monoculture control”).

This chapter specifically tests the following hypotheses (i) parasitism of *H. zea* eggs will be higher in the biocontrol-enhanced plots compared to the monoculture controls and similar between corn-intercropped plots and *Trichogramma* released plots, (ii) abundance of *Orius* spp. as influenced by prey availability will be higher in the corn-

intercropped plots than the monoculture controls and, (iii) higher yield will be achieved in the biocontrol-enhanced plots than the monoculture controls and the corn-intercropped- and *Trichogramma* released plot will produce equal yields.

## Methods

Field experiments were conducted to examine effects of sunn hemp intercropping on abundance of *Orius* spp. as influenced by prey (thrips and *H. zea* eggs) availability, and parasitism of *H. zea* eggs by *T. pretiosum*. Parasitism of *H. zea* eggs was also examined in corn monoculture with release of *T. pretiosum*, and compared with a corn - sunn hemp intercropping system. Three experiments were conducted at the University of Hawaii's experimentation station, Waimanalo Research Station at Waimanalo. One experiment was conducted at the Pioneer Hi-Bred International Inc. field station at Mililani Agricultural Park, Mililani. The experimental sites were located on the island of Oahu in Hawaii and characterized by diversified agricultural systems, including fruits, vegetables, ornamentals and parent seed-corn farming. The experiments were planted at the stations at randomly chosen planting seasons during the study period from October 2010 to December 2011. Each experiment at the Waimanalo site consisted of three treatment plots, corn monoculture, corn intercropped with sunn hemp and corn monoculture with release of *T. pretiosum*. The experiment conducted at the Mililani site consisted only two treatment plots, corn monoculture and corn intercropped with sunn hemp.

## **Crop planting**

A total of five strips of sunn hemp, each measured 30.5 m x 1.8 m were planted in a field plot of 30.5 m x 30.5 m area. Six rows of corn were planted into four areas between two pre-established strips of sunn hemp, to make 24 rows of corn plants in a corn-intercropped plot. Continuous 34 rows of corn were planted in an area of 30.5 m x 30.5 m in a corn monoculture plot. A sweet corn variety, Passion (Seminis Vegetables) and a fodder variety, P0125xR (Pioneer Hibred International Inc.), were used at the Waimanalo and Mililani sites, respectively. The experiments were planted on October 5 (fall 2010) at the Waimanalo, November 23, 2010 (spring 2011) at the Mililani followed by April 14 (summer 2011) and October 11 (fall 2011) at the Waimanalo.

## **Release of parasitoids**

The *T. pretiosum* originally obtained from parasitized eggs of *L. boeticus* from sunn hemp flowers, were reared captively on the eggs of *Ephestia kuehniella* (Zell.). The parasitoids were mass-reared on the factitious host eggs before the field release, using the methods described by Morrison (1985). The parasitized eggs of *E. kuehniella* were held in the laboratory until approximately 1 day before anticipated emergence of the wasps.

In each experiment, a single release of *T. pretiosum* was made at the equivalent rate of 250,000 females per hectare when 40% of corn plants reached silking stage, followed by another release of 150,000 females per hectare five days later. For releases, parasitized eggs of *E. kuehniella* were glued on 10.2 cm x 10.2 cm size Post-it paper using white Elmer's glue (Elmer's Product, Inc., Columbus, OH). The papers with parasitized eggs were glued in a 130 ml paper disposable drinking water cups

(Sweetheart Cup Company, Inc., Owings Mills, MD) to serve as the standard release unit. Each cup was fastened with thread at the base and suspended upside down on an individual corn plant to prevent flooding of release cups. Six - eight such paper cups were randomly placed in the released plot.

### **Ear sampling**

Developing corn ears were sampled from all treatment plots three times at five-day intervals during the corn silking stage. The first two samplings of corn ears was timed a day after the first and second release of *T. pretiosum* in the release plot, respectively. On each sampling date, 40 ears were randomly sampled from each plot and brought to the laboratory to isolate *H. zea* eggs on the corn silks. Each *H. zea* egg found on the silks was capsulated in a gel cap and observed for emergence of either a *H. zea* larva or parasitoid. Observations of number of eggs, and emergence of larvae or parasitoids were recorded to calculate percentage parasitism and proportions of eggs that emerged to yield either larvae or parasitoids. Additionally, each corn ear was beaten by hand 30-40 times to dislodge thrips and *Orius* spp. on to a clean surface for capture and quantification.

### **Ear yield and quality assessment**

Sweet corn ears were harvested at the dry silk stage at the Waimanalo site. At the Mililani site where fodder corn was planted, ears were harvested at the appropriate maturity for seed-corn production. For quantifying corn ear yield, two (fall 2010 and spring 2011) and four (summer 2011) rows of corn plants were randomly selected in each treatment plot. In fall 2011 experiment, one row of corn was selected due to continuous

rain during the week of harvest. At the harvesting stage, all corn ears were harvested from plants of the selected row. Total number of ears and weight harvested for each row were recorded. Total weight of corn ears in tons per hectare was estimated from each row data assuming corn monoculture and corn intercropped plots would have similar planting density for the corn plants.

Further, quality of corn ears in terms *H. zea* infestation was assessed in spring 2011 and summer 2011 experiments by randomly sampling 40 corn ears from the harvested corn in each treatment plot. Corn ears were assessed qualitatively by grading on the basis of depth of *H. zea* larvae that bored from the tip of the ear. Here, “undamaged” indicated an ear with no evidence larval presence, “moderately damaged” indicated up to 5 cm of the kernels on the corn ear bored by larvae from the tip and “damaged” indicated more than 5 cm of the kernels on the corn ear bored from the tip. Thus percentage of each category of corn ear was calculated for each treatment plot.

### **Data analyses**

Data for *H. zea* egg counts, arthropods counts, percentage parasitism of *H. zea* eggs and corn yield (tons / ha) was analyzed using mixed model analysis of variance (PROC MIXED, SAS Institute) by each planting season and site. The pooled analysis for each variable from all four experimental data sets was performed in default settings to evaluate overall effect of treatments on variables. In addition, a model was constructed to examine the main effect of treatment, with planting season designated as a random factor to examine if the results are consistent throughout the year irrespective of planting season. Effect of treatments on percentage parasitism of *H. zea* eggs was also analyzed by

date with planting season as a random factor to examine parasitism during corn silking. Any variable measured in all three treatments were compared using pre-planned orthogonal contrasts: monoculture control vs. biocontrol-enhanced plots (corn-intercropped plot + *Trichogramma* release plot), and corn-intercropped plot vs. *Trichogramma* release plot.

The proportion of *H. zea* eggs, which produced either larvae or parasitoid were compared between treatment plots using Fisher exact test (PROC FREQ, SAS Institute) to further support the results from percentage parasitism. Assuming each thrips and a *H. zea* egg, as a prey, and *Orius* spp. is the only predominant predator in a corn ear assemblage, the predator - prey relationship was analyzed using correlation and covariate analyses. Relationships between ‘*Orius*’, ‘thrips’, ‘*Hz*’ and ‘thrips + *Hz*’ were examined using Pearson’s correlation coefficient (PROC CORR, SAS Institute). Here, ‘*Orius*’, ‘*Hz*’, ‘thrips’ and ‘*Hz* + thrips’ indicated numbers of *Orius* spp., *H. zea* eggs, thrips, and sum of *H. zea* eggs and thrips on silks of corn ear, respectively. In most cases, abundance of predator may depend on prey availability and there relationship may vary between the treatments. In such cases, an analysis of *Orius* spp. abundance considering prey availability as covariate could explain the actual effects of treatments on *Orius* spp. abundance. Effect of treatments on abundance of *Orius* spp. in treatment habitats was analyzed with their most correlated variable as a covariate using mixed model analysis of covariance (PROC MIXED, SAS institute). The combined data from four experiments were fitted to the three regression models to test the hypotheses whether the slopes are zero, unequal or parallel. A zero slope indicates treatments do not have significant effect on abundance of *Orius* spp. and not influenced by prey availability. Unequal slopes

indicate treatments have a significant effect on abundance of *Orius* spp. as influenced by prey availability. Parallel slopes indicate that the treatments may not have significant effect on abundance of *Orius* spp. as influenced by prey availability. In such case, if the regression lines do not overlap, the significance of distance between two parallel regression lines is tested to examine any differences between treatments at a given prey density. The following regression models were used in order to compare change in *Orius* densities with respect to prey abundance in the treatment plots:

$$1. Y_{ij} = \alpha_i + \beta_i X_{ij} + b_j + e_{ij}$$

$$2. Y_{ij} = \alpha_2 + (\alpha_i - \alpha_2) + \beta_2 X_{ij} + (\beta_i - \beta_2) X_{ij} + b_j + e_{ij}$$

$$3. Y_{ij} = \alpha_i + \beta X_{ij} + b_j + e_{ij}$$

Where,  $i = 1, 2$ , (treatments)

$j = 1, 2, 3, 4$  (planting season)

$Y$  = dependent variable (densities of *Orius* spp.)

$X$  = independent variable (levels of *Hz* + thrips, treatment x *Hz* + thrips)

$\alpha_i$  = the intercept of  $i^{\text{th}}$  treatment model

$\beta_i$  = the slope of  $i^{\text{th}}$  treatment model

$\beta$  = the common slope model

$b_j$  = the effect of  $j^{\text{th}}$  “planting season”

$e_{ij}$  = the experimental error

*Model 1:* The type 3 *F*-statistics corresponding to ‘treatment x *H<sub>Z</sub>* + thrips’ tests the hypothesis (Slopes equal to zero),  $H_0: \beta_1 = \beta_2 = 0$  i. e. slopes are most likely equal to zero versus  $H_a$  (not  $H_0$ ). The type 3 *F*-statistics corresponding to ‘treatment’ tests the hypothesis  $H_0: \alpha_1 = \alpha_2 = 0$  versus  $H_a$ : (not  $H_0$ ) at ‘*H<sub>Z</sub>* + thrips’ = 0.

*Model 2:* The type 3 *F*-statistics corresponding to ‘treatment x *H<sub>Z</sub>* + thrips’ tests the hypothesis (Unequal slopes),  $H_0: \beta_1 - \beta_2 = 0$  i. e. slopes are most likely equal versus  $H_a$  (not  $H_0$ ). The type 3 *F*-statistics corresponding to ‘treatment’ tests the hypothesis  $H_0: \alpha_1 - \alpha_2 = 0$  versus  $H_a$ : (not  $H_0$ ) at ‘*H<sub>Z</sub>* + thrips’ = 0.

*Model 3:* The type 3 *F*-statistics corresponding to ‘*H<sub>Z</sub>* + thrips’ tests the hypothesis for common slope model (Parallel slopes),  $H_0$ : common slope = 0 versus  $H_a$ : common slopes are significantly different from zero. The type 3 *F*-statistics corresponding to ‘treatment’ tests the hypothesis that the distance between two regression lines are not significantly different.

All the statistical analysis procedures involving mixed models in SAS were conducted as described by Littell et al. (2006).

## Results

### Parasitism of *H. zea* eggs

A combined analysis of the two experiments in the fall planting season showed parasitism of *H. zea* eggs by *T. pretiosum* was significantly greater in biocontrol-enhanced plots (corn-intercropped- and released plots) than monoculture controls ( $F_{1,15} =$

15.44,  $P < 0.01$ ) and was greater in released- than intercropped plots ( $F_{1,15} = 21.41$ ,  $P < 0.01$ ). Similarly, a combined analysis of three experiments at the Waimanalo site showed parasitism was significantly greater in biocontrol-enhanced plots than controls ( $F_{1,24} = 19.34$ ,  $P < 0.01$ ) and was greater in released- than intercropped plots ( $F_{1,24} = 13.45$ ,  $P < 0.01$ ). The pooled analysis over all planting seasons showed parasitism was significantly greater in biocontrol-enhanced plots than controls ( $F_{1,30} = 21.55$ ,  $P < 0.01$ ) and was greater in released- than intercropped plots ( $F_{1,30} = 11.46$ ,  $P < 0.01$ ). Considering random effects of planting seasons, parasitism was significantly greater in biocontrol-enhanced plots than controls ( $F_{1,3} = 13.87$ ,  $P < 0.01$ ), while no significant difference was found between the two biocontrol-enhanced treatments ( $P > 0.05$ ) (Table 3.1). The pooled analysis showing trends of parasitism during the corn silking stage showed significantly greater parasitism in biocontrol-enhanced plots than monoculture controls on mid- to final sampling dates (mid:  $F_{1,8} = 10.93$ ,  $P = 0.01$ ; final:  $F_{1,8} = 16.04$ ,  $P < 0.01$ ) and was significantly greater in released- than intercropped plots on mid-sampling dates ( $F_{1,8} = 18.01$ ,  $P < 0.01$ ) (Figure 7.1). The contingency table analysis using Fisher exact tests, showed proportion of *H. zea* eggs from which parasitoids emerged was greater in biocontrol-enhanced plots (corn-intercropped- or released plots) than controls ( $P < 0.05$ ), and was greater in released- than intercropped plots on each sampling date ( $P < 0.05$ ) (Figure 7.2).

### **Predator - prey densities**

Individual analyses combining experiments by planting season and site showed significant lower abundance of *H. zea* eggs on corn silks in corn-intercropped plots than monoculture controls in the fall plantings ( $F_{1,473} = 14.28$ ,  $P < 0.01$ ) and at the Waimanalo

site ( $F_{1,705} = 5.08$ ,  $P = 0.02$ ). The pooled analysis over the planting seasons showed abundance of *H. zea* eggs was significantly lower in corn-intercropped plots ( $8.77 \pm 0.34$ ) than monoculture controls ( $7.78 \pm 0.31$ ) ( $F_{1,945} = 5.04$ ,  $P = 0.03$ ). Similarly, densities of thrips on the corn silks were significantly lower in corn-intercropped plots than monoculture controls on the fall plantings ( $F_{1,473} = 15.89$ ,  $P < 0.01$ ) and at the Waimanalo site ( $F_{1,705} = 4.60$ ,  $P = 0.03$ ). In the pooled analysis, densities of thrips was lower in corn-intercropped plots ( $5.78 \pm 0.28$ ) than monoculture controls ( $6.63 \pm 0.28$ ) ( $F_{1,945} = 5.27$ ,  $P = 0.02$ ). On the other hand, abundance of *Orius* spp. on corn silks were significantly higher in corn-intercropped plots than monoculture controls on the fall plantings ( $F_{1,473} = 9.69$ ,  $P = 0.002$ ) and at the Waimanalo site ( $F_{1,705} = 10.09$ ,  $P < 0.01$ ). In the pooled analysis, abundance of *Orius* spp. was significantly higher in corn-intercropped plots ( $0.62 \pm 0.04$ ) than monoculture controls ( $0.48 \pm 0.04$ ). Considering the random effect of planting seasons on these pooled analyses, the treatments did not have a significant effect on *H. zea* egg abundance, thrips densities and *Orius* spp. frequency ( $F_{1,2} = 0.60 - 1.99$ ,  $P > 0.05$ ) (Table 3.2). In addition, Pearson's correlation analysis showed no significant relationship between abundance of *Orius* spp. and *H. zea* eggs ( $r = 0.06$ ,  $P = 0.06$ ) and a moderately positive relationship between *Orius* spp. and combined thrips and *H. zea* eggs together, or thrips alone ( $r = 0.20$ ,  $P < 0.01$ ).

### **Predator - prey relationship**

Fitting the *Orius* and prey data to model (1), the  $F$ -statistics corresponding to 'treatment x *H. zea* + thrips' ( $F_{2,676} = 11.16$ ,  $P < 0.01$ ) indicate that the slopes are not all equal to zero. The  $F$ -statistics corresponding to 'treatment' ( $F_{2,5,2} = 6.2$ ,  $P = 0.04$ ) indicate that the intercepts are most likely not all equal to zero, at '*H. zea* + thrips' = 0.

Fitting data to model (2), the  $F$ -statistics corresponding to ‘treatment x  $H_z$  + thrips’ ( $F_{1,945} = 14.39$ ,  $P < 0.01$ ) indicate there is sufficient evidence that the treatments resulted in responses with unequal slopes (Table 3.3). Pairwise comparisons among the treatment models at mean density of ‘ $H_{ze}$  + thrips’ = 8.25 showed a significant difference in distance between the two regression lines for corn-intercropped- and monoculture plots ( $\beta = -0.1645$ ,  $t_{944} = -3.03$ ,  $P < 0.01$ ). This result indicated that abundance of *Orius* spp. was significantly different between corn-intercropped plots and monoculture controls at the given density of prey, and was significantly higher in corn-intercropped plots compared to monoculture control at the mean density of prey.

### **Ear yield and quality assessment**

A combined analyses for the two fall season experiments showed that biocontrol-enhanced plots produced significantly greater corn ear yield (t / ha) than monoculture controls ( $F_{1,8} = 7.47$ ,  $P = 0.03$ ) and released plots produced significantly greater ear yield than corn-intercropped plots ( $F_{1,8} = 13.23$ ,  $P < 0.01$ ). The treatment effect was not significant for corn ear yield ( $F_{2,6} = 1.23$ ,  $P = 0.36$ ) in an experiment in the summer planting at the Waimanalo site. The pooled analysis for all seasons showed biocontrol-enhanced plots produced significantly greater corn ear yield than controls ( $F_{1,19} = 4.45$ ,  $P = 0.04$ ) and released plots produced significant greater corn ear yield than intercropped plots ( $F_{1,19} = 4.96$ ,  $P = 0.04$ ) (Figure 7.3).

Further, quality assessments of corn ears from the combined yield data of spring and summer 2011 experiments showed similar percentages of undamaged, moderately damaged and damaged ears in corn-intercropped plots and monoculture controls.

Released plots yielded 16 % increase in undamaged ears, 6.4% increase in moderately damaged ears and 22.6% decrease in damaged ear than in intercropped plots and monoculture controls (Figure 7.4).

## Discussion

### Parasitism of *H. zea* eggs

Results showed that the enhancement of the biocontrol agent, *T. pretiosum*, through either intercropping corn with sunn hemp, or inundative release of *T. pretiosum* in corn monoculture significantly increased parasitism of *H. zea* eggs compared to monoculture controls. Further, inundative release of *T. pretiosum* in corn monoculture resulted in significantly increased parasitism of *H. zea* eggs compared to corn-intercropped plots. Greater parasitism in released plots was due to higher abundance *T. pretiosum* achieved through inundation of parasitoids at the susceptible stage of *H. zea*. In contrast, increase in parasitism in intercropped plots was due to enhanced activity of naturally occurring parasitoids probably encouraged by floral resources provided by of sunn hemp.

Floral resources enhance parasitoid survival, longevity, fecundity and activity of natural enemies to increase their effectiveness in the agriculture systems (Landis et al. 2000). A greenhouse experiment showed that survival of the egg parasitoid *T. carverae* Oatman and Pinto was greater in presence of flowering shoots of *Lobularia maritima* L. or *F. esculentum* Moench or *Borago officinalis* L. than in without flowering shoots and control treatment (Begum et al. 2006). Similarly, a study showed that floral resources

provided by buckwheat or fennel enhanced longevity and fecundity of a parasitoid *T. exiguum* Pinto and Platner in a caged experiment (Bissinger – Witting et al. 2008). However, Gurr and Nicol (2000) suggested that because *Trichogramma* spp. are proovigenic, food availability will not likely increase fecundity of the adult parasitoids, if any increase in parasitism is due to increase in fitness of the parasitoids. Additionally, Hossain et al. (2001) showed that enhanced parasitism of *Helicoverpa* spp. egg by *Trichogramma* spp. and their predation in strip-harvested than completely harvested lucerne fields that provided refuge for the natural enemies. Therefore greater parasitism of *H. zea* eggs in intercropped plots in the current experiments is likely mainly due to sunn hemp flowers that provided alternative survival sites and energy sources for the parasitoids.

Studies have shown that the inoculative release of *T. ostriniae* Pang and Chen parasitized > 40% of *O. nubilalis* Pang egg masses, significantly higher than in control plots in field corn and sweet corn fields (Wright et al. 2001 and 2002, Hoffman et al. 2002, Gardener et al. 2007). These studies suggested although inoculative releases of *T. ostriniae* effectively suppress *O. nubilalis* infestation, refinement in timing, rate of release and frequency of release could enhance the benefits of using these parasitoids inoculatively. In the current study, higher rates of *T. pretiosum* were released twice targeting eggs of *H. zea* during the corn silking stage. As hypothesized, augmentation of *T. pretiosum* resulted in ~ 30 % and 15% increase in parasitism of *H. zea* eggs over monoculture controls and intercropping corn with sunn hemp, respectively. A study comparing efficacy of *T. ostriniae* release and habitat modification with inter-planting buckwheat on parasitism of sentinel *O. nubilalis* eggs in bell peppers showed similar

results with higher parasitism in the released plot than buckwheat inter-planted and control plots (Russell and Bessin 2009). The current results showed that the augmentative biological control was more efficient than the conservation biological control.

The pooled analysis for *H. zea* parasitism from all four experiments with planting season as a random factor showed similar results, indicating this pattern will hold true irrespective of planting season. Although parasitism during corn silking in the pooled analysis was not exclusively significant on each sampling date, a rapid increase in parasitism was observed in released plots, while a gradual increase occurred in intercropped plots, and no change in monoculture controls. In a separate analysis, the proportion of *H. zea* eggs that yielded *T. pretiosum* was significantly greater in released- or intercropped plots than monoculture controls on each sampling date. Additionally, the proportion was significantly higher in released- than intercropped plots on each sampling date. The results of proportion of eggs that emerged as either larvae or parasitoids are congruent with the results of percentage parasitism, showing that there were no other sources of egg mortality. Additionally, *H. zea* parasitism and proportion of eggs that yielded *T. pretiosum* were greater after the second release (on the second sampling date) in released plots indicating that more frequent and a higher rate of *T. pretiosum* releases were desired at the corn silking stage targeting the *H. zea* egg stage to achieve optimal parasitism of *H. zea* eggs.

### **Predator - prey relationship**

Abundance of prey, either *H. zea* eggs or thrips, on the corn silks was significantly lower in the corn-intercropped plots compared to the monoculture controls

in the fall plantings or at the Waimanalo site. In similar conditions, predator (*Orius* spp.) densities were higher in corn-intercropped plots compared to monoculture controls. These results suggested that higher densities of predators may have reduced the abundance of prey items in the intercropped plots and vice versa in monoculture controls, and thus the treatments that were applied may have produced a density dependent relationship. Other studies have demonstrated density dependent numerical responses between *Orius* predators and their prey. For example, Sansone and Smith (2001) showed a positive correlation between *Orius* spp. numbers and abundance of *H. zea* (Boddie) and *H. virescens* (F.) eggs on cotton flowers at blooming. Similarly, the numeric relationship between predators [*O. insidiosus* (Say) and *O. pumilio* (Champion)] and prey [*F. bispinosa* (Morgan)] were also found to be density dependent on the flowers of *Daucus carota* L. and *Ammi majus* L. (Shirk et al., 2012). In the current study, the predator (*Orius* spp.) and prey (*H. zea* eggs and thrips) densities were moderately correlated, indicating moderate density dependence. The results of this chapter were consistent with the results of the previous experiment reported in this dissertation, examining effects of cover crops on predator – prey relationships in corn – cover crop intercropping systems (Chapter III).

Results of covariate analysis in this study showed the abundance of *Orius* spp. was significantly higher in the intercropped treatment compared to the monoculture control at given densities of prey. This indicated the relationship between prey and predator was significantly stronger in the intercropped treatment compared to the monoculture. This result was also consistent with the previous results, which showed that the predator – prey relationship was significantly stronger in buckwheat and sunn hemp-intercropped treatments compared with cowpea-intercropped treatment and the corn

monoculture. This study suggested sunn hemp provided alternate prey (thrips) resources that inhabit in their flowers, resulting enhanced abundance of *Orius* spp. in the intercropped plots. Thus, it is suggested that significant numbers of *Orius* spp. from sunn hemp may have moved to corn plants and their predation resulted in reduced densities of prey items on corn silks.

### **Ear yield and quality assessment**

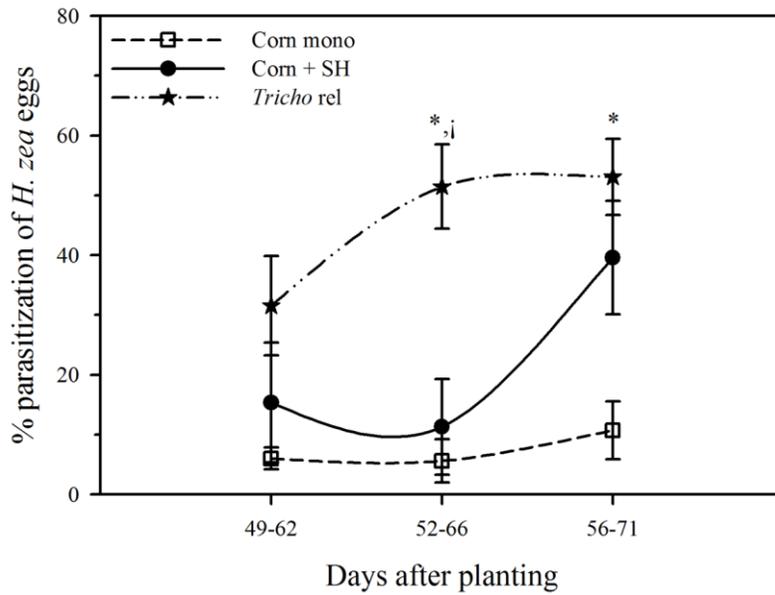
Results of combined analysis of the two fall experiments at Waimanalo showed sweet corn yield per hectare was higher in biocontrol-enhanced plots than monoculture controls and was higher in the released- than the intercropped plots. The pooled analysis from all four experiments showed consistent results, indicating significant increase in corn ear yield in biocontrol-enhanced plots compared to monoculture controls. A similar trend was observed in the previous experiment, in evaluating effect of intercropped cover crops on sweet corn yield compared to corn monoculture showed highest sweet corn yield in corn intercropped with sunn hemp plots than the others (Chapter III). Various previous studies have shown positive effects on yield, of *Trichogramma* releases. For example, percentage of *O. nubilalis* infested bell pepper fruits was significantly less in plots receiving releases of *T. ostrinae* and buckwheat inter-planted plots compared to non-release- and monoculture plots (Russell and Bessin 2009). Similarly, release of *T. ostrinae* significantly reduced plant and ear damage caused by *O. nubilalis* compared to non-release plots in sweet corn (Wright et al. 2002, Hoffman et al. 2006). Similar to these results, the current study has shown that releases of *T. pretiosum* resulted in an increase in undamaged and moderately damaged ears, and a concomitant decrease in damaged corn ear yield caused by *H. zea* over sunn hemp intercropping and corn monoculture

control treatments. These results indicated that biocontrol-enhanced treatments, particularly augmentative releases of *T. pretiosum* in corn monoculture could increase quantity and quality of corn ear yield compared to monoculture controls.

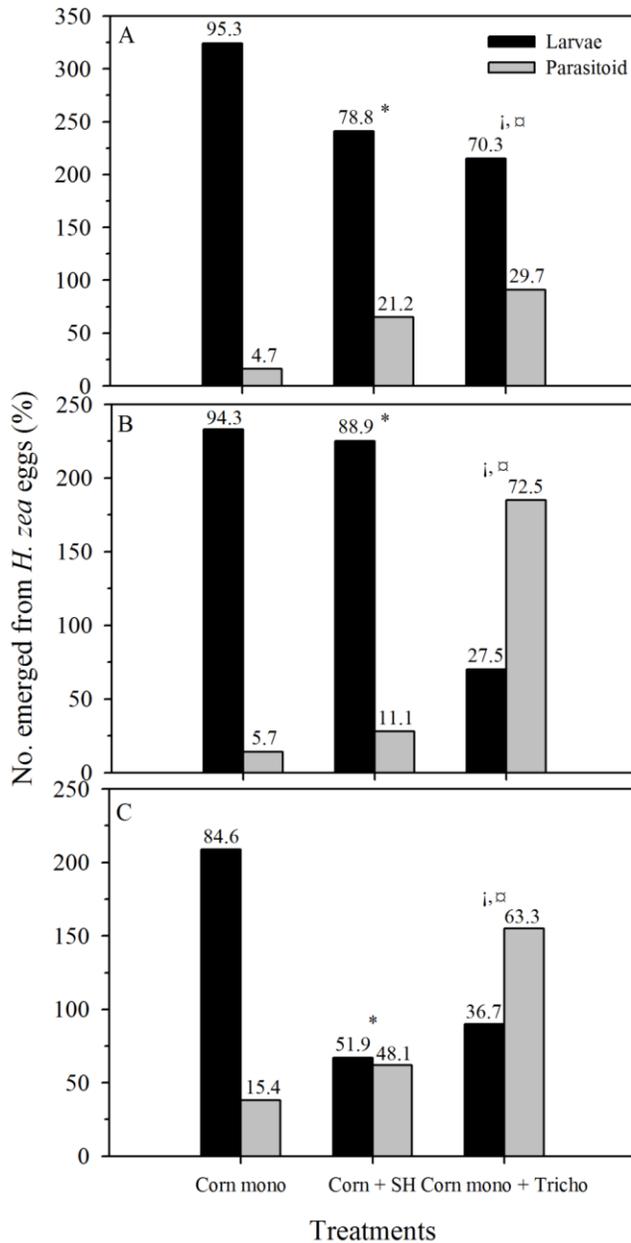
Although corn ear yield might have been affected by *H. zea* and thrips (primarily *F. occidentalis*) in corn ears, several other pest related factors might have influenced corn yield, such as *P. maidis* and other thrips species, *F. williamsi* transmitting MMV and *Maize chlorotic mosaic virus*, MCMV, respectively (Chapters IV and V). Considering these factors, greater parasitism of *H. zea* eggs achieved through inundative release of *T. pretiosum* may have played a significant role in higher yield in the released plots. On the other hand, although multiple factors favored the intercropped treatment over the released treatment (e.g. moderate parasitism of *H. zea* eggs, higher predation of *H. zea* eggs and thrips, and lower incidence of MMV), they contributed minimally in influencing corn ear yield. These results indicated management of *H. zea* by inundative release of *T. pretiosum* may have played an important role in increasing quantity and quality of corn yield, if other pest related problems remained constant. Inundative release of *T. pretiosum* is a more efficient and economic management tool for *H. zea* control than habitat management techniques with sunn hemp as an intercrop in cornfields. An analysis of the economics of each system will provide a quantitative measure of the benefits of either approach.

**Table 4.1:** Comparison of parasitism of *Helicoverpa zea* eggs between biocontrol-enhanced plots (corn intercropped with sunn hemp, corn + SH and corn monoculture with release of *Trichogramma*, *Tricho rel*) and monoculture control (corn monoculture, corn mono), and corn + SH and *Tricho rel* on three planting seasons (fall, spring and summer) at two sites (Waimanalo and Mililani). The results showed pooled effects of treatments over all season evaluated in four experiments with default settings and planting seasons as a random factor. *Contrast 1* and *2* represent orthogonal contrasts between biocontrol-enhanced plots and corn mono, and Corn + SH and *Tricho rel* plots, respectively.  $P < 0.05$  indicates significant difference between two treatment combinations.

Effects	Treatments			Error <i>d.f.</i>	<i>Contrast1</i>		<i>Contrast2</i>	
	Corn mono	Corn + SH	<i>Tricho rel</i>		<i>F</i> ratio	<i>P</i> -value	<i>F</i> ratio	<i>P</i> -value
<b>1. Planting season</b>								
Fall	4.55 ± 1.7	12.96 ± 5.9	48.70 ± 7.2	15	15.44	< 0.01	21.41	< 0.01
Spring (Mililani site)	4.78 ± 3.0	24.46 ± 20.5	-	4	0.90	0.40	-	-
Summer	15.77 ± 4.5	37.86 ± 3.6	38.61 ± 3.6	6	21.83	< 0.01	0.02	0.90
<b>2. Site</b>								
Waimanalo	8.29 ± 2.5	21.26 ± 5.7	45.34 ± 5.0	24	19.34	< 0.01	13.45	< 0.01
<b>3. Pooled (default)</b>								
Pooled (random)	7.41 ± 2.0	22.06 ± 6.1	45.34 ± 5.0	30	21.55	< 0.01	11.46	< 0.01
				3	13.87	0.03	6.15	< 0.09



**Figure 7.1:** Patterns of parasitism of *Helicoverpa zea* eggs ( $\pm$  SE) by *Trichogramma pretiosum* during corn silking in biocontrol-enhanced plots (corn intercropped with SH, Corn + SH and corn monoculture with *T. pretiosum* release, *Tricho rel*) and monoculture controls (corn monoculture, Corn mono). \* Indicates biocontrol-enhanced plots are significantly higher than control. j Indicates *Tricho rel* is significantly higher than Corn + SH.



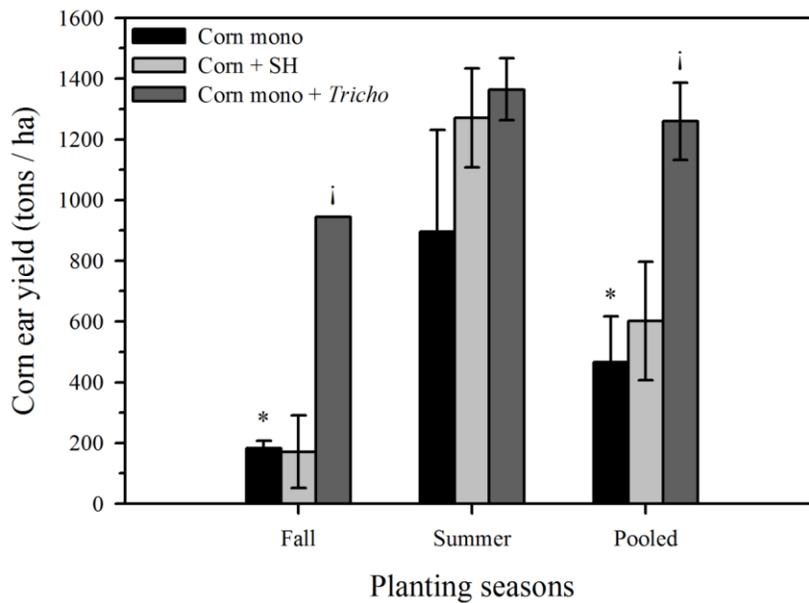
**Figure 7.2:** Proportion of *Helicoverpa zea* eggs emerged into larvae or parasitoids in bicontrol-enhanced plots (corn intercropped with sunn hemp, Corn + SH and corn monoculture with release of *T. pretiosum*, *Tricho* rel) and monoculture controls (corn monoculture, Corn mono). Larvae and parasitoids indicate *H. zea* larvae and *Trichogramma pretiosum* emerged from parasitized *H. zea* eggs, respectively. \* Indicates Corn + SH is significantly higher than corn mono, j indicates *Tricho* rel is significantly higher than Corn mono and □ indicates *Tricho* rel is significantly higher than Corn + SH, using Fisher Exact tests.

**Table 4.2:** Comparisons on abundance of *Helicoverpa zea* eggs, thrips and *Orius* spp. between corn intercropped with sunn hemp plots, corn + SH and corn monoculture controls, corn mono in three planting seasons (fall, spring and summer) at two sites (Waimanalo Research Station, Waimanalo and Mililani Agriculture Park, Mililani). The results showed pooled effects of treatments over all seasons evaluated in four experiments with default settings and planting seasons as a random factor.  $P < 0.05$  indicates significant difference between two treatment combinations.

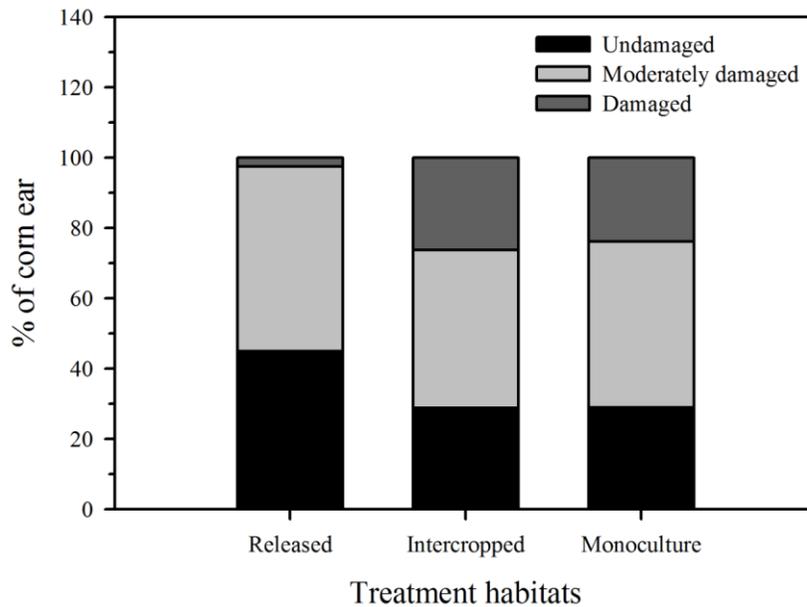
Effects	Treatments		Error <i>df</i>	<i>F</i> ratio	<i>P</i> -value
	Corn mono	Corn + SH			
<b>I. <i>H. zea</i> eggs</b>					
1. Planting season					
Fall	10.26 ± 0.41	8.31 ± 0.30	473	14.28	< 0.01
Spring (Mililani site)	1.36 ± 0.14	1.34 ± 0.12	240	0.02	0.89
Summer	13.08 ± 0.71	13.78 ± 0.71	230	0.47	0.49
2. Site					
Waimanalo	11.20 ± 0.37	10.07 ± 0.34	705	5.08	0.02
Pooled (default)	8.77 ± 0.34	7.78 ± 0.31	945	5.04	0.03
Pooled (random)			2	0.37	0.60
<b>II. Thrips</b>					
1. Planting season					
Fall	7.26 ± 0.28	5.77 ± 0.25	473	15.89	< 0.01
Spring (Mililani site)	0.48 ± 0.07	0.35 ± 0.06	240	2.13	0.15
Summer	11.41 ± 0.64	11.80 ± 0.70	230	0.18	0.68
2. Site					
Waimanalo	8.65 ± 0.30	7.71 ± 0.32	705	4.60	0.03
Pooled (default)	6.63 ± 0.28	5.78 ± 0.28	945	5.27	0.02
Pooled (default)			2	0.66	0.50
<b>III. <i>Orius</i> spp.</b>					
1. Planting season					
Fall	0.42 ± 0.05	0.68 ± 0.06	473	9.69	< 0.01
Spring (Mililani site)	0.29 ± 0.05	0.23 ± 0.05	240	0.61	0.44
Summer	0.77 ± 0.08	0.93 ± 0.10	230	1.66	0.20
2. Site					
Waimanalo	0.54 ± 0.04	0.76 ± 0.05	705	10.09	< 0.01
Pooled (default)	0.48 ± 0.04	0.62 ± 0.04	947	6.70	< 0.01
Pooled (random)			2	1.99	0.29

**Table 4.3:** Linear relationship between variables predator, ‘*Orius*’ and prey, ‘*Hz* + thrips’ fitted into two regression models to test hypotheses whether the slopes of treatment models are either equal to zero or equal. Table shows intercept and slopes for each model with type 3 *F*-statistics and *P*-values for intercepts and slopes for each model. Corn mono and Corn + SH represent plots receiving corn monoculture control and corn intercropped with SH treatments. ‘*Orius*’ indicates numbers of *Orius* spp. is the response variable and ‘*Hz* + thrips’ indicates total of *H. zea* eggs and thrips is the indicator variable.

Model	Effect	Intercept			Slopes		
		treatment	<i>Hz</i> + thrips	<i>Hz</i> +thrips x treatment			
1. $Y_{ij} = \alpha_i + \beta_i X_{ij} + b_j + e_{ij}$	Corn mono	0.46		0.01			
	Corn + SH	0.38		0.03			
<i>F</i> ratio (df)		6.2 (2, 5.2)		11.16 (2, 676)			
<i>P</i> value		0.04		<0.01			
2. $Y_{ij} = \alpha_2 + (\alpha_i - \alpha_2) + \beta_2 X_{ij} + (\beta_i - \beta_2) X_{ij} + b_j + e_{ij}$	Corn mono	0.46		-0.03			
	Corn +SH	0.38		0			
			0.03				
<i>F</i> ratio (df)		6.20 (2, 5.2)	11.48 (1, 452)	14.39 (1, 945)			
<i>P</i> value		0.04	<0.01	<0.01			



**Figure 7.3:** Mean corn ear yield (t / ha) ( $\pm$  S. E.) in biocontrol-enhanced plots (corn intercropped with sunn hemp, Corn + SH and corn monoculture with *T. pretiosum* release, *Tricho* rel) and monoculture control (corn monoculture, Corn mono) in two planting seasons (fall and summer) at two sites (Waimanalo Research Station, Waimanalo and Mililani Agriculture Park, Mililani). \*Indicates biocontrol-enhanced plots are significantly higher than control. <sup>j</sup>Indicates *Tricho* rel is significantly higher than corn + SH.



**Figure 7.4:** Mean percentage of corn ears categorized as undamaged, moderately damaged and damaged (severe damage rating) by *H. zea* infestations in the released (corn monoculture plot with release of *T. pretiosum*), intercropped (corn intercropped with sunn hemp plots) and monoculture (monoculture control plots) pooled for two planting seasons (spring and summer 2011) at the Waimanalo Research Station, Waimanalo. The results showed a relatively lower percentage of undamaged ears in released plots than intercropped plots and monoculture control

## GENERAL CONCLUSIONS

Crop diversification is an important component of ecological pest management in agricultural systems. Diverse cropping systems provide environmentally benign and sustainable pest management options that can help reduce pesticide dependency in agricultural production. Diversified agricultural systems researched for this dissertation were created either through strip-tilled or intercropped cover cropping methods, which allowed the impact of these cultural tactics on pest management to be quantified. The pest suppressive nature of these systems was realized either through the cover crops causing changes in the host seeking behavior of insects or supporting beneficial insects. The overall aims of this dissertation are to examine the impacts habitat management in the form of cover crops on pest- and beneficial insect populations, and plant pathogenic viruses vectored by the study insects and compare habitat management techniques with augmentative biological control for their ability to manage pest populations in corn.

In chapter I, the strip tilled cover cropping systems reduced densities of thrips on cucurbit plants and resulted in lower thrips damaged fruits compared to bare-ground monoculture. However, benefits of pest suppressive strip-tilled cover cropping systems have been nullified due to inter-specific competition between cucurbits and cover crops.

In subsequent chapters, cover crop intercropping systems were explored to suppress multiple pest and disease complexes of corn. Chapter II suggested a tall-statured sunn hemp intercropped with corn increased within-field activity of corn planthopper, *Peregrinus maidis*, which in turn reduced *P. maidis* settling and colonization of corn

plants, and ultimately resulted in lower incidence of hopperburn and *Maize mosaic virus*, MMV symptomatic plants. Conversely, cowpea intercropping increased the incidence of hopperburn and MMV symptoms in corn plants. The results showed that the use of a suitable intercrop such as sunn hemp has the potential to be useful for managing insect vectors of persistent viruses, which are usually considered unmanageable by cultural modifications to cropping systems.

Furthermore, sunn hemp and cowpea flowers provided oviposition sites for *Lampidus boeticus*, the eggs of which were heavily parasitized by *Trichogramma* spp. Thus, intercropping corn with sunn hemp and cowpea increased *Trichogramma* spp. abundance and resulted in greater parasitism of corn earworm, *Helicoverpa zea* eggs on the corn silks. On the other hand, intercropping corn with buckwheat produced a stronger relationship between *Orius* spp. and *H. zea* eggs and thrips as prey items. This suggested buckwheat might have provided various prey resources (eg. thrips, aphids, whiteflies) and food from floral resources (eg. pollens) for a predator, *Orius* spp. that increased their fitness and abundance. The intercropping ratio of corn to cover crop (4:1) eliminated inter-specific competition between corn and cover crops, which resulted in similar corn growth and ear yield compared to monoculture plantings (Chapter III).

Prompted by the results of Chapters II and III, a sunn hemp intercrop, which reduced incidence MMV symptomatic corn plants and increased parasitism of *H. zea* eggs on the corn silks was chosen for further studies (Chapter IV - VI). Chapter IV suggested within-field dispersion of *P. maidis* was mediated by their population densities and was most distinct at peak densities of the insect. The dispersion pattern of *P. maidis* showed temporal trends from initially random, progressing to aggregated in the mid-crop

growth stages, and then random to uniform on the later growth stages. The stronger dispersion pattern in the sunn hemp intercropped treatment at mid-growth stages suggested restriction in within-field movement of *P. maidis* compared to corn monoculture. This may have caused higher *P. maidis* densities within the restricted areas and resulted in lower numbers of plants infected with MMV. The spatial distribution patterns of *P. maidis* were unassociated with patterns of severity of hopperburn and MMV symptoms in both treatments.

The effect of sunn hemp intercropping on *P. maidis* activity and incidence of MMV was consistently similar to results that were obtained from small field experiments with a lower intercropping ratio. These results suggested MMV could be managed with higher intercropping densities in large-scale corn production. This strategy may be an important component of IPM in reducing spread of persistently transmitted viruses by vectors in large-scale cropping systems. On the other hand, sunn hemp intercropping increased within-field activity of corn thrips, *Frankliniella williamsi*, and resulted in greater movement of thrips and rapid spread of *Maize chlorotic mottle virus*, MCMV in cornfields. Thus, using sunn hemp as an intercrop in corn plantings should be avoided in areas with severe MCMV problems (Chapter V).

In chapter VI, biocontrol-enhanced treatments either through intercropping corn with sunn hemp or inundative releases of *Trichogramma pretiosum* in corn monoculture enhanced parasitism of *H. zea* eggs on corn silks compared to monoculture controls. The rate of parasitism was higher in corn monoculture receiving inundative release of *T. pretiosum* compared to sunn hemp intercropping treatments. In addition, predator-prey relationships were stronger in corn intercropped with sunn hemp treatments compared to

monoculture controls. As a result, corn ear yield was greater in biocontrol-enhanced treatments compare to monoculture controls, and was higher in corn monoculture with inundative release of *T. pretiosum* compared to corn intercropped with sunn hemp treatments. These results suggested that the inundative release of *T. pretiosum* is a more effective tool for *H. zea* management than the habitat management technique tested here, in cornfields.

Putting results obtained in this dissertation in context to results found by research in other systems, the following findings are supported:

- Strip-tilled cover cropping suppresses thrips on cucurbit plants (Theunissen and Schelling 1996 and 1998, Olson et al. 2006, Toews et al. 2010).
- The pest suppressive nature of strip-tilled cover cropping is nullified by interspecific competition between cash crop and cover crops (Altieri et al. 1985, Letourneau 1986, Andow et al. 1986, Gold et al. 1990, Bukoviszky et al. 2004).
- Suppression of thrips densities in strip-tilled cover cropping reduces thrips damaged cucurbit fruits (Theunissen and Schelling 1996, Hooks and Johnson 2002).
- Intercropping corn with sunn hemp increases within-field activity of *P. maidis*, which reduces colonization on corn plants, and results in lower incidence of MMV, a persistently transmitted virus (Power 1987, 1988 and 1991).
- Intercropping corn with cover crops such as sunn hemp and cowpea provides shelter for *Trichogramma* spp., which increases their abundance and results in higher rates of *H. zea* parasitism on the corn silks (Gurr and Nicol 2000, Hossain et al. 2001)

- Intercropping corn with buckwheat supports stronger relationship between predator (*Orius* spp.) and prey (*H. zea* eggs and thrips) on corn silks (Pumarino and Alomar 2012, Bickerton and Hamilton 2012).
- Spatial distribution pattern of *P. maidis* is stronger in corn intercropped with sunn hemp compared to corn monoculture treatments. Strong spatial pattern in corn-intercropped treatments at mid-crop growth stages suggested restricted movement of *P. maidis* and resulted in reduced incidence of MMV compared to corn monoculture treatments. This is the first report to my knowledge comparing spatial patterns of a vector insect in two different cropping systems using SADIE.
- Patterns of *P. maidis*, and severity of hopperburn and MMV symptoms is unassociated (Reay-Jones et al. 2010).
- Inundative releases of *T. pretiosum* in corn monoculture enhanced parasitism rate of *H. zea* eggs compared to corn intercropped with sunn hemp treatments (Russell and Bessin 2009).
- Inundative releases of *T. pretiosum* in corn monoculture result in greater and higher quality ear yield compared to corn intercropped with sunn hemp treatments (Russell and Bessin 2009).
- Intercropping corn with sunn hemp enhances within-field activity of *F. williamsi* and results in rapid spread MCMV. To my knowledge this is the first case examining effects of an intercrop on spread thrips transmitted MCMV, a semi-persistent virus.

## Recommendations

- A planting configuration in strip-tilled cover cropping systems that accommodate multiple rows of cash crops between standing cover crop rows and provide an adequate spacing between cash crop and cover crop is required to mitigate inter-specific competition and benefit from pest suppressive nature of strip-tilled cover cropping.
- Intercropping corn with cowpea in the areas with high *P. maidis* densities is not desired for their high infection rates of MMV.
- Intercropping corn with cowpea and sunn hemp enhances parasitism rates of *H. zea* eggs, and is thus recommended in areas with high infestations of *H. zea*.
- Intercropping corn with buckwheat enhances generalist predators (such as *Orius* spp.), and is thus recommended in areas with *H. zea* and thrips infestations.
- At least 4:1 corn to cover crop intercropping ratio is required to eliminate inter-specific competition between the corn and cover-crop plants.
- Inundative releases of *T. pretiosum* to suppress *H. zea* population in cornfields is recommended whenever feasible.
- Intercropping corn with sunn hemp in the areas with high inoculum of MCMV must be avoided.

## Contributions of this dissertation

- Marigold and sunn hemp strip-tilled cover cropping systems suppress multiple pests (nematodes, weeds and insects) and enhance soil health, thus can be an integrated crop management package for organic cucurbit production, provided that interspecific competition among crops is avoided.
- The level of protection against *P. maidis* transmitted MMV is similar among each corn – sunn hemp intercropping densities (corn: sunn hemp = 2:1, 4:1 and 6:1). Therefore, sunn hemp intercropping with higher intercropping densities may offer a valuable tool for MMV management in cornfields. As such the proposed cropping practice allocates large areas to corn cultivation, thus the loss of corn production through the area allocated for sunn hemp is minimized. This proposed cropping practice could be compatible for the commercial corn production without altering their existing cropping practices.
- Higher densities of insects may not always relate to the greater plant injury in a cropping system. Therefore a carefully planned precision sampling scheme needs to be designed to a specific pest in a cropping system. At high densities, of *P. maidis*, intercropping corn with sunn hemp produces an aggregated distribution pattern compared to monoculture planting, that results in lower incidence of MMV symptomatic corn plants. The aggregated spatial pattern *P. maidis* in the intercropping system should be included in statistical procedures when developing precision sampling schemes to detect *P. maidis* patches in cornfields (Binns et al. 2000). Significant spatial pattern with larger gaps than patches in insect dispersion should enable growers to locate patches effectively. Thus

identified *P. maidis* patches could be spot-sprayed with insecticides at peak density, which may reduce frequency of treatments and quantity of insecticide applied. In large-scale corn farming, intercropping corn with sunn hemp, combined with appropriate integrated pest management practices may provide valuable options for reducing the impacts of insect-borne plant pathogens such as MMV.

- Cover crops (such as buckwheat, cowpea, sunn hemp) grown in strips or patches in a large-scale corn landscape provide sources of natural enemies (such as *T. pretiosum*, *Orius* spp.) that can suppress *H. zea* and thrips populations. The strategy of growing strips or patches of cover crops may be appropriate for parent seed-corn production, where cornfields are isolated by a distance to avoid cross contamination among varieties. In these conditions, a suitable cover crop grown in the area between the cornfields enhances abundance of natural enemies that move into the cornfield and results in pest suppression. In addition, a population of natural enemies can be sustained, even at the time of insecticide spray in cornfield for other pest related problems, such as epidemics of MCMV.
- Sunn hemp planted after summer in Hawaii, flowers for a long period of time (~2 months). Pruning of flowering branches of sunn hemp gives new floral shoots, which can extend its flowering period even longer. Growing sunn hemp in the fallow or refuse land in seed-corn industries or in a commercial corn farm provides a natural insectary for *T. pretiosum* for longer period of time. Thus, a continuous population of *T. pretiosum* can be produced in an area targeting multiple planting seasons to suppress *H. zea* populations.

- The efficacy of inundative releases of *T. pretiosum* in suppressing *H. zea* populations is greater than that achieved through habitat management in the cornfield. Thus, the inundative release of *T. pretiosum* contributes to effective and economically viable management practice of *H. zea* in cornfields.

### **Future research**

- Effects of isolated patches of *T. pretiosum* insectary plants (such as sunn hemp, cowpea etc.) on the dispersal of *T. pretiosum* within the agricultural landscape as indicated by parasitism rates of *H. zea* eggs in cornfields.
- Effect of habitat modification coupled with inundative release *T. pretiosum* on parasitism rates of *H. zea* in cornfields.
- Exploring alternative management options for corn thrips, *F. williamsi*, such as application of biopesticides.

### **Drawbacks of cover crops used in this study systems**

- The vigorous vegetative growth and delayed flowering of sunn hemp and cowpea plants in summer months does not enhance natural enemies in corn plantings.
- Shade created by sunn hemp plants attracts melon flies, *Bactocera cucurbitae* (Coquillett) in the corn-intercropped treatment. Thus, intercropping sunn hemp with melon fly prone crops may be risky.
- Bird damage in consecutive sunn hemp plantings in the same plots is common. The birds, for example *Geopelia striata* (L.) cause damage either by pulling recently emerging seedlings from the soil or feeding on the growing tip of sunn hemp plants. The damage was intense at the fourth planting that needed reseeding

at several places and employment of 'scarecrow' devices to re-establish sunn hemp plantings. This damage delayed the establishment of sunn hemp at by at least three weeks.

- The problems associated with the stem borer pests (Order: Coleoptera) occurred at the third consecutive sunn hemp planting in the same field plot. The grubs of this pest bore through the root and moves upward by feeding internally in the stem, causing plant death at flowering stage of the crop. The stem borer infestation was severe at the fourth planting causing 30 - 40% of plant mortality at the flowering stage.
- Infestation of powdery mildew, *Erysiphe polygoni* was common in cowpea plantings, ranging from 90-100% at pod bearing stage.
- Infestation of phytophagous mites, *Tetranychus* spp. may occur on foliage of sunn hemp and marigold plants at flowering stages. The movement of mites to the adjacent cash crop may be a problem.

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