ABSTRACT

Title of Thesis: EVALUATING THE IMPACTS OF
CROTOLARIA JUNCEA, SUNN HEMP, ON
ARTHROPOD POPULATIONS AND PLANT
GROWTH AND DEVELOPMENT IN A
ZUCCHINI INTER-CROPPED
AGROECOSYSTEM

Jermaine L. Hinds, Master of Science, 2012

Thesis directed By: Professor Cerruti Hooks
Department of Entomology

Recently, studies have shown that crop diversification strategies can be effective and sustainable means of suppressing pests, improving crop growth and enhancing beneficial soil organisms. Experiments were conducted in 2009, 2010 and 2011 to investigate the impacts of the tropical cover crop sunn hemp (SH), *Crotalaria juncea*, utilized as a living mulch and green manure on insect populations, crop growth and the nematode community. When inter-planted as a living mulch, SH reduced populations of cucumber beetles (*Acalymma* spp. and *Diabrotica* spp.) compared to monoculture zucchini, *Cucurbita pepo*, plots. When SH was utilized as an organic mulch, SH treatment plots resulted in significantly larger zucchini plant biomass and yield than monoculture plots. In 2011 when SH was strip-tilled, this resulted in a more nutrient enriched soil as indicated by nematode abundances and calculated soil health indices compared to monoculture plots. Organic fertilizer application resulted in late-season increases in bacteria feeding nematodes.

EVALUATING THE IMPACTS OF *CROTOLARIA JUNCEA*, SUNN HEMP, ON ARTHROPOD POPULATIONS AND PLANT GROWTH AND DEVELOPMENT IN A ZUCCHINI INTER-CROPPED AGROECOSYSTEM

By

Jermaine L. Hinds

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Advisory Committee:
Assistant Professor Cerruti Hooks, Chair
Associate Professor William Lamp
Associate Professor Paula Shrewsbury

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Introduction

The purpose of this review is to evaluate the impacts of crop diversification on arthropod communities associated with cucurbit cropping systems. Crop diversification, or multiple cropping, is defined as the growth of two or more crops in the same field (Vandermeer 1989). Multiple cropping systems are generally categorized as sequential cropping or intercropping. Sequential cropping refers to multiple crops grown in succession. Intercropping refers to multiple crops grown concurrently in close proximity. Crop diversification methods are aimed at exploiting physical and chemical properties of cropping species to manipulate insect populations, crop growth, and soil health. Cropping practices relating to crop diversification include a wide range of techniques such as cover cropping, intercropping, trap cropping, barrier cropping, and exploitation of plant volatiles. The objective of this review is to examine the influences of crop diversification on arthropod populations found in cucurbit agroecosystems.

The Cucurbitaceae and their importance

The Cucurbitaceae, or cucurbit, family consists of plants that are characterized as herbaceous, viny, tendril-bearing, frost sensitive annuals. Characteristic features of cucurbit plants are non-woody, herbaceous, hollow stems, singular taproot with many branching secondary roots spread laterally, deeply lobed leaves arranged one leaf per stem (Robinson and Decker-Walters 1997). Cucurbit plants produce large showy white to yellow unisexual flowers to attract important pollinator species. The most important pollinator species of cultivated cucurbit crops are the squash and gourd bees *Peponapis spp.* and *Xenoglossa spp.* (Hurd et al. 1971, Tepedino 1981). Fruits from cucurbit plants are extremely diverse and vary in size, shape, color, and ornamentation. Cucurbits consist of a wide range of fruits and vegetables including cucumbers, melons, and gourds.

Cucurbits all produce varying amounts of the secondary metabolite cucurbitacin, an oxygenated tetracyclic triterpene (Metcalf et al. 1980). Cucurbitacins are a class of extremely bitter compounds that are highly toxic to a variety of species ranging from arthropod to mammalian herbivores (David and Vallance 1955, Metcalf et al. 1980). Cucurbitacins are hypothesized to be an evolutionary response to deter herbivory. Several studies suggest that insect toxicity, mortality, and other detrimental effects are due to cucurbitacin when sufficient amounts of the compound is ingested (Da Costa and Jones 1971, Ferguson and Metcalf 1985). For example, after exposure to cucurbitacin rich cucumbers, two-spotted spider mites, *Tetranychus urticae*, experienced 96-98% mortality (Da Costa and Jones 1971). Though toxic to many species, cucurbitacins have been found to elicit kairomonal responses in some herbivorous insects. Beetles from the Luperini tribe have been shown to be highly attracted to the cucurbitacins even in miniscule quantities (Metcalf et al. 1980). Laboratory experiments suggest that quantities as low as 3 nanograms were sufficient enough to arrest searching and initiate feeding behavior (Metcalf et al. 1980). Luperine beetles selectively sequester ingested cucurbitacins into their elytra and other body tissue as an evolutionary adaptation to deter predation and parasitism (Nishida et al. 1994, Tallamy et al. 1997, Tallamy et al. 2000)..

Cucurbitaceous crops represent the one of world's earliest cultivated and most important crop families. Cucumbers are thought to have originated in southwest Asia and were domesticated approximately 1500 BC (Robinson and Decker-Walters 1997). Cucurbit ancestors of melon and watermelon originated on the African continent. Evidence of cultivation of melons date back as far as 3000BC in Egypt (Wang et al. 2011b). The squash, pumpkin, and gourd species originated in the New World, more specifically in Mexico and other regions in South America (Robinson and Decker-Walters 1997). The oldest evidence of cucurbit crop domestication date back 9000 years ago to Mexico's Oaxaca valley (Decker 1988).

This diverse group of fruits and vegetables are grown worldwide in a variety of climates ranging from tropical, to subtropical and temperate climate zones. Cucurbit crops native to temperate climates are generally frost sensitive annuals susceptible to winter kills. Notable cultivated cucurbit species belong to a variety of genera within the family Cucurbitaceae. These species include, *Cucumis sativus* L. (cucumber), *Cucurbita pepo* L (squash and zucchini),*Cucurbita maxima* Duch., (pumpkin), *Citrullus lanatus* Thunb. (watermelon), and *Cucumis melo* L. (muskmelon) (Robinson and Decker-Walters 1997).

Today, a significant portion of farmland is devoted to the production of cucurbit crops. Ranked by acreage, production, and value, cucurbits comprise 4 of the top 15 vegetables grown in the United States (Cantliffe et al. 2007). According to USDA's Annual Vegetable Summary, cucurbit crops make up 330,040 acres, or 20% of the 1.7 million total acres of U.S farmland devoted to vegetable production (USDA 2012). Cucurbit crops are of even greater importance worldwide. According to the Food and Agricultural Organization of the United Nations, in 2010, approximately 1 million hectares or 52% of the world's cucumbers and gherkins were grown in Asia (FOA 2010). Numbers are similar for other cucurbit crops grown worldwide including watermelon, muskmelon, squash, pumpkin, and gourds.

Notable insect pests of cucurbits

A host of economically important pest species have been reported to feed on cultivated cucurbit crops. Serious damage, defoliation, and disease transmission result from feeding by herbivorous pest species. Notable pests of cucurbit crops include the melon and green peach aphids (*Aphis gossypii* Glover and *Myzus persicae* Sulzer), silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring), squash bug (*Anasa tristis* De Geer), striped cucumber beetle (*Acalymma vittatum* Fab.), spotted cucumber beetle (*Diabrotica undecimpuctata howardi* Barber), pickleworm (*Diaphania nitidalis* Stoll), and squash vine borer (*Melittia cucurbitae* Harris) (Cartwright et al. 1990, McKinlay 1992, Summers et al. 2004, Frank and Liburd 2005, Brust 2009, Manandhar et al. 2009).

The melon aphid and peach aphid, both found worldwide, have been reported to feed on over 200 cultivated crop plants. In temperate regions melon aphid overwinters as eggs in greenhouses and on cold tolerant plants then subsequently emerge in spring where they become serious pests of cereal, vegetable and ornamental crops (McKinlay 1992, Robinson and Decker-Walters 1997, Summers et al. 2004, Frank and Liburd 2005, Brust 2009, USDA 2012). These aphids colonize the base of suitable host plants and undersides of leaves in large numbers. Feeding by aphids often results in stunted growth, shed blossoms, and virus transmission of plants (McKinlay 1992, Robinson and Decker-Walters 1997, Brust 2009). Melon and green peach aphids were found to be the most efficient vectors of non-persistent watermelon mosaic virus and zucchini yellow mosaic virus in laboratory and field studies (Adlerz 1974, Castle et al. 1992, Manandhar and Hooks 2011).

Silverleaf whitefly, *B. argentifolii*, is another important insect pest of many cropping species. Cucurbit crops including squash, cucumbers, and melons are among its most common hosts in Maryland (Chen et al. 2004, Summers et al. 2004, Nyoike et al. 2008, Brust 2009). The immature stages of silverleaf whitefly are most damaging to host plants. However, both immature and adult stages pose serious threats to host plants. Silverleaf whiteflies aggregate on the undersides of host plant leaves and pierce into minor veins using stylet-like mouthparts to feed on phloem (Cohen et al. 1998, Brust 2009). Intensive feeding by silverleaf whitefly often includes chlorosis, wilting, fungal infection, induction of phytotoxemias, and disease transmission (McKinlay 1992, Hooks and Wright 2008, Brust 2009). Honeydew substances produced by silverleaf whiteflies serve as a substrate for subsequent colonization of sooty mold fungi (Brust 2009). Silverleaf whitefly has been found to induce phytotoxemias, adverse reactions to herbivore-injected toxins during feeding, in host plants (Manandhar et al. 2009). Among the most common, is squash silver leaf disorder, a disorder largely dependent on intensity of immature whitefly feeding, which results in silvering of plant leaves and ultimately a reduction in photosynthetic output, plant vigor, and fruit production (Hooks et al. 1998, Manandhar et al. 2009). Silverleaf whitefly is also responsible for the transmission of cucurbit viruses such as

Cucurbit Leaf Crumple Virus, which is characterized by crumpling, curling, and discoloration of cucurbit plant leaves and fruit (Nyoike et al. 2008).

Squash bug, *A. tristis* De Geer¸ usually limited to the western hemisphere, can damage cucurbit crops, especially squash and zucchini crops. Unmated females emerge from overwintering sites (fence rows and crop debris) in early summer months and locate host plants to begin feeding (McKinlay 1992, Brust 2009). Shortly after feeding, squash bug mating occurs, followed by oviposition continuing through August. As such, there are generally 1-1.5 generations of squash bug per year in temperate regions (McKinlay 1992). *A tristis* larval and adult life stages aggregate on the undersides of leaves and fruit then use their piercing-sucking mouthparts to feed on plant phloem. Feeding damage symptoms include chlorosis around leaf margins that progress into the leaves and wilting. Damage inflicted to cucurbit crops due to squash bug feeding is often referred to as "Anasa wilt", characterized by yellowing of leaves, wilting, and death if feeding pressure is intense (McKinlay 1992, Robinson and Decker-Walters 1997, Brust 2009). Squash bugs are also reported to transmit the Cucurbit Yellow Vine Disease, which is a bacterial infection whose symptoms are characterized by stunting, yellowing, and decline. Intense feeding damage often results in severe stand loss of cucurbit crops (Bruton et al. 2003).

Lepidopteran pests such as the squash vine-borer (*M. cucurbitae*) pose a serious threat to cucurbit crops. Squash vine borers emerge as adults during the early summer months after overwintering in soil as cocoons (McKinlay 1992, Robinson and Decker-Walters 1997). Larvae emerge from eggs laid on leaf petioles approximately 7 days after oviposition and immediately burrow into stems of their host plants, typically *Cucurbita pepo* (Robinson and Decker-Walters 1997). Stem feeding, which is evident by sawdust-like frass extrusions near entrance holes, by squash vine borer larvae is especially damaging and often fatal to cucurbit crops (Canhilal et al. 2006, Brust 2009). Larval feeding causes disruption of xylem and phloem lines and subsequent wilting and death of infested plants (McKinlay 1992, Brust 2009). Wounding resulting from squash vine-borer feeding damage also serve as entry points for fungal and bacterial pathogens (Brust 2009).

Cucumber beetles, which are generally restricted in distribution to the western hemisphere, are regarded as the most important pests of cucurbit crops. Both spotted and striped cucumber beetles, emerge from overwintering sites as unmated adults in spring in search of host plants (McKinlay 1992). *A. vittatum*, the striped cucumber beetle, and *D. undecimpunctata howardi*, the spotted cucumber beetle, for example, are serious pests of all cucurbit crops, though they exhibit the ability to preferentially feed on certain species and cultivars. Generally, cucumber beetles prefer cucurbit species that are richer in cucurbitacin. Upon host plant location, cucumber beetles inflict injury on cucurbit plant seedlings via stem girdling, cotyledon defoliation, and larval root feeding (McKinlay 1992, Robinson and Decker-Walters 1997, Brust 2009). Cucumber beetles remain serious pests of cucurbits throughout the growing season. On more developed plants, cucumber beetles feed on essentially all plant tissue including leaf, stem, floral, pollen, and fruit tissue. Herbivory can be tolerated to some degree, however transmission of viral and other disease by cucumber beetles agents are devastating to cucurbit plants. Both, spotted and striped cucumber beetles are known vectors of the causative agent of bacterial wilt, *Erwinia tracheiphila* (Leach 1964). The bacterial pathogen overwinters in the gut of their cucumber beetle vectors and can be transmitted directly or indirectly, via saliva during feeding or via frass through wounds opened by feeding (McKinlay 1992, Brust 1997, Brust 2009). Upon infection, *E. tracheiphila*, colonizes and expands in plant tissue, impeding the flow of water and nutrients in the xylem and phloem (Brust 2009, Sasu et al. 2010). Colonization by bacterial wilt results in wilting of cucurbit plants and is usually fatal (Brust 2009). Other diseases transmitted and facilitated by cucumber beetles include Squash mosaic virus and Fusarium wilt (Robinson and Decker-Walters 1997).

Traditional management practices for cucurbit pests

Management strategies for insect pests of cucurbit crops are largely centered on the application of broad spectrum insecticides. Cultural and biological control methods have proven successful in certain pest species, *A. gossypii* for example, but are generally regarded as unreliable to manage the more serious specialist pests such as cucumber beetles, squash bugs, and squash vine borer (Brust 2009).

Current recommendations for squash bug management requires several applications of pesticides at 7-10 day intervals beginning when plot surveys suggest one egg mass per plant on average. Recommended insecticides include pyrethroids such as bifentrhin (Brigade), lambda-cyhalothrin (Warrior II), and zeta-cypermethrin (Mustang MAX); neonicotinoids such as acetamiprid (Assail), clothianidin (Belay), and dinotefuran (Scorpion); and carbamates such as carbaryl (Sevin XLR) (Pair 1997, Dogramaci et al. 2004, Service 2012). Chemical pesticides are targeted at nymphs due to resistance exhibited by egg and adult stages of *A. tristis.* Palumbo et al (1993) report some success with well-timed applications of cypermethrin in keeping populations below damaging levels (Palumbo et al. 1993). Recommended mechanical methods of control for squash bug infestations include the destruction of crop residues as well as removal of nearby overwintering sites. Row coverings are also useful in excluding squash bugs from cucurbit crops. However, row coverings have to be removed upon cucurbit crop blossom to allow pollination to occur (Cartwright et al. 1990).

Similarly, recommendations for *M. cucurbitae*, the squash vine borer, advise weekly applications of pesticides upon detection of adults. Chemical applications should be directed at stems and bases of cucurbit plants to target and deter larval entrance. Recommended insecticides include flubendiamide (Asana XL), permethrin (Perm-Up), and zeta-cypermethrin + bifentrhin (Hero EC)(Service 2012). Chemical methods are often ineffective for larval vine borer management. Once stem boring and subsequent larval infestation has occurred in host plants, little can be done to save infected plants. Mechanical management techniques consist of implementation of floating row covers tightly anchored to the soil to impede oviposition and colonization of vine-borers (Brust 2009). Additionally, cultural controls such as removal of crop residues and overwintering sites can reduce population numbers. Crop rotation has also been suggested to reduce the likelihood of colonization by overwintering adults (Brust 2009).

Current strategies for the management of cucumber beetles, the most important of the cucurbit crop pests, are also largely focused on pesticide application. Because cucumber beetles vector the bacterial wilt pathogen, prevention tactics have been advised to reduce persistence of beetles and transmission of bacterial wilt. Where cucumber beetles and cucurbit diseases have historical abundance, early season soil applications of insecticides such as imidacloprid (Admire PRO) and dinotefuran (Scorpion) are recommended to reduce the number of root feeding larvae (Cavanagh et al. 2009, Service

2012). Weekly applications of foliar insecticides have also been recommended to prevent early season stem-girdling and defoliation. Effective foliar insecticides suggested are acetamiprid (Assail), Beta-cyfluthrin (Baythroid XL), clothianidin (Belay), and zeta-cypermethrin (Mustang MAX) (Cavanagh et al. 2009, Service 2012). Cultural and mechanical methods of management include row covers as well as removal of overwintering sites.

Pesticide applications have varying efficacies on insect pests associated with cucurbit crops. Studies conducted by Jasinski et al. (2009), suggest high levels of *A. vittatum* mortality resulting from a precision-based soil application of the carbamate, Furadan, on pumpkin, zucchini, and cucumber crops. Mortality rates of up to 100% were reported in cucumber beetles 72 hours after exposure. Although, the efficacy of Furadan seems promising, the authors reported that there were incidents of beetles recovering from their “dead” state (8%), which implies beetles have the capacity to become resistant (Jasinski et al. 2009). Foster and Brust (1995) reported lower yields in watermelon plots treated with carbofuran when compared to untreated plots. Results from their studies showed a significant yield decrease in carbofuran sprayed plots (Foster and Brust 1995). Pollinator decline was implicated, as broad spectrum insecticides often have lethal effects on non-target species such as bee pollinators in this case.

Over-reliance on chemical methods of control, especially broad spectrum insecticides, have been shown to have significant ecological impacts. Routine application of pesticides can result in resistant pest species, non-target effects, pest resurgence, secondary pest outbreaks, and groundwater contamination (Metcalf and Luckmann 1994). Important socioeconomic impacts such as human health and welfare also have to be considered. Increasing interest in conservation, sustainability, and the development of integrated pest management programs have resulted in a number of studies aimed at developing alternative methods of control.

Insect responses to crop diversification

Crop diversification or multiple cropping is defined as the growth of two or more crops in the same field per year (Vandermeer 1989). Crop diversification methods are aimed at exploiting physical and chemical properties of cropping species to manipulate insect populations and improve growing conditions, thus resulting in lower amounts of off farm inputs and long term sustainability. Cropping practices include a wide range of techniques ranging from cover cropping, intercropping, trap cropping, barrier cropping, and exploitation of plant volatiles. Ever increasing interest in organic production and environmental conservation has inspired research aimed at investigating the underlying mechanics responsible for the purported benefits of increased crop diversification. Multiple cropping agro-ecosystems offer many advantages including increased productivity, more efficient use of resources (land, labor, and nutrients), reduction in pest species influence (insect, disease, and weeds), as well as socioeconomic advantages.

Several important ecological hypotheses form the basis of multiple cropping. The *associational resistance hypothesis* described by Elton (1958) and Tahvanainen and Root (1972) suggests that taxonomically simple habitats are more likely to be colonized by pests and are therefore more susceptible to damage than taxonomically complex habitats. By being in close proximity, non-host plants confer “an associational resistance”, or various benefits to host plants (Elton 1958). Tahvanainen and Root (1972) put forward two additional hypotheses to explain the mechanisms behind herbivore responses to diversified habitats; *resource concentration* - insect herbivores are more likely to locate and colonize host plants that are more concentrated and *natural enemy* hypothesis - higher abundances of natural enemies are more likely in complex ecosystems (Tahvanainen and Root 1972). Several mechanisms have been proposed to explain observed insect responses to crop diversification. These mechanisms include, reduced colonization rates, reduced tenure time, increased predation and parasitization, disruption of host location cues, repellant chemical stimuli, barriers, and microclimatic influences (Vandermeer 1989).

Inter-planting

Inter-planting, the agricultural practice of growing two or more crops simultaneously, has been shown by several studies to have significant impacts on both pest and natural enemy populations when implemented in cucurbit agro-ecosystems. A series of field experiments conducted by Bach (1980) investigated the impacts of plant diversity and density in cucumber agro-ecosystems. Insect and plant growth parameters of monoculture cucumber (*Cucumis sativa*) plots varying in host plant densities were compared to cucumber intercropped with corn (*Zea mays* L) and broccoli (*Brassica oleracea* L). Populations of *A. vittatum*, the striped cucumber beetle, were found to be 10-30 fold higher in monocultures compared to intercropped systems. Bach argued that the mechanism underlying the difference in *A. vittatum* counts was not likely due to difficulty in locating host plants or lower fecundity in polycultures, but by reduced tenure time and increased emigration rates. Mark-recapture trials suggested that beetles in polycultures were more likely to emigrate to monoculture plots. Increased immigration to monoculture plots was attributed to the microclimatic differences between plot designs. Shading provided by intercropped plant species seemed to contribute to higher emigration rates from intercropped cucumber plots. Results from the study seem to support the resource concentration hypothesis. Specialist herbivores tended to be more influenced by habitat diversity and microclimatic differences rather than biotic influence (Bach 1980a). Other studies exhibited similar patterns in specialist herbivore populations and colonization rates in intercropped cucurbit agro-ecosystems (Bach 1980a, b, Risch 1980, Letourneau 1986, Letourneau 1987, Manandhar et al. 2009).

Generalist herbivores are expected to be less influenced by intercropping due to the presence of alternative food sources (Sheehan 1986). Impacts of intercropping on generalist herbivores in cucurbit systems are similar. Populations of the generalist herbivore *B. argentifolii* were found to be significantly lower on zucchini plants when intercropped with white clover. Similar reductions in *B. argentifolii*, with varying intensities, were found in zucchini-buckwheat, zucchini-okra, and zucchini-sunn hemp intercropped systems. Sunn hemp (*Crotalaria juncea*) was reported to be more effective in reducing whitefly numbers compared to the okra, white clover, and buckwheat inter-planted plots (Manandhar et al. 2009).

Empirical support for the natural enemies hypothesis in cucurbit agroecosystems is mixed. Results from studies conducted by Andow and Risch (1985) contradict the expectation of higher natural enemy abundances in intercropped plots. *Coleomegilla* *maculata* (DeGeer), a generalist coccinelid predator, was found to be more abundant in a corn monoculture than that of a corn-bean-squash polyculture (Andow and Risch 1985). The authors argued that *C. maculata* population numbers differed due to higher emigration from polycultures, which was attributed to reduced food encounter rates (Andow and Risch 1985). Conversely, increased natural enemy abundances have been found in a squash-cowpea-maize tricultures compared to squash monocultures (Letourneau 1987). During the study, several parasitoid species were more commonly found and displayed higher rates of parasitism in polyculture squash arrangements than in their monoculture counterparts. Egg parasitism of *D. hyalinata,* the melon worm, was approximately 30% in the squash triculture compared to 11% in squash monoculture plots. Similarly, larval parasitism rates were higher in polycultures (60% in triculture vs. 30% in monoculture plots). Letourneau reported significantly higher rates of parasitism of the melon worm by parasitoids in squash plots containing maize. Higher parasitoid visitation rates were attributed to benefits provided by the presences of maize.

Trap Cropping

Trap cropping is the growing of apparent plants concurrently with cash crops to divert key insect pests away from the cash crop, thus protecting the cash crop from herbivory and disease transmission. Trap crops function by attracting and maintaining pest populations, thus reducing colonization and migration into the main cash crop. Relative success of trap cropping in particular systems depends on several ecological factors including, but not limited to, insect dispersal ability and mobility, insect oviposition preferences, plant apparency, plant insect interactions, etc (Shelton and Badenes-Perez 2006). Several studies have investigated the impacts of trap cropping in cucurbit systems on specialist and generalist herbivores of cucurbits (Pair 1997, Boucher and Durgy 2004, Adler and Hazzard 2009, Cavanagh et al. 2009). *A. vittatum*, the most important cucurbit crop pest in the northeastern United States has been shown to be highly attracted to cucurbitacin (Metcalf et al. 1980). Cucurbit crops containing higher concentrations cucurbitacin seem to be more preferable host plants for *Diabrotica and Acalymma spp.* (Metcalf et al. 1980).

Few studies have shown the effectiveness of trap cropping in managing striped cucumber beetles in cucurbit systems. Cavanagh et al (2009) conducted a study aimed at investigating the impacts of Blue Hubbard squash (*C. maxima*) as a trap crop around butternut squash (*C. moschata*) on populations of *A. vittatum*. They found that when implemented as a perimeter trap crop, *C. maxima* significantly reduced numbers of *A. vittatum* in butternut squash (*C moschata*) main crop plots. *A. vittatum* exhibited a strong preference toward *C. maxima* as evidenced by an 8-fold higher incidence in the perimeter trap crops than in the conventional crop plots (Cavanagh et al. 2009). Although there was higher incidence of striped cucumber beetle in trap cropped plots, defoliation ratings were not significantly different between treatments. Since beetles were more concentrated in trap crop borders, pesticide use decreased 50 percent and 94 percent, respectively, due to smaller area needed to apply pesticide. Further studies report similar success when a Blue Hubbard squash trap crop was used to effectively protect summer squash, cantaloupe, and watermelon main crops (Pair 1997, Boucher and Durgy 2004). A study conducted by Adler and Hazzard (2009) reported similar results. Their field trials suggested that in addition to

Blue Hubbard squash, other varieties such as zucchini and buttercup squash are equally attractive to *A. vittatum* and are thus equally viable as trap crop candidates.

Perimeter trap cropping, when used appropriately, has been shown to greatly reduce the frequency and volume of insecticide applications needed to protect main crops. However, the cultural practice of perimeter trap cropping along with insecticide application may result in non- target effects. Natural enemies drawn to trap crops by higher abundance of prey items will be susceptible to broad spectrum insecticides, which can result in decline of natural enemies, pest resurgence, and secondary outbreaks.

Living Mulches

Living mulches, while similar to inter-planting, differ in that living mulches are not harvested along with the main crop. Living mulches are cover crops primarily employed for the purpose of weed suppression and preventing soil erosion. Recent research has been aimed at investigating potential impacts that living mulches can have on arthropod pests and natural enemies. A study by Hooks et al. (1998) designed to evaluate the impacts of living mulches on arthropod populations suggested increased natural enemy populations when buckwheat (*Fagopyrum esculentum*) and yellow mustard (*Sinapis alba*) were utilized as living mulches in a zucchini agro ecosystem. Hooks et al. (1998) reported significant decline of both alate and apterous melon aphids (*Aphis gossypii*) in all treatments containing living mulch on all dates sampled. At this site, sampled predator abundances did not differ significantly among treatments. However, the same study conducted at another site suggested increased abundances of coccinelid natural enemies in bare ground treatments. Hooks et al. (1998) suggested coccinelid predators were lured to bare ground plots by higher abundances of aphid prey. Additional results suggested significantly higher biomass and production from zucchini plants grown among buckwheat living mulch compared to bare ground plots (Hooks et al. 1998).

Another study conducted by Nyoike et al. (2008) examined the effects of living mulches used alone and in tandem with imidacloprid pesticide application on whitefly-transmitted cucurbit leaf crumple virus in a zucchini system. Study treatments included white mulch (control), reflective mulch, reflective mulch with imidacloprid, buckwheat, and buckwheat with imidacloprid applications. Significantly fewer whitefly (*B. argentifolii*) numbers were found on living and reflective mulches compared to the white mulch control. Results also suggested enhanced protection when living mulches were used in tandem with imidacloprid application. Living and reflective mulch treatments with pesticide applications contained the fewest whitefly individuals. Whitefly adults were highest on the white mulch control. The authors reported a similar correlation for the incidence of cucurbit leaf crumple virus. White mulch plots exhibited highest incidence of the virus while living and reflective mulch treatments with imidacloprid applications exhibited lowest virus incidence (Nyoike et al. 2008).

Crop Rotation

Crop rotation, the practice of altering crop plantings spatially or temporally, is commonly suggested to help reduce pest populations in a variety of agro-ecosystems. Alternating crop species in a particular field may prevent the buildup of overwintering herbivores and soil dwelling pathogens. Rotating cropping fields may also make it more difficult for pest species to find their host plants. As such, crop rotations are best suited to suppressing sessile or relatively immobile monophagous herbivore species. In cucurbit agro-ecosystems, crop rotation may have limited effectiveness due to the biology and ecology of its suite of pests. For example, *.A vittatum* relies on cucurbitacin-based kairomonal cues to detect host plants (Metcalf et al. 1980). This species have been found to be highly sensitive to small concentrations of cucurbitacins (Metcalf et al. 1980). In addition, *A. vittatum* is a relatively mobile insect. Efficient host searching ability and mobility limits the efficacy of crop rotation as a cultural control tool. Similarly, *A. tristis* and *M. cucurbitae*, are both strong flyers with the ability to travel long distances to locate host plants (McKinlay 1992, Robinson and Decker-Walters 1997, Brust 2009).

Conclusion

Cucurbits are one of the world's oldest and most economically important crop families. They are colonized by a unique suite of generalist and specialist herbivore species. This review attempted to examine current management strategies as well as sustainable methods of crop diversification and the subsequent responses of arthropod populations to changes in plant diversity and density in cucurbit based agro ecosystems. The "resource concentration" and "natural enemies" hypotheses proposed by Elton (1958) and Root and Tahvanainen (1972) described the disadvantages of monoculture stands and inspired several studies aimed at investigating the influences of crop diversification on arthropods in agro-ecosystems. Crop diversification can include a variety of different cropping practices including, intercropping, trap cropping, barrier cropping, utilization of living mulches, crop rotation, and companion planting.

Studies aimed at examining the impact of crop diversification on cucurbit pests, though limited, generally reported significant declines in generalist and specialist herbivore populations. Many of these herbivore declines were attributed to factors such as reduced tenure time, microhabitat variability, increased emigration rate, and increased difficulty locating host plants (Bach 1980b, Vandermeer 1989). Although most studies reported decline in herbivore populations with increased plant diversification, natural enemy response to crop diversification seems to be more variable. Studies specifically examining insect responses to crop diversification in cucurbit agro ecosystems are limited, however, there have been numerous studies reporting similar trends in other plant taxa such as the Cruciferae (Hooks and Johnson 2003). A review conducted by Hooks and Johnson (2003) examined the impacts of agricultural diversity on arthropod populations in cruciferous crops. The review proposed several mechanisms underlying particular responses to agricultural diversification in cruciferous cropping systems as well as several tactics for manipulating pest densities (Hooks and Johnson 2003).

The recent increased interest in reducing pesticide use, organic farming and adopting more sustainable agricultural practices demands a more concerted effort in investigating the effectiveness of more sustainable methods for manipulating arthropod populations. Crop diversification practices such as inter-planting, trap cropping, and utilization of living mulch not only has the potential to reduce insect pests but offer a host of other benefits such as more efficient land use, reduction of off farm inputs, and improved soil health.

Chapter 2: Impact of inter-planting Sunn Hemp, *Crotalaria juncea*¸ as a living mulch on insect populations in zucchini *Cucurbita pepo* L.

Abstract

Zucchini, *Cucurbita pepo* L., is often colonized by specialist and generalist insect herbivores including the spotted, *Acalymma vittatum,* and striped, *Diabrotica undecimpunctata howardi,* cucumber beetles. To evaluate the impact of cover cropping on insects associated with zucchini, sunn hemp (*Crotalaria juncea)* was inter-planted with zucchini as a living mulch and compared with monoculture bare-ground zucchini during the 2009, 2010, and 2011 growing seasons. Foliar counts of arthropods conducted on zucchini plants showed significantly lower numbers of *A. vittatum* in sunn hemp compared to bare-ground treatment plots. Striped cucumber beetles were also lower on zucchini plants in sunn hemp plots. When present, squash bugs (*Anasa tristis*) were less abundant in sunn hemp treatment plots. Among predators, spider numbers were significantly higher in sunn hemp treatment plots. Potential reasons for difference in arthropod populations among the two treatments are discussed.

Introduction

In the northeastern United States, there are several economically important insect pests of zucchini (*Cucurbita pepo* L.). Of these, the striped cucumber beetle, *Acalymma vittatum.* and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, (Coleoptera: Chrysomelidae) are the most common and economically important among cucurbit crops (McKinlay 1992, Robinson and Decker-Walters 1997, Brust 2009). Cucumber beetles overwinter as unmated adults, emerge in spring and begin their search for suitable host plants (McKinlay 1992). Larval and adult life stages can potentially inflict serious damage and/or mortality to zucchini seedlings (Brust 2009). Larval root feeding by cucumber beetles can stunt and delay the growth and development of zucchini seedlings (Hoffman et al. 2000, Brust 2009). Adult cucumber beetles girdle zucchini seedling stems causing early season mortality. They remain a serious threat throughout the season due to damage inflicted via defoliation, stem and pollen feeding, and direct fruit damage (McKinlay 1992, Hoffman et al. 2000, Brust 2009). Additionally cucumber beetles are known to vector an important cucurbit disease, bacterial wilt (Leach 1964, Yao et al. 1996, Brust 1997). Other pests such as the squash vine borer (*Melittia cucurbitae*), squash bug (*Anasa tristis*), melon aphids (*Aphis gossypii*) and silver leaf whiteflies (*Bemisia argentifolii*) have the potential to cause stand loss and yield reduction through the transmission of persistent and non persistent viruses and plant phytotoxemias (McKinlay 1992, Robinson and Decker-Walters 1997, Manandhar et al. 2009, Manandhar and Hooks 2011).

Currently, successful control regimens for these pests rely on frequent applications of broad spectrum insecticides such as pyrethroid, neonicotinoid, and carbamate (Foster and Brust 1995, Zhu et al. 2005, Service 2012). However, reliance on chemical controls is unsustainable and may result in herbivores developing resistance to insecticides, secondary pest outbreaks, and negative impacts on non-target organisms. To mitigate this, sanitation, mechanical and other cultural control practices are often suggested to manage cucurbit insect pests (Pair 1997, Boucher and Durgy 2004, Shelton and Badenes-Perez 2006, Brust 2009, Cavanagh et al. 2009).

Crop diversification is an agricultural practice that was commonplace in earlier civilizations and more recently it has been suggested that multiple crops be grown in close proximity (Francis 1986, Horrocks et al. 2004). The *associational resistance*, *resource concentration* and *enemy* hypotheses put forth by Elton (1958) and Root and Tahvanainen (1972) suggest that crop diversification can be used to manipulate insect populations. The associational resistance hypothesis posits that susceptible plants are “protected” by nearby non-host plants due to reduced host-plant density and increased natural enemy abundance. Root and Tahvanainen (1972) furthered the hypothesis by presenting the resource concentration and natural enemy hypotheses. Resource concentration suggests that monocultures are more susceptible to herbivory due to the assumption that larger, more concentrated food sources are easier to locate and exploit. The natural enemies hypothesis suggests that natural enemies will be more abundant in complex ecosystems due to the increased likelihood of alternate prey items and microhabitats. These hypotheses inspired several studies aimed at investigating the influences of increased crop diversification on insect suppression (Bach 1980b, a, Andow and Risch 1985, Letourneau 1986, Andow 1991, Hooks et al. 1998, Hooks and Wright 2008, Adler and Hazzard 2009, Cavanagh et al. 2009, Manandhar et al. 2009). The relative success of crop diversification strategies in particular agricultural ecosystems can depend on a variety of factors. Plant architecture of living mulches, for example, may reduce insect colonization by impeding movement onto a main crop.

Sunn hemp, *Crotalaria juncea* (Fabaceae), is a tropical, drought tolerant, herbaceous, nitrogen-fixing, annual legume native to India (USDA-NCRS 1999). Sunn hemp is most widely grown for use as a green manure, livestock feed, and fiber production (USDA-NCRS 1999). However, when implemented as a living mulch, sunn hemp is a viable candidate for insect and weed suppression due to its ability to protect cucurbits from insect caused plant impairments (USDA-NCRS 1999, Manandhar et al. 2009, Manandhar and Hooks 2011). Rapid accumulation of large amounts of biomass contributes to the plants architectural complexity which is a characteristic that allows plants to serve as a barrier to insect pests(Vandermeer 1989). Additionally, increased habitat complexity afforded by sunn hemp may provide favorable microhabitats for natural enemies such as ground dwelling lycosid spiders and carabid beetles (Vandermeer 1989). In Hawaii, studies have shown that sunn hemp, inter-planted as a living mulch with zucchini, is effective in reducing populations of silverleaf whitefly and their associated diseases and phytotoxemias (Manandhar et al. 2009). However, the insect pest complex that colonizes cucurbits grown in Hawaii differs from pest complexes in Mid-Atlantic states. Thus, field studies were conducted to investigate the potential use of sunn hemp as an intercropped living mulch to manage economically important cucurbit pest found in the Mid-Atlantic states. Specifically, investigating the impacts of sunn hemp living mulch on predominant cucurbit pest insects such as the striped and spotted cucumber beetles. To investigate the impacts of fertilizer type on insect populations, organic and synthetic fertilizers were compared.

Materials and Methods

Field Experiments. To investigate impacts of sunn hemp living mulch on insect populations, field experiments were conducted at the University of Maryland's Central Maryland Research and Education Center (CMREC) in Upper Marlboro, MD and Wye Research and Education Center (WREC) in Queenstown, MD during the 2009, 2010, and 2011 growing seasons. Because of its susceptibility to insect pests, zucchini was used as the study crop during the 2009 and 2010 growing seasons (variety Fortune; Seedway; Hall, New York)(Cavanagh et al. 2009) and zucchini variety Gold Star (Syngenta Inc.) was used during 2011. Sunn hemp "Tropic Sun" was used as an intercropped living mulch for each study year.

Experimental Design. Each year, soil type, land preparation, amendments, and seeding method were replicated four times in a randomized complete block design. Treatments consisted of zucchini planted into a sunn hemp living mulch (SH) and zucchini planted directly into bare-ground (BG). Each plot measured 14.6 m x 14.6 m with an inter- and intra- row spacing of zucchini at 1.2 m. Each year, sunn hemp seeds were seeded at a rate of 44.8 kg/ha and each SH treatment plot consisted of 12 sunn hemp rows so that each row of zucchini was surrounded on either side by a row of sunn hemp. Main plots were split into subplots to compare the effects of synthetic ammoniated fertilizer (5-10-10 Southern States) (SF) and organic pelletized chicken manure (7-1-2 Perdue AgriRecycle Seaford, DE) (OF). Each treatment plot contained 143 zucchini plants.

Plot Establishment. During the 2009 growing season, SH treatment plots were established on June 2 and 15 at the Queenstown and Upper Marlboro sites, respectively.

SH treatment plots were allowed 6-7 weeks to accumulate biomass prior to cutting to an approximate height of 45 cm. SH treatment plots were clipped bi-weekly On July 16 and 23, two-week old greenhouse grown zucchini seedlings were transplanted directly into bare ground in BG plots and directly into sunn hemp surface mulch in SH plots at the Queenstown and Upper Marlboro field sites, respectively.

During the 2010 growing season sunn hemp seeds were sown on June 2 and 17 at Upper Marlboro and Queenstown, respectively. Due to the rapid growth of sunn hemp plants during the preceding study year, sunn hemp was clipped weekly as opposed to every 2 weeks. Two-week old zucchini seedlings were planted on June 24 and July 24 at Upper Marlboro and Queenstown, respectively. Sunn hemp rows were cut to a height of 45 cm.

Sunn hemp plot establishment during 2011 differed from the preceding years of the study in that 24 rows of sunn hemp were established. Prior to zucchini transplant, sunn hemp plots were flail mowed to a height of ~ 20 cm following 6 weeks of growth. Alternating sunn hemp rows were then strip-tilled and zucchini seedlings were transplanted into tilled strips on July 15 and 27 at Queenstown and Upper Marlboro, respectively.

During each year of the study subplots received their respective fertilizer application approximately 28 days after zucchini transplant, Drip and overhead irrigation were used at the Upper Marlboro and Queenstown sites, respectively, to maintain zucchini plants. When necessary, plots and border areas were weeded via hoeing, hand weeding and spot applications of glyphosate (Roundup; Monsanto, St. Louis, MO, USA) applied with a backpack sprayer.

Foliar counts. Beginning 14 days after planting (DAP) and continuing at 7 day intervals, 12 randomly chosen zucchini plants, per subplot, were visually inspected for the presence of arthropods such as melon aphids, silverleaf whiteflies, squash vine-borers, squash bugs, striped and spotted cucumber beetles, and natural enemies such as lacewings, lady beetles, and spiders. Visual inspections consisted of identifying, counting and recording arthropods found on the upper and lower surfaces of all leaves, stems, and flowers. The areas of soil immediately surrounding the base of the plants were also inspected for the presence of insects. During sampling sessions, foliage was gently turned to avoid disturbance. Foliar counts at the Queenstown site were initiated on July 30, August 4, and July 28 in 2009, 2010, and 2011, respectively and on August 5, July 8, and August 11 in 2009, 2010, and 2011 at Upper Marlboro**.** In addition to foliar counts, yellow sticky cards were placed randomly within each field plot to help quantify insect numbers and movement into plots. Sticky card sampling was initiated 7 DAP and remained in the field at 7 day intervals until final harvest.

Statistical Analyses. Statistical analyses of collected insect count data by species were analyzed using a split plot repeated measure analysis of variance (Proc Mixed) (SAS-Instutute 2002). However, since the sub plot factor was insignificant, the split plot factor was averaged to compare across main treatments. A model was constructed to determine the effects of main plot factors over the course of the growing season. Block was incorporated as random factors. Whenever there was a significant date by treatment interaction for a particular species, a separate analysis was conducted to examine main effect on particular sampling occasions.

Results

During each growing season, prior to zucchini planting, SH plots produced, on average, 2330 kg/ha of dry biomass. Given the growth periods, sunn hemp biomass accumulation was comparable to growth in warmer climates (Marshall 2002). Pest species found across all years of the study included striped cucumber beetles, spotted cucumber beetles, squash bugs, squash vine-borers, melon aphids, and silverleaf whiteflies. Predators included coccinellids, lacewings, carabid beetles, and web-building and ground spiders. Only cucumber beetles, aphids, and spiders were consistently abundant enough for statistical assessment. Each year, at both sites, cucumber beetle abundances exceeded the action threshold of 1 beetle per plant. However in 2011 at Upper Marlboro, beetle abundances were low and never approached threshold levels.

2009 Growing Season. During the 2009 growing season, at the Queenstown study site, striped cucumber beetles were initially encountered at 14 and 21 DAP, in BG and SH treatment plots, respectively. Striped cucumber beetle counts were highest between the 28 and 35 DAP sampling period and declined thereafter. Throughout the growing season, SH plots consistently had lower abundances of striped cucumber beetles. These differences became significantly different on late season sampling occasions. SH treatment plots contained significantly lower numbers of striped cucumber beetle compared to BG plots on 35 ($F_{1,\,3} = 19.13$, $P = 0.022$), and 42 DAP ($F_{1,\,3} = 22.48$, $P = 0.018$) (Fig. I-1a). Although fewer spotted cucumber beetles were found overall in SH plots, the differences were not significant until 42 DAP where densities were significantly lower in SH plots ($F_{1,\,3} = 75.27$, $P = 0.003$) (Fig. I-2). Results of sticky card collections indicated that melon aphid populations were generally lower in SH plots compared to BG plots. Differences were only significant at 35DAP ($F_{1, 3} = 35.41$, $P = 0.001$) DAP (Fig. I-3). With regard to natural enemies, only spiders were found in high enough densities for statistical analysis. There were consistently more spiders on zucchini plants in SH plots than BG plots. Abundances were significantly different on 35 ($F_{1,3} = 18.00$, $P = 0.024$) and 42 DAP ($F_{1,3} = 21.60$, $P = 0.019$) (Fig. I-4a).

At the Upper Marlboro site striped cucumber beetle populations were not significantly different until 35 ($F_{1, 3} =35.24$, $P = 0.001$) and 42 ($F_{1, 3} = 97.14$, $P = 0.002$) DAP where populations were significantly lower in SH plots (Fig. I-1b). Spotted cucumber beetle numbers were not significantly different between treatment plots ($P > 0.05$). Aphid populations, unlike Queenstown, were surprisingly higher in SH plots and lower in BG plots. However, these differences were not significant. ($P > 0.05$). Spider populations were similar to those found at the Queenstown field site. Spider predators were more abundant in SH plots. Differences were significant on 21 ($F_{1,3} = 25.0$, $P = 0.015$), 28 ($F_{1,3} = 16.33$, $P = 0.027$), 35 ($F_{1,3} = 24.0$, $P = 0.016$), and 42 ($F_{1,3} = 16.75$, $P = 0.026$) DAP (Fig. I-4b).

2010 Growing Season. In 2010 at Queenstown, striped cucumber beetles were initially found in both treatment plots 21 DAP. Numbers of striped cucumber beetles were generally higher in BG plots, however differences were only significant 49 DAP ($F_{1, 3} =13.23$, $P= 0.035$) (Fig. I-5a). Populations of the spotted cucumber beetle were similar among treatments during the 2010 growing season ($P > 0.05$). Aphid populations at the Queenstown site were too low to be statistically assessed during the 2010 growing season. Although spider predators were more abundant in SH treatment plots, differences were not significantly different across treatment types ($P > 0.05$).

At the Upper Marlboro field site striped cucumber beetle populations were initially found at 21 and 28 days in BG and SH plots, respectively. Generally, striped cucumbers were more abundant in BG plots compared to SH plots. Differences in striped cucumber populations significant 28 ($F_{1,3}$= 30.34, $P = 0.012$) and 35 ($F_{1,3}$= 36.61, $P = 0.009$) DAP (Fig. I-5b). Similarly, populations of spotted cucumber beetles were higher in BG plots on 35 ($F_{1,3}$ = 19.65, $P = 0.021$), 42 ($F_{1,3}$= 19.46, $P = 0.022$), and 49 ($F_{1,3}$ = 106.7, $P = 0.002$) DAP (Fig. I-6). Aphid populations were consistently lower in SH plots throughout the growing season. However, these differences were never found to be statistically significant across treatment habitats.. With regards to predators, spider populations were not found to be significantly different between treatments at Upper Marlboro ($P > 0.05$).

2011 Growing Season. At the Queenstown field site, striped cucumber beetle populations were similar until 28 ($F_{1,3}$ = 11.44, $P = 0.043$) and 35 ($F_{1,3}$= 83.54, $P = 0.003$) DAP where abundances of striped cucumber beetles were significantly higher in BG plots compared to SH plots. Striped cucumber beetle abundances were not significantly different when sampled 42 DAP. (Fig. I-7). According to analyses, spotted cucumber beetle, alate aphid, squash bug, and spider predator numbers were not significantly different among treatment habitats ($P >0.05$). Results were similar at the Upper Marlboro field site. During the 2011 growing season, there was no significant treatment effect on numbers of striped and spotted cucumber beetles, melon aphid, and spiders ($P > 0.05$).

Discussion

The objective of this study was to investigate the influences of sunn hemp, inter-planted as a living mulch, on arthropod populations present in a zucchini agroecosystem. SH inter-planted plots typically contained significantly lower numbers of striped and spotted cucumber beetles and aphids.

In 2009 and 2010, SH treatment plots contained significantly lower numbers of striped cucumber beetle, on zucchini plants approximately 28 days after planting. Though spotted cucumber beetle numbers were usually less abundant than striped cucumber beetles, their numbers were lower in SH than BG treatment plots. Bach (1980) found significantly lower populations of both striped and spotted cucumber beetles in cucumber-corn-broccoli tricultures compared to monoculture plots. Similarly, Amirault and Caldwell (1998) found that living mulch strips of buckwheat, *Fagopyrum esculentum,* reduced populations of striped cucumber beetles up to 60% in monoculture treatments. Reduction in cucumber beetle populations in the current study was likely due to microclimatic differences between SH and BG plots rather than host location impediments. Cucumber beetles were found to be extremely sensitive to cucurbitacins, reacting to concentrations as low as 0.001µg (Metcalf et al. 1980). Due to their sensitivity to cucurbitacins it is unlikely that cucumber beetles would have difficulty locating cucurbits inter-cropped into living mulches. Bach (1980) found that microclimatic differences between polyculture and monoculture plots resulted in reduced tenure time and increased immigration rates of cucumber beetles in polyculture plots. In 2011, other than the late-season sampling dates at the Queenstown field site, there were no significant treatment differences in cucumber beetle populations. This may have been due to the implementation of an alternate plot establishment strategy. Because the mowed sunn hemp never recovered to exceed 30 cm in height, it is unlikely that sunn hemp rows created adequate barriers or microclimatic differences to differentiate it from BG treatment plots. In addition, larger, more apparent zucchini plants in SH plots may have contributed to higher densities of cucumber beetles compared to BG during the 2011 growing season.

Melon aphid populations were variable during the study years. In 2009, SH plots contained fewer aphids than BG plots at the Queenstown study site. However, during that same season at the Upper Marlboro site, populations did not differ significantly. In studies conducted in Florida and Hawaii, buckwheat and white clover mulches significantly reduced populations of whiteflies and aphids in zucchini plantings (Hooks et al. 1998, Frank and Liburd 2005). Hooks and Wright (2008) also found that buckwheat and white clover mulches inter-planted into zucchini significantly reduced adult whitefly and aphid densities compared to BG treatments. Reduced densities of aphids in diverse cropping systems are attributed to exploitation of their host-finding strategy in which they locate host plants by contrasting plants against the soil background (Kennedy et al. 1959, 1961). Companion plants can effectively increase the ratio of plant to soil background thereby camouflaging plants from aphids (Hooks and Johnson 2003).

Spiders were found at similar or greater abundance in SH compared to BG plots. This is consistent with the proposed natural enemies hypothesis in which predators and parasitoids will be more abundant in botanically diverse ecosystems due to increased habitat complexity and alternate prey items (Tahvanainen and Root 1972) and the findings uncovered in the review conducted by Sunderland and Samu (2000) that spider abundance is increased by plant diversification. Cucumber beetles are known to exhibit anti-predator behaviors when they encounter spiders. Laboratory and field studies revealed that cucumber beetle feeding rates and emigration rates increased in response to visual and tactile cues produced by spiders (Williams and Wise 2003).

In cucurbit agroecosystems, the application of broad spectrum insecticides is used commonly to manage arthropod pests. However, this practice is less sustainable. The plant diversification strategies present a more sustainable approach by exploiting behavioral aspects of pest and arthropod species to manipulate their densities. The current study was aimed at investigating the impact of sunn hemp, grown as a living mulch, on arthropods in zucchini plantings. Results from this study showed that striped and spotted beetle cucumber populations were consistently lower in SH treatment plots. Sunn hemp intercropping may serve as a cultural practice to suppress cucumber beetle colonization to levels below the action threshold of 1 beetle per plant. Because aphid populations were variable during the three study years, the impact of sunn hemp on their densities could not be adequately accessed. Although, spiders were generally more abundant on zucchini in SH plots, their role in pest suppression remains unclear. Future studies could be directed to elucidating their interactions with other arthropods found on zucchini to determine if conservational practices should be directed towards increasing their numbers on zucchini plants.

Appendix I

Figure Captions

Figure I-1. Mean (±SEM) number of striped cucumber beetles recorded in bare ground (BG) and sunn hemp (SH) plots at (a) Queenstown, MD and (b) Upper Marlboro during the 2009 growing season. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05.

Figure I-2. Mean (±SEM) number of spotted cucumber beetles recorded in bare ground (BG) and sunn hemp (SH) plots at Queenstown, MD during the 2009 growing season. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05.

Figure I-3. Mean (±SEM) number of alate aphids recorded in bare ground (BG) and sunn hemp (SH) plots at (a) Queenstown, MD. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05.

Figure I-4. Mean (±SEM) number of spiders recorded in bare ground (BG) and sunn hemp (SH) plots at (a) Queenstown, MD and (b) Upper Marlboro during the 2009 growing season. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05.

Figure I-5. Mean (±SEM) number of striped cucumber beetles recorded in bare ground (BG) and sunn hemp (SH) plots at (a) Queenstown, MD and (b) Upper Marlboro during the 2010 growing season. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05.

Figure I-6. Mean (±SEM) number of spotted cucumber beetles recorded in bare ground (BG) and sunn hemp (SH) plots at Upper Marlboro, MD during the 2010 growing season. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05.

Figure I-7. Mean (±SEM) number of striped cucumber beetles recorded in bare ground (BG) and sunn hemp (SH) plots at Queenstown, MD during the 2011 growing season. BG plots represent zucchini plants planted into bare ground. SH plots represent zucchini inter-planted into sunn hemp strips. * Denotes significant difference between treatment means P < 0.05

Figures

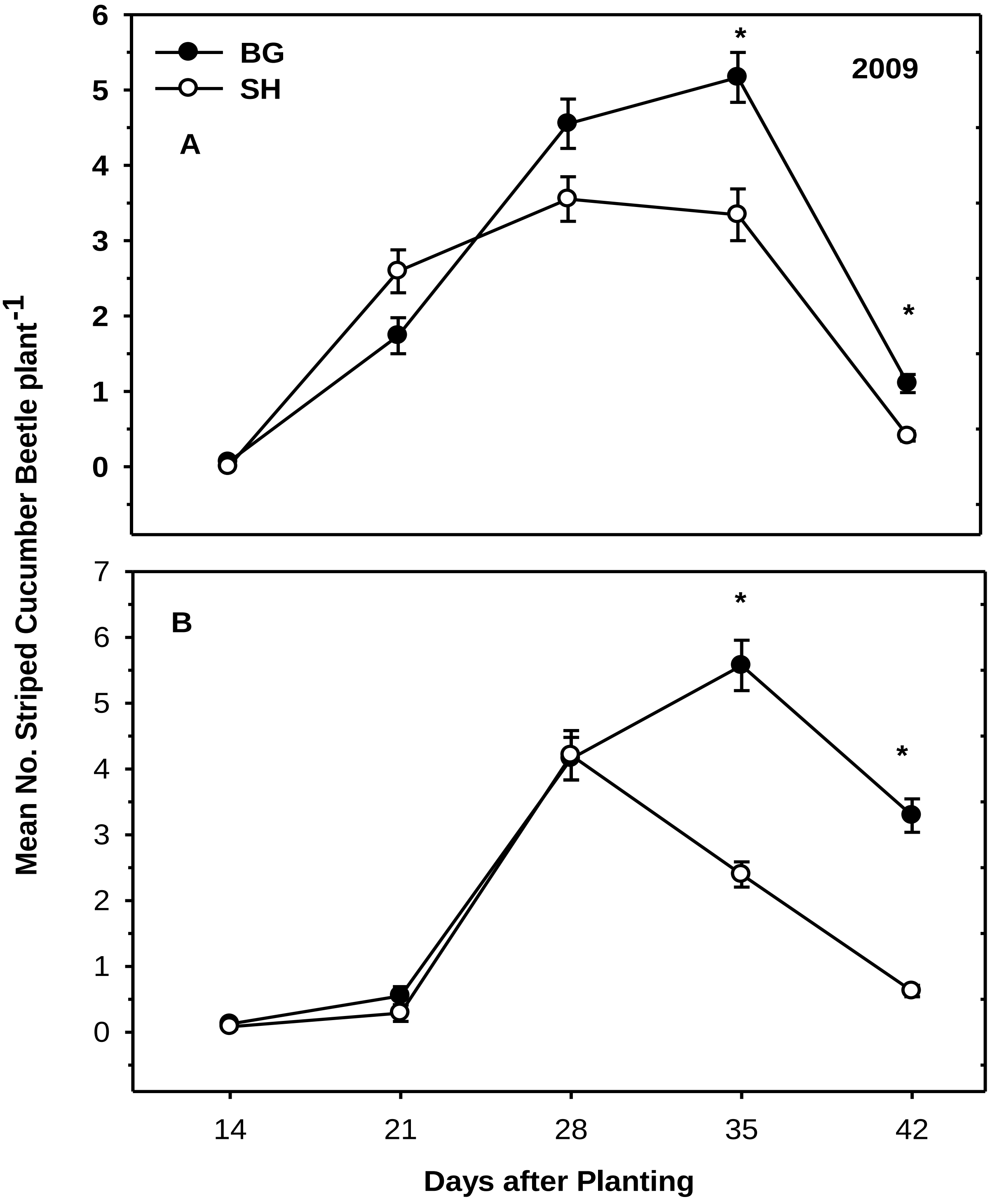


Figure I- 1

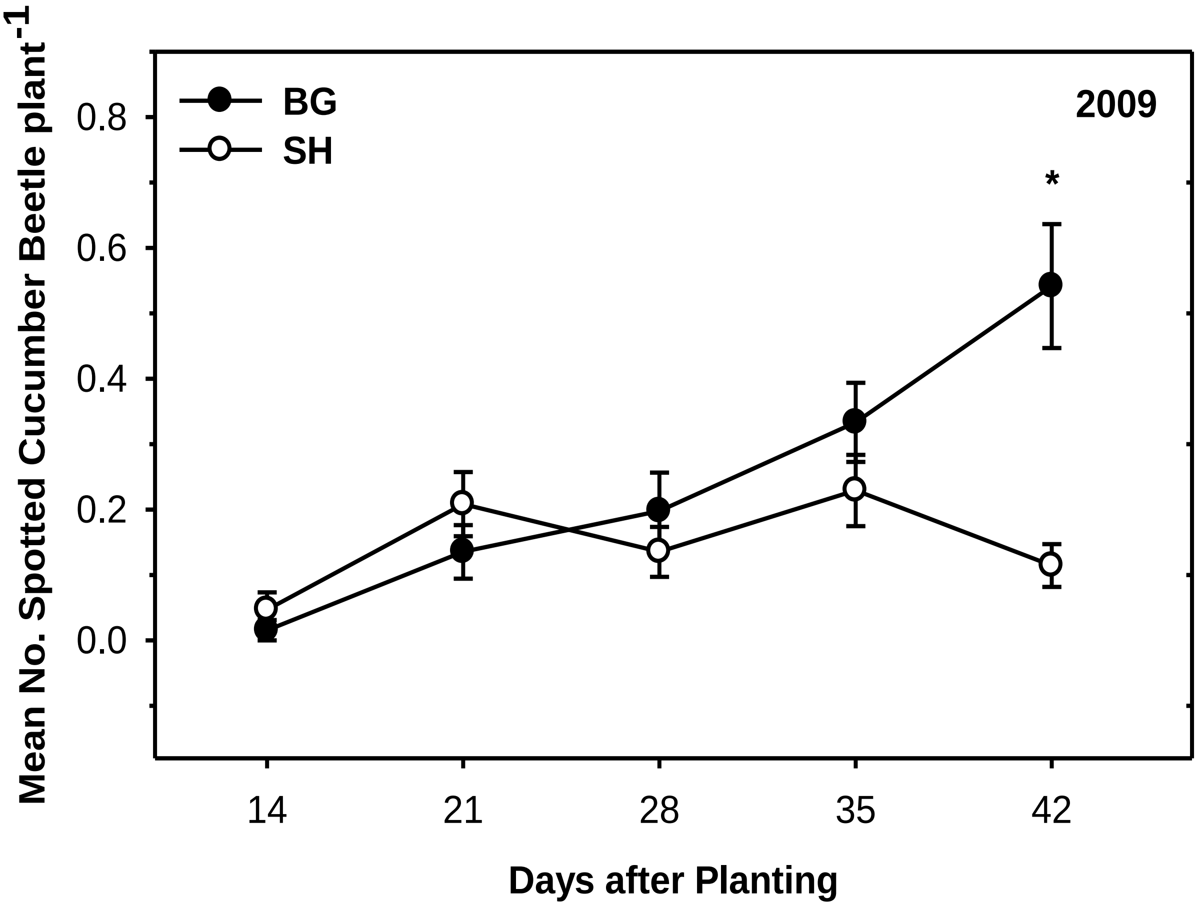

Mean No. Spotted Cucumber Beetle plant^{-1}
0.8
0.6
0.4
0.2
0.0
BG
SH
2009
*
14
21
28
35
42
Days after Planting

Figure I-2

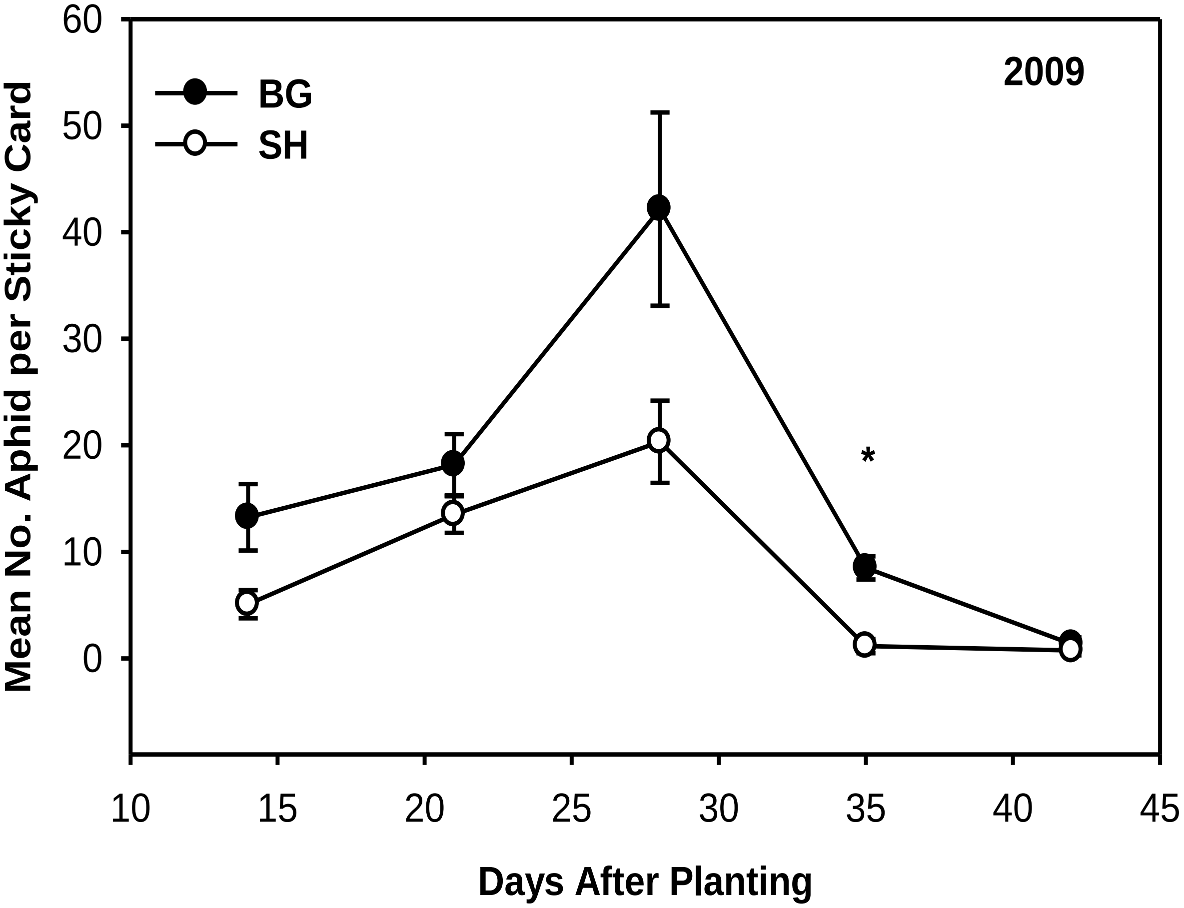
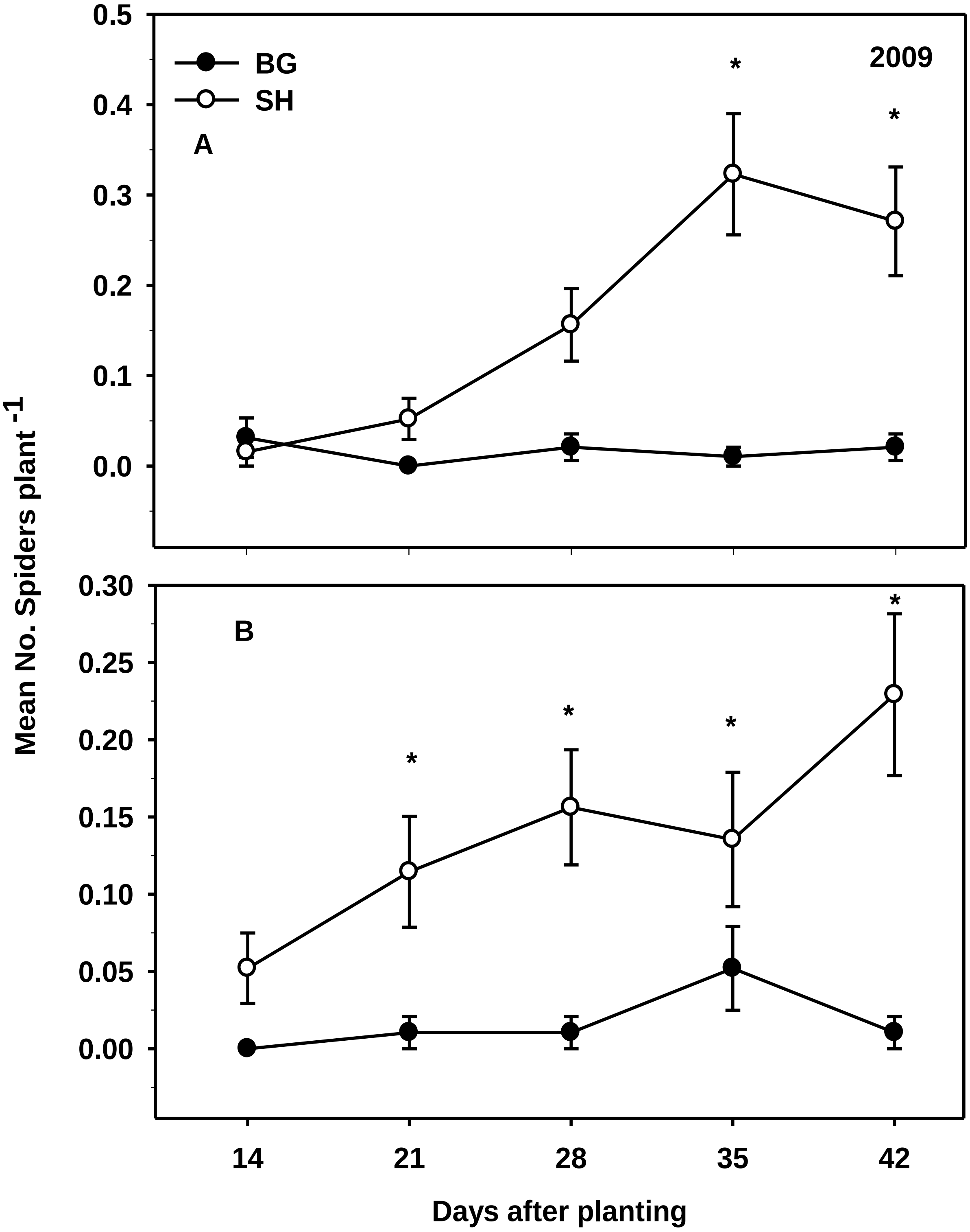
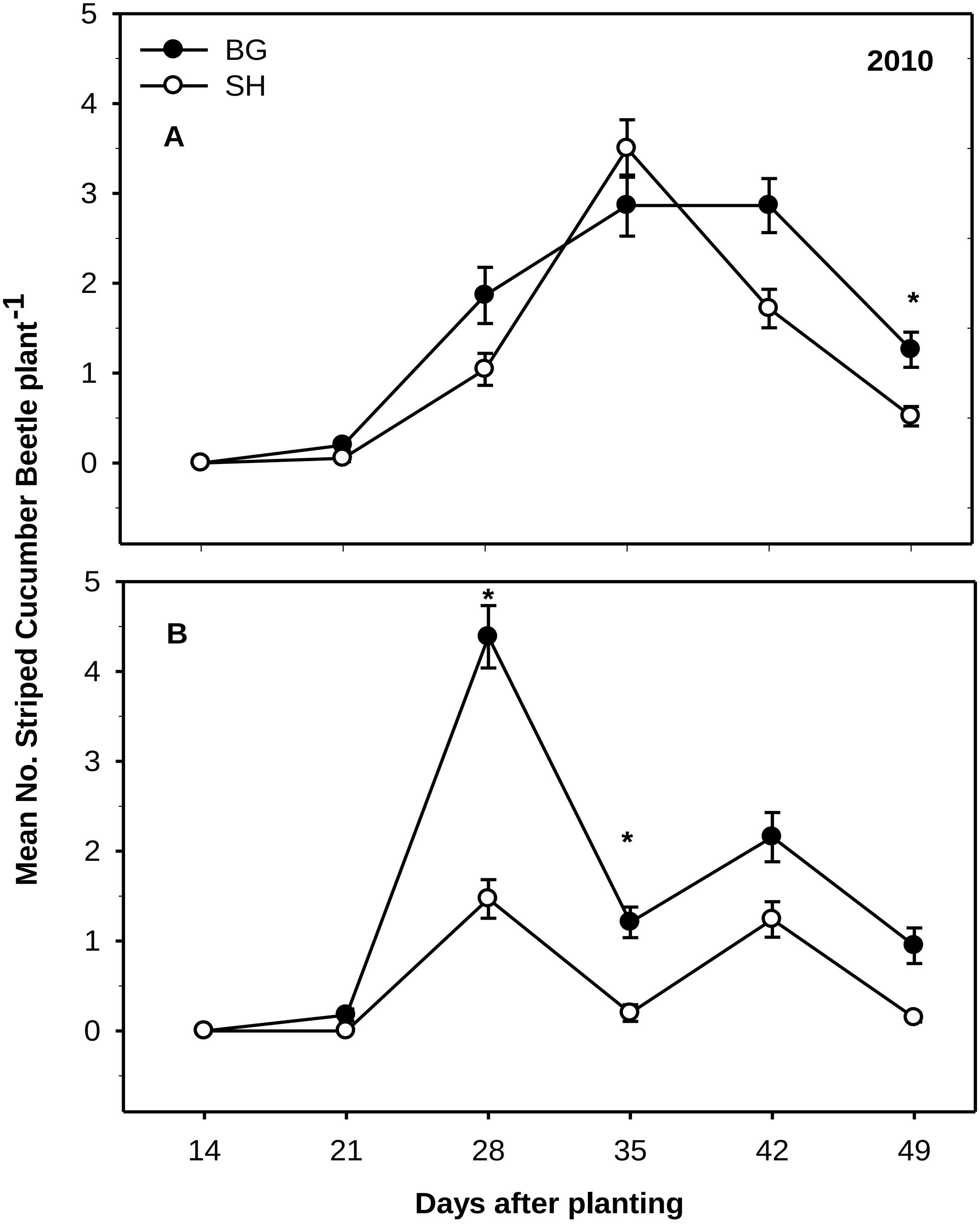

2009
BG
SH
Mean No. Aphid per Sticky Card
60
50
40
30
20
10
0
*
10
15
20
25
30
35
40
45
Days After Planting

Figure I-3



2009
BG
SH
A
B
*
Mean No. Spiders plant $^{-1}$
0.5
0.4
0.3
0.2
0.1
0.0
0.30
0.25
0.20
0.15
0.10
0.05
0.00
14
21
28
35
42
Days after planting

Figure I-4



BG
SH
2010
A
B
Mean No. Striped Cucumber Beetle plant^{-1}
0
1
2
3
4
5
*
*
*
14
21
28
35
42
49
Days after planting

Figure I-5

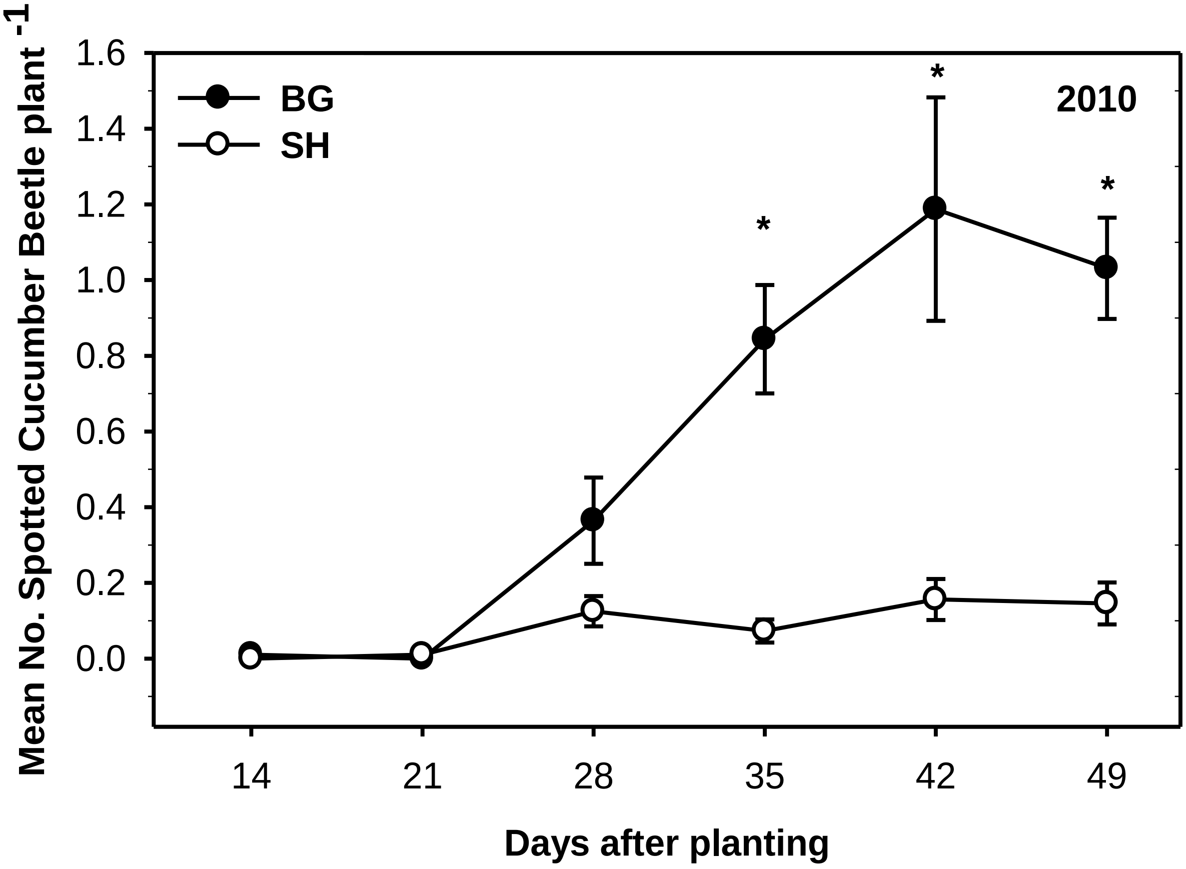

Mean No. Spotted Cucumber Beetle plant -1
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BG
SH
2010
*
*
*
14
21
28
35
42
49
Days after planting

Figure I-6

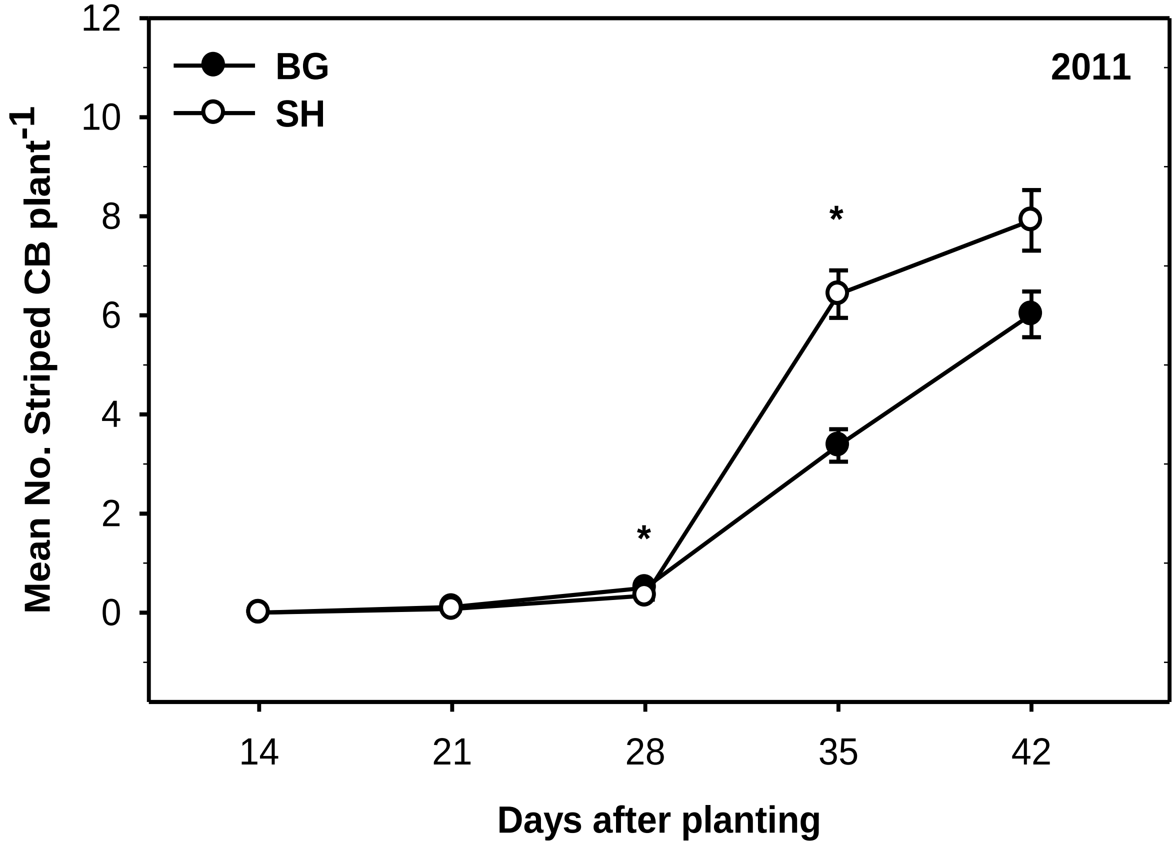

BG
SH
2011
Mean No. Striped CB plant^{-1}
12
10
8
6
4
2
0
14
21
28
35
42
Days after planting
*
*

Figure I-7

Chapter 3: The influence of sunn hemp, *Crotalaria juncea*, as living mulch on growth, development, and yield production in a *Cucurbita pepo* L. agroecosystem.

Abstract

To investigate the influence of sunn hemp (*Crotalaria juncea*) living mulch on zucchini (*Cucurbita pepo L.*) plant growth parameters, experiments were conducted at field sites in Queenstown and Upper Marlboro, MD during the 2009, 2010 and 2011 growing seasons. Sunn hemp plots were established at each experimental site and mowed prior to zucchini transplanting. Plant growth measurements, dry biomass, and yield data were taken from zucchini inter-planted into sunn hemp living mulch (SH) and zucchini planted into bare-ground (BG). In 2009 and 2010 when clipped and used concurrently as a living and organic surface mulch, SH treatment resulted in severe reductions in zucchini growth and yield. This reduction was attributed to competition for sunlight. In 2011, SH rows were flail mowed and rows where zucchini plants were transplanted strip-tilled. This resulted in similar or significantly greater zucchini growth and yields in SH plots than BG plots. The potential use of sunn hemp as a companion plant in vegetable plantings is discussed.

Introduction

In the United States, crop production is often severely reduced due to insect caused damages, weather extremes, plant diseases, and weed competition. In cucurbit crops, stunted plants, mortality and yield loss often results from intense herbivory by insect pests such as *Acaylmma vittatum*, the striped cucumber beetle, and *Diabrotica undecimpunctata howardi*, the spotted cucumber beetle (Brewer et al. 1987, Hoffmann et al. 2000). Though plants have some capacity to compensate for defoliation, early season colonization and intense herbivory can significantly reduce plant vegetative growth, pollen and subsequent fruit production (Brewer et al. 1987, Quesada et al. 1995, Hoffman et al. 2000). Persistent and non-persistent insect-vectored diseases and phytotoxemias such as bacterial wilt, squash silver leaf disorder, cucurbit yellow vine virus, and cucurbit leaf crumple virus often result in retardation of growth, reduced fruit set and mortality (Leach 1964, McKinlay 1992, Robinson and Decker-Walters 1997, Nyoike et al. 2008, Brust 2009, Manandhar et al. 2009, Sasu et al. 2010). Weed competition for nutrients, water, and sunlight are known causes of stand and yield reduction in cropping systems (Monaco et al. 1981). Current crop protection recommendations suggest that prevention is the best way to mitigate factors causing yield reductions.

Crop diversification strategies such as companion planting, cover cropping, and mulching are often suggested as sustainable methods for managing insect pests, diseases, and weeds in cropping systems. Companion plants have been shown to reduce pest populations and damage by functioning as barriers, camouflaging the host plant, and deterring pest colonization by altering the microclimatic environment (Bach 1980a, Letourneau 1986, Hooks et al. 1998, Boucher and Durgy 2004, Frank and Liburd 2005, Adler and Hazzard 2009, Cavanagh et al. 2009, Manandhar et al. 2009). Additionally, several studies have shown that crop diversification strategies may be effective in delaying the onset of both persistent and non-persistent plant viruses and phytotoxemias (Frank and Liburd 2005, Hooks and Wright 2008, Nyoike et al. 2008, Manandhar and Hooks 2011). Studies have shown also that crop diversification strategies can function to outcompete and suppress weed species in cropping systems (Creamer et al. 1996, Creamer and Bennet

1997, Akemo et al. 2000). Although companion plants have been shown to reduce the occurrence of insect, disease, and weed pests, in some instances companion plants have been shown to compete directly with the main crop thus making the use of this tactic less feasible (Vandermeer 1989, Walters and Young 2008, Hooks et al. 2012). However, careful selection, maintenance, and use of leguminous companion plants have the potential to result in increased crop growth compared to monoculture plantings. For example, in a study conducted by Hooks et al. (2012), despite significant reduction in Colorado potato beetle pests (*Leptinotarsa decemlineata*), eggplant yields in a strip-mowed crimson clover dying mulch plot were significantly lower than in a bare-ground conventional plot. However, in a subsequent study year when crimson clover was strip tilled, pest suppression was maintained and eggplant yield was not significantly different compared to bare-ground plots (Hooks et al., unpublished data). Data from other studies also suggest improved main crop performance when crop diversification strategies were implemented (Hooks et al. 1998, Hooks et al. 2007, Manandhar et al. 2009).

Sunn hemp (*Crotalaria juncea*) is a tropical/sub-tropical leguminous plant native to India and is commonly used for fiber production or as a green manure and organic mulch (USDA-NCRS 1999, Wang and McSorley 2004). Several characteristics of sunn hemp such as nitrogen fixation, rapid growth, and biomass production, make sunn hemp a desirable candidate for use as a living mulch in vegetable cropping systems. Under ideal conditions, sunn hemp has been found to produce up to 164 kg/ha of nitrogen (Rotor and Joy 1983). When used as a green manure or organic mulch, sunn hemp can function as a slow release fertilizer (Wang and McSorley 2004). Under optimal growing conditions, sunn hemp has been found to produce up to 6,725 kg/ha and 5,380 kg/ha dry biomass in Hawaii and northern Florida, respectively (Rotor and Joy 1983, Marshall 2002). Thus, sunn hemp when used appropriately can increase plant growth, development, and yield in vegetable cropping systems. Objectives of this study were to evaluate the impacts of sunn hemp used as a living mulch on the growth, development, and yield of zucchini (*Cucurbita pepo L.*).

Materials and Methods

Field Experiments. To evaluate the impacts of sunn hemp as a living mulch on zucchini growth, development and yield, field experiments were conducted at the University of Maryland's Central Maryland Research and Education Center (CMREC) in Upper Marlboro, MD and at Wye Research and Education Center (WREC) in Queenstown, MD during the 2009, 2010 and 2011 growing seasons. During the 2009 and 2010 growing seasons, zucchini (variety Fortune; Seedway; Hall, New York) and in 2011 variety Gold Star (Syngenta Inc.) were used as test crops. Sunn hemp cultivar "Tropic Sun" was used as the companion living mulch each study year. Sunn hemp plots were seeded approximately one month prior to planting the zucchini seedlings. Prior to planting, sunn hemp rows were cut and its foliage allowed to fall in the inter-row spacing between zucchini plants to function as a surface mulch (Manandhar and Hooks 2011, Wang et al. 2011a).

Experimental Design. At each field site, experiments consisted of eight plots arranged in a randomized complete block design. Each study year, main plots were replicated four times and consisted of two zucchini treatments; zucchini planted into sunn hemp living mulch (SH) and zucchini planted into bare-ground (BG) plots. Each plot measured 14.6 m x 14.6 m with an inter and intra-row spacing of 1.2 m. Main plots were split into subplots to compare the effects of synthetic ammoniated fertilizer (5-10-10 Southern States) (SF) and organic pelletized chicken manure (7-1-2 Perdue AgriRecycle Seaford, DE) (OF) fertilizers on zucchini growth and yield production. Each year, sunn hemp seeds were seeded at a rate of 44.8 kg/ha and each SH treatment plot consisted of 12 sunn hemp rows so that each row of zucchini was surrounded on either side by a row of sunn hemp. Zucchini transplants were planted between sunn hemp rows. Each treatment plot contained 143 zucchini plants. Synthetic and organic fertilizers were applied to their respective sub plots approximately 28 days after transplanting at rates of 35g and 58g, respectively. These rates were used so that plants would receive the same level of N from both fertilizer types.

Plot Establishment. During the 2009 growing season, SH treatment plots were established on June 2 and 15 at the Queenstown and Upper Marlboro sites, respectively. The sunn hemp in SH treatment plots were allowed to grow 6.5 weeks to accumulate biomass prior to being cut at an height of approximate 45 cm. On July 16 and 23, two-week old greenhouse grown zucchini seedlings were transplanted into each treatment plot at the Queenstown and Upper Marlboro field sites, respectively. Immediately after transplanting, sunn hemp rows were manually cut to a height of 45 cm to prevent excessive shading.

During the 2010 growing season sunn hemp seeds were sown on June 2 and 17 at Upper Marlboro and Queenstown sites, respectively. Due to the rapid growth of sunn hemp plants during the preceding study year, sunn hemp was clipped at weekly intervals as opposed to every two weeks. Two-week old zucchini seedlings were planted on June 24 and July 24 at Upper Marlboro and Queenstown sties, respectively. Sunn hemp rows were cut to a height of 45 cm.

Sunn hemp plot establishment during differed in 2011 in that 24 rows of sunn hemp were established at an inter-row spacing of 61 cm. Sunn hemp was allowed to grow for 5 weeks prior to being flail mowed to a height of ~ 20 cm. Alternating sunn hemp rows were then strip-tilled to make rows for the zucchini seedlings which were transplanted into the tilled strips on July 15 and 27 at Queenstown and Upper Marlboro, respectively. Plants were either drip (Upper Marlboro) or overhead (Queenstown) irrigate to compensate for periods of low rainfall. When necessary, plots and border areas were weeded via manual hoeing, hand weeding and spot applications of glyphosate (Roundup; Monsanto, St. Louis, MO, USA) applied with a backpack sprayer.

Plant Measurements. Initiating 14 days after planting (DAP), eight randomly chosen plants per subplot were marked and numbered with field flags. Zucchini plant growth parameters were measured at 10 day intervals beginning 14 DAP. Measured parameters include canopy width, length and width of largest leaf, leaf area, leaf number, total surface area estimate, flower number, and chlorophyll content. Canopy length measurements consisted of measuring the distance across the plant from leaf apex to furthest leaf apex. Plant leaf area and total leaf surface area were estimated by multiplying leaf length by leaf width of the largest leaf and total number of plant leaves by leaf area, respectively. Chlorophyll content was recorded using a Chlorophyll Meter SPAD 502 (Konica Minolta Optics, Inc.) on the youngest fully expanded leaf. When conducting chlorophyll measurements, six readings were taken per leaf and averaged.

Zucchini Yield. Zucchini fruit harvest began approximately 35 DAP or when fruit approached marketable size. After initial harvest, subsequent harvests were conducted every two days for approximately 10 harvest periods. At harvest, zucchini fruit were visually inspected for the presence of insect damage, disease symptoms, and disfigurement. After visual inspection, zucchini fruits produced were counted, weighed, and categorized as marketable, cull (disfigured), insect-damaged, and jumbo (over marketable weight).

Dry Biomass. At the end of each growing season, the flagged zucchini plants were stripped of all fruit and flowers, cut and collected to obtain dry biomass measurements. Zucchini plants were held in large paper bags until completely dry then weighed**.**

Statistical Analyses. Statistical analyses of plant growth parameters were initially analyzed using a split plot repeated measure analysis of variance (Proc Mixed, SAS Institute 2002). Non-significant treatment effects of the split-plot factor resulted in were averaged to compare main effects. A model was constructed to determine the effect of main plot treatment factors on plant parameters, biomass, or zucchini yield over time. Block was designated as random factors. Whenever an interaction between main plot factor and days after planting was present, a separate analysis was conducted to determine main plot effects by date. Effects were considered significant when $P < 0.05$.

Results

Plant Measurements

2009 Growing Season. At the Queenstown study site, zucchini canopy length was significantly greater in SH plots at 14 ($F_{1,3} = 82.97$, $P = 0.003$) and 24 DAP ($F_{1,3} = 56.57$, $P = 0.005$) but was similar by 34 DAP ($P > 0.05$) (Fig. II-1). Average leaf area was also significantly higher in SH plots compared to BG plots at 14 ($F_{1,3} = 26.48$, $P = 0.014$) and 24 DAP ($F_{1,3} = 22.30$, $P = 0.018$) (Fig. II-2) and similar at 34 DAP

(P >0.05). Correspondingly, leaf surface area estimates were significantly higher in SH plots at 14 ($F_{1,3}$ =20.96, $P = 0.020$) and 24 DAP ($F_{1,3}$ =12.84, $P = 0.038$) (Fig. II-3). However, at 34 DAP, total surface area estimates were significantly higher in BG treatment plots ($F_{1,3}$ =23.10, $P = 0.017$). Chlorophyll content was similar across treatment plots on each sampling date ($P > 0.05$).

At the Upper Marlboro field site, there were no significant differences across treatment plots for any measured parameter (P >0.05).

2010 Growing Season. At the Queenstown study site zucchini growth and development was lower relative to plantings from the previous growing season and across both study sites. Zucchini growth was lower in SH plots for all parameters measured compared to BG treatment plots. However, these differences were not significant on any sampling occasion ($P > 0.05$).

At the Upper Marlboro field site, results were similar to those found at the Queenstown location in that all measured plant morphological features were reduced in zucchini grown in SH compared to BG treatment plots. Zucchini plant canopy length was significantly lower for zucchini plants grown in SH compared to BG plots at 24 ($F_{1,3}$ =15.35, $P = 0.030$) and 34 DAP ($F_{1,3}$ =126.71 $P = 0.002$) (Fig. II-4). Plant canopy lengths were 35 to 50% lower throughout the growing season in SH compared to BG plots. Leaf area measurements were similarly lower in SH compared to BG plots on 24 ($F_{1,3}$ = 28.14, $P = 0.013$), and 34 DAP ($F_{1,3}$ = 80.73, $P = 0.003$). (Fig. II-5). Total leaf surface estimates were also significantly lower in SH than BG plots on 24 **(**$F_{1,3}$ =37.68, P = 0.009) and 34 DAP ($F_{1,\ 3}$ =1695, $P < 0.0001$)(Fig. II-6). At 24 ($F_{1,3}$ =16.60, $P = 0.027$) and 34 DAP ($F_{1,3}$ =95.41, $P < 0.002$), chlorophyll levels in zucchini grown in SH treatment plots were significantly lower than those grown in BG plots (Fig. II-7). At 44 DAP there was a sharp decline in chlorophyll content in all treatment plots and no significant differences occurred ($P > .005$).

2011 Growing Season. During the 2011 growing season, sunn hemp was flail mowed and every other row incorporated prior to zucchini transplanting. At the Queenstown site, zucchini plants in SH appeared to develop more vigorously than in BG treatment plots. For all measured parameters including canopy length, leaf area, leaf surface area estimate, and chlorophyll content, zucchini grown in SH plots were marginally greater. However these differences were not significant at $P > 0.05$.

In comparison to previous growing seasons, zucchini growth in SH compared to BG plots at the Upper Marlboro site improved overall. All measured plant growth parameters were generally similar among treatment types. Canopy length, leaf area, leaf surface area estimate, and chlorophyll levels were higher in SH plots but not significantly different compared to BG ($P > 0.05$).

Dry Biomass and yield.

2009 Growing Season. During the 2009 growing season, plant samples collected at the Queenstown site did not dry adequately and thus were discarded. However, average marketable zucchini yield in kilograms per hectare was significantly higher in BG plots compared to SH plots. Average marketable zucchini yield was significantly higher in BG plots compared to SH plots on 33, 37, 39, 41, 43, 45, and 46 DAP ($F_{1,3} = 25.40\text{-}183$, $P < 0.015$) (Fig. II-8a). Zucchini fruit graded as cull were generally higher in BG plots than in SH. Differences were significant on 43 ($F_{1,3} = 73.53$, $P < 0.003$) and 46 DAP ($F_{1,3} = 12.89$ $P < 0.037$) (Fig. II-9). Insect-damaged zucchini fruit was higher in BG plots compared to SH and significant 28 ($F_{1,3} = 33.86, P < 0.010$) and 45 DAP ($F_{1,3} = 31.90$, $P < 0.011$)(Fig II-10a.). There were no significant difference between BG and SH for zucchini graded as jumbo ($P > 0.05$).

At the Upper Marlboro site, zucchini plant biomass was significantly greater in BG than SH plots ($F_{1,3} = 32.14, P = 0.011$) (Table 1). On average, plant dry biomass was twofold greater in BG than in SH plots. Zucchini fruit production was noticeably lower at Upper Marlboro compared to the Queenstown site. Marketable yields at Upper Marlboro were significantly higher in BG compared to SH plots on 37 ($F_{1\ 3} = 191.5, P < 0.001$), 39 ($F_{1,3} = 125.37, P < 0.002$), 41 ($F_{1,3} = 63.87, P < 0.004$), and 43 DAP ($F_{1,3} = 19.74, P < 0.021$) (Fig. II-8b). Zucchini grown in BG plots produced significantly more fruit classified as insect-damaged than zucchini grown in SH plots on 25 ($F_{1,3} = 57.32, P < 0.005$), 29 ($F_{1,3} = 52.31, P < 0.005$), 39 ($F_{1,3} = 17.44, P < 0.022$), and 41 DAP ($F_{1,3} = 17.96, P < 0.037$) (Fig. II-10b). No significant differences existed among the two treatments for cull or jumbo zucchini fruit ($P > 0.05$).

2010 Growing Season. Zucchini dry weight samples collected from the Queenstown field site were significantly lower in SH plots compared to their counterparts in BG plots ($F_{1,3}=14.51, P < 0.032$) (Table 1). On average, plants from SH plots were approximately 50% smaller and similar to the previous study year. Harvested marketable zucchini was generally higher in BG plots compared to SH plots. Differences between treatment plots were significant on 48 ($F_{1,3} = 10.15, P < 0.049$) and 51 DAP ($F_{1,3} = 18.30$, $P < 0.024$) (Fig. II-11a). There were also significantly higher zucchini fruit produced classified as insect-damaged in BG compared to SH plots on 36 ($F_{1,3} = 10.15, P < 0.049$), 46 ($F_{1,3} = 10.15$, $P < 0.049$), and 51 DAP ($F_{1,3} = 10.15$, $P < 0.049$)(Fig. II-12). Zucchini classed as cull and jumbo were similar across treatments ($P > 0.05$).

At the Upper Marlboro field site, zucchini production overall was higher than the previous growing season. Zucchini plant dry biomass was significantly lower in SH than BG plots ($F_{1,3} = 431.53$, $P = 0.0002$) at the Upper Marlboro site (Table 1). BG plots produced significantly more marketable fruit by weight than SH plots ($F_{1,3} = 11.93\text{-}45.68$, $P < 0.041$) (Fig. II-11b). Zucchini fruit graded as cull were significantly higher in BG plots compared to SH plots on 37, 45, 48, 49 and 50 DAP ($F_{1,3} = 15.30\text{-}27.77$, $P < 0.036$) (Fig. II-13). There was no significant difference in the amounts of insect-damaged or jumbo zucchini fruit produced between treatment plots ($P > 0.05$).

2011 Growing Season. Zucchini biomass samples from the Queenstown field site were on average, approximately 25% larger in SH plots compared to BG plots. However, this difference was not significant ($P > 0.05$) (Table 1). Overall, total zucchini yields at the Queenstown site were lower compared to the 2012 study. However, unlike 2010, more marketable zucchini by weight were harvested from SH than BG plots. These differences were significant on 34 ($F_{1,3} = 11.55$, $P < 0.043$) and 36 ($F_{1,3} = 14.52$, $P < 0.032$) DAP and marginally higher 38 DAP ($F_{1,3} = 9.33$, $P < 0.055$) (Fig. II-13). Harvested zucchini graded as cull, insect-damaged, and jumbo did not significantly differ across treatment plots ($P > 0.05$).

Although collected dry biomass samples were higher in SH treatment plots compared to BG, differences were not significant ($P > 0.05$). Yields across both treatment plots were also lower at the Upper Marlboro field site compared to the previous years. Marketable zucchini fruit harvested were higher in SH plots but not significant ($P > 0.05$).

Zucchini graded as cull, insect-damaged, and jumbo were similar between SH and BG treatment ($P > 0.05$).

Discussion

.The objective of this study was to investigate the impact of sunn hemp on zucchini plant growth, development and yield when used as interplanted living mulch. During the initial two study years, growth parameters of zucchini plants were significantly reduced in SH compared to BG counterparts. However, in 2011, after the sunn hemp was initially flail mowed to a height of 20 cm as opposed to clipped at a height of 45 cm, zucchini plants grown in SH were significantly larger than those in BG plots.

During the 2009 and 2010 growing seasons, SH plots were allowed to grow for 6.5 and 4 weeks, respectively before clipping it; and prior to transplanting, SH rows were manually trimmed and its foliage allowed to fall on the soil surface to function as organic mulch. At Queenstown in 2009, plant canopy length, mean leaf area and leaf surface area were significantly higher in SH than BG plots. Though these plant parameters were larger, plant biomass and fruit yields were severely reduced in SH compared to BG zucchini plots. There was a 52% reduction in dry biomass from samples and a 34-56% reduction in marketable fruit in SH than BG plots. In 2010, results were similar, dry biomass and zucchini fruit yields were 43-63% and 47-73% lower respectively in SH than BG plots. These reductions are likely due to shading. Competition for nutrients and water is unlikely as both treatments plots were fertilized and irrigated. Reduced growth due to increased insect defoliation is also unlikely as herbivore numbers were significantly lower in SH plots (Hinds unpublished data). Although sunn hemp rows were routinely clipped, aggressive regrowth resulted in shading of zucchini plants.

Competition is a common factor to consider when investigating the feasibility of using a living mulch as a companion crop in vegetable systems (Hooks and Johnson 2003). Living mulches can potentially compete with main crops for water, nutrients and light. Anderson and Roberts (1994) found that when inter-planted with rye living mulch, bell pepper production was significantly lower compared to black mulch, straw mulch, white mulch, and bare ground treatment plots. This reduction was attributed to the interference of sunlight by the rye living mulch.

Excess nitrogen could also contribute to yield reductions in vegetable crops. Over supply of nitrogen has been found to prolong vegetative growth, leading to larger more vigorous plants (Wittwer et al. 1947, Huett 1989, Wang and Li 2004, Eifediyi and Remision 2010). This, however, may also delay flower maturation and poor fruit production of plants grown in soils containing excess nitrogen (Ruiz and Romero 1998). Although SH grown zucchini plants were characterized by higher leaf morphological characteristics and lower yields at the Queenstown 2009 site, it is unlikely that resulted from nitrogen provided by decomposing SH foliage. According to nutrient analyses conducted on dry SH biomass obtained from the field site, SH foliage and stem tissue consisted of approximately 2.6% nitrogen by mass.

In 2011, sunn hemp rows were flail mowed and rows in which zucchini were planted were strip-tilled. This crop husbandry strategy improved overall performance of zucchini grown in SH plots compared to prior study years. At the Queenstown site, all growth parameters measured were significantly larger in SH compared to BG plots and plant biomass and fruit yield were similar among the two treatments. At Queenstown, dry biomass and marketable yields were 35% and 40% higher in SH plots, respectively; and at the Upper Marlboro site, dry biomass was 23% higher in SH plots but marketable yields were similar in SH and BG plots. Other studies have shown increased growth and yield of cash crops when grown with a living mulch. For example, in a study conducted by Manandhar et al. (2009), yields produced by zucchini grown in buckwheat and white clover were significantly higher in comparison to bare-ground plots.

Results from this study suggest, that when implemented appropriately, sunn hemp may result in greater zucchini growth and marketable yields. However, care must be taken to reduce possible avenues for competition. Sunn hemp presence seemed to have a larger impact on zucchini yield than insect pest loads. For example, in 2011 although striped cucumber beetles (*Acalymma vittatum*) were higher in SH plots (Hinds unpublished data), zucchini fruit yields were significantly higher in SH compared to BG plots. Future studies can be directed at determining ways to incorporate SH into zucchini plantings in ways that minimize competition, maintain pest suppression and improve crop growth thorough increased soil and associated plant nutrient levels.

Appendix II

Figure Captions

Figure II-1. Mean zucchini canopy length recorded at Queenstown, MD during the 2009 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-2. Mean zucchini largest leaf area recorded at the Queenstown, MD field site during the 2009 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-3. Mean zucchini surface area estimates recorded at the Queenstown, MD field site during the 2009 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-4. Mean zucchini canopy length recorded at the Upper Marlboro, MD field site during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-5. Mean largest leaf area recorded at the Upper Marlboro, MD field site during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH).
* Denotes significance between treatment means $P < 0.05$.

Figure II-6. Mean leaf surface area estimates recorded at (a) Queenstown, MD and (b) Upper Marlboro, MD field sites during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-7. Mean chlorophyll levels recorded at the Upper Marlboro, MD field site during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-8. Mean marketable zucchini fruit harvested at (a) Queenstown, MD and (b) Upper Marlboro, MD field sites during the 2009 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$.

Figure II-9. Mean cull zucchini fruit harvested at the Queenstown, MD field site during the 2009 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$

Figure II-10. Mean insect-damaged zucchini fruit harvested at (a) Queenstown, MD and (b) Upper Marlboro, MD field sites during the 2009 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$

Figure II-11. Mean marketable zucchini fruit harvested at (a) Queenstown, MD and (b) Upper Marlboro, MD field sites during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$

Figure II-12. Mean insect-damaged zucchini fruit harvested at the Queenstown, MD field sites during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$

Figure II-13. Mean cull zucchini fruit harvested at the Upper Marlboro, MD field site during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$

Figure II-14. Mean marketable zucchini fruit harvested at the Queenstown, MD field site during the 2010 growing season. Closed circles represent zucchini planted into bare-ground (BG) while open circles represent zucchini inter-planted with sunn hemp (SH). * Denotes significance between treatment means $P < 0.05$

Figures

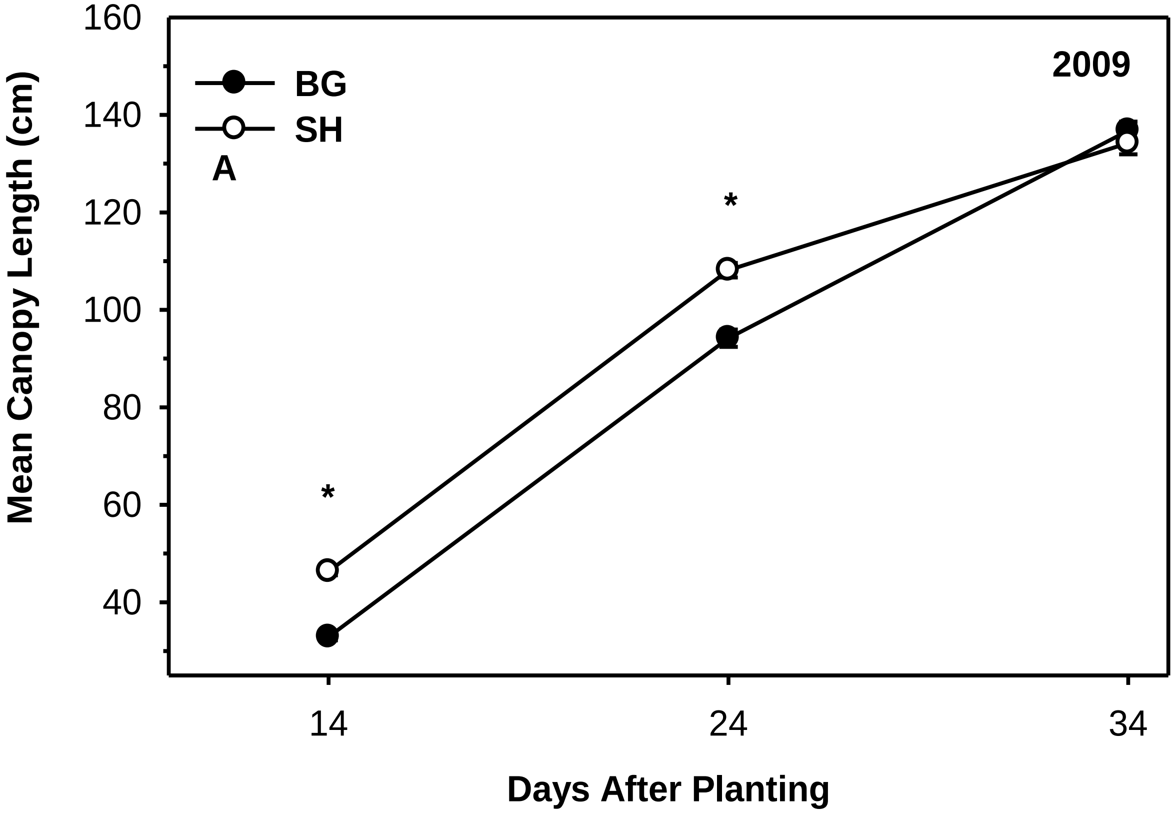
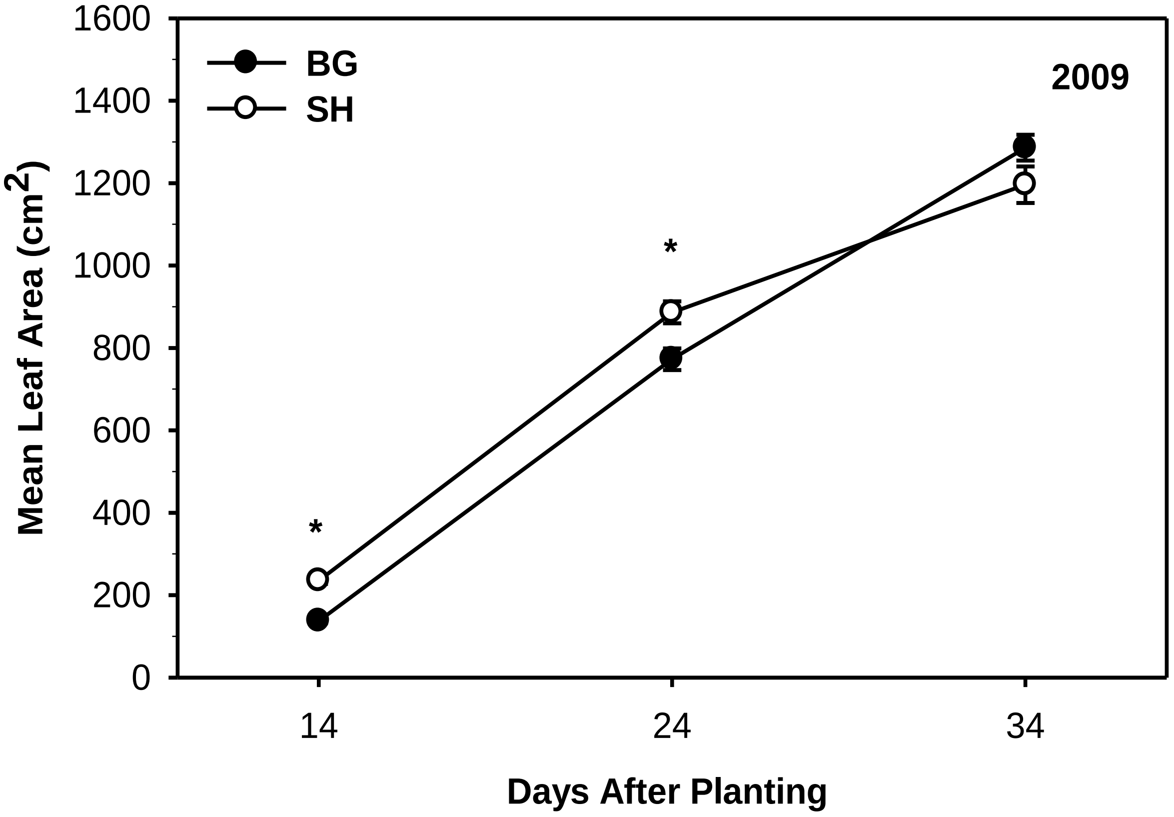


Figure II-1



Mean Leaf Area (cm^2)
0
200
400
600
800
1000
1200
1400
1600
BG
SH
2009
*
*
14
24
34
Days After Planting

Figure II-2

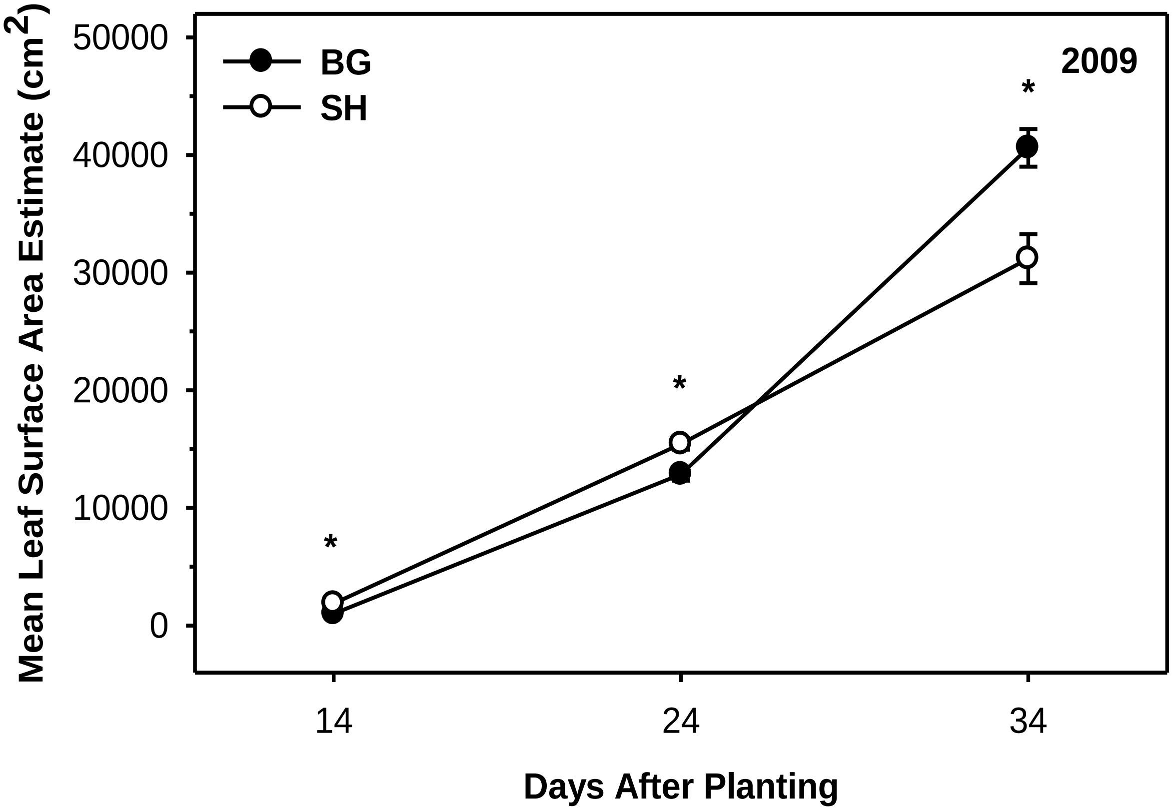

Mean Leaf Surface Area Estimate (cm^2)
50000
40000
30000
20000
10000
0
BG
SH
2009
*
*
*
14
24
34
Days After Planting

Figure II-3

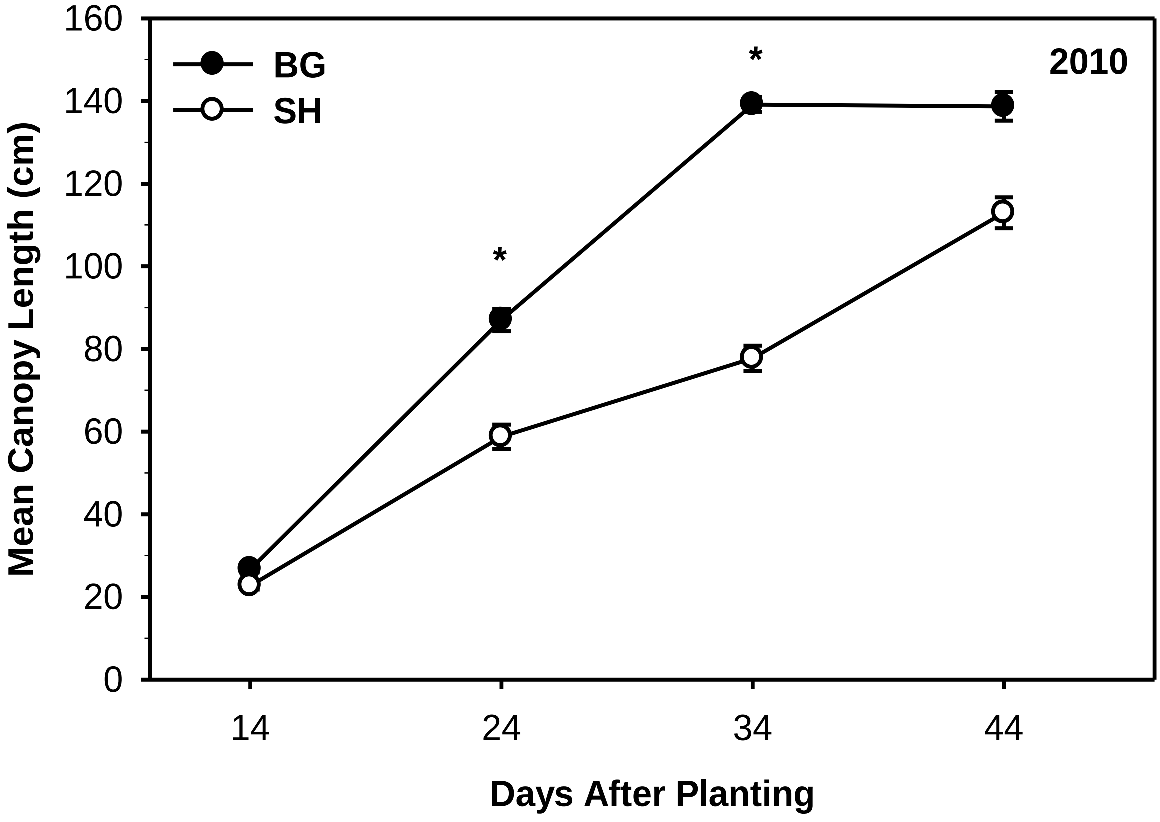

160
140
120
100
80
60
40
20
0
Mean Canopy Length (cm)
14
24
34
44
Days After Planting
● BG
○ SH
2010
*
*

Figure II-4

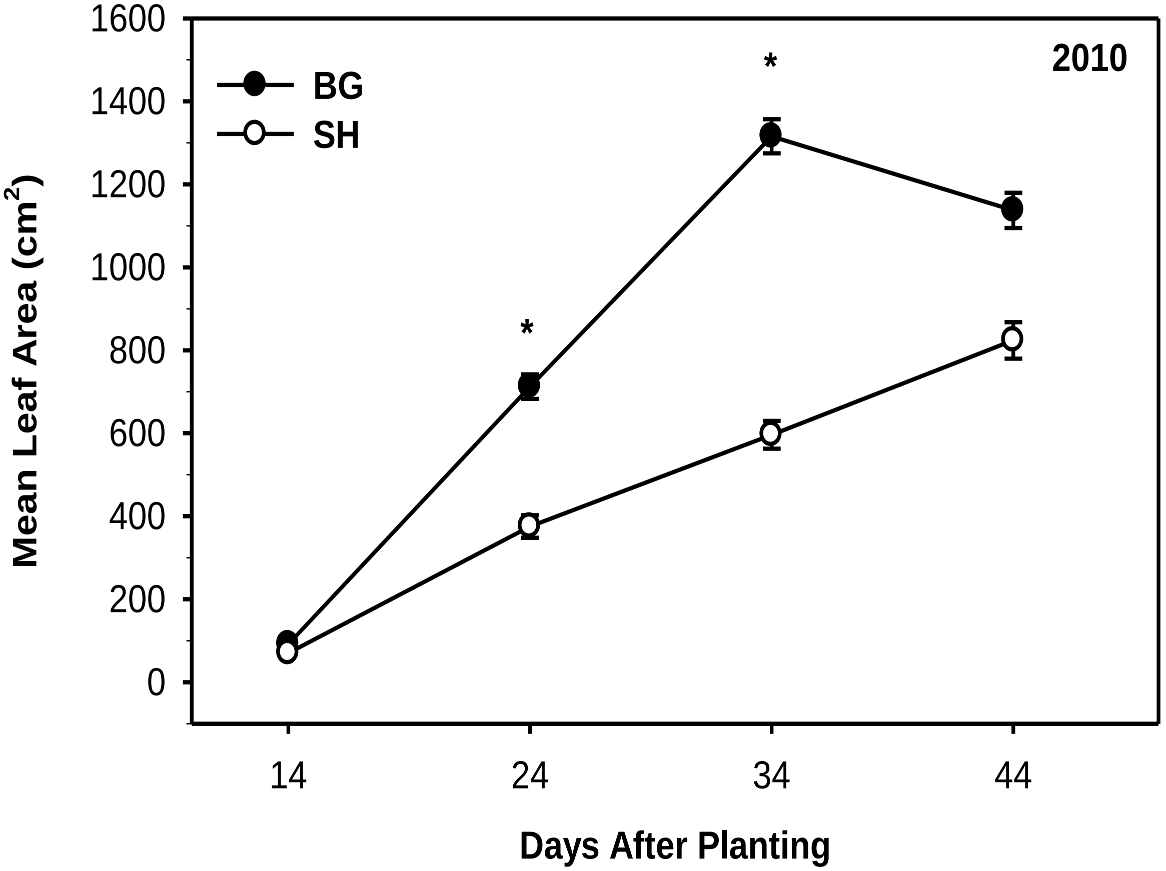
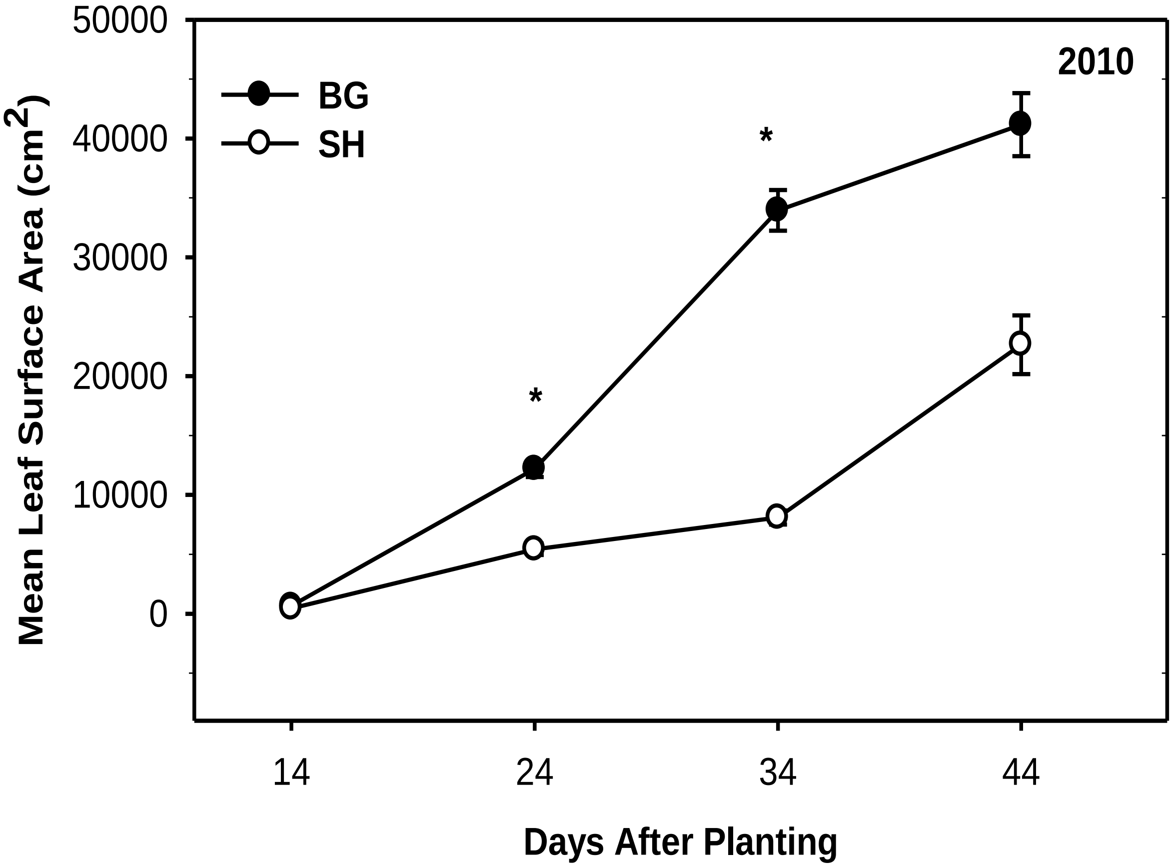

1600
1400
1200
1000
800
600
400
200
0
Mean Leaf Area (cm²)
14
24
34
44
Days After Planting
BG
SH
2010
*
*

Figure II-5



Mean Leaf Surface Area (cm²)
50000
40000
30000
20000
10000
0
2010
BG
SH
*
*
14
24
34
44
Days After Planting

Figure II-6

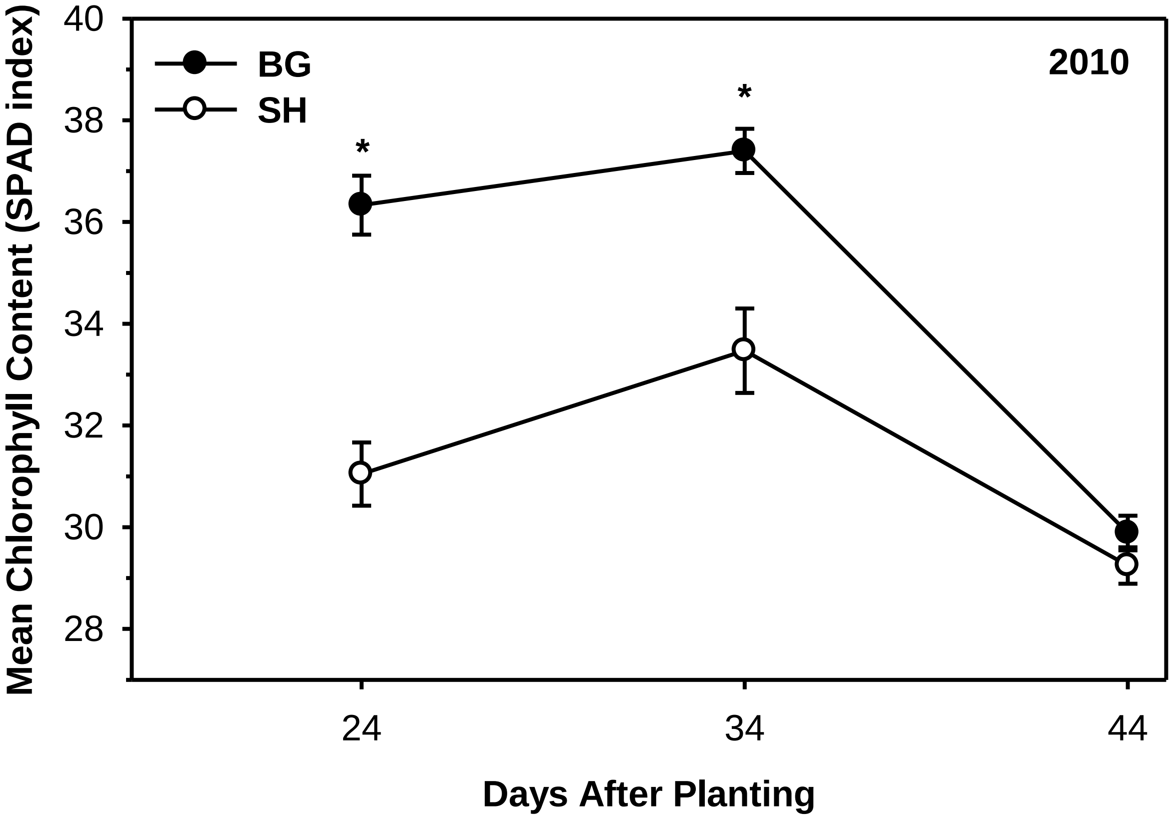

Mean Chlorophyll Content (SPAD index)
40
38
36
34
32
30
28
BG
SH
2010
*
*
24
34
44
Days After Planting

Figure II-7

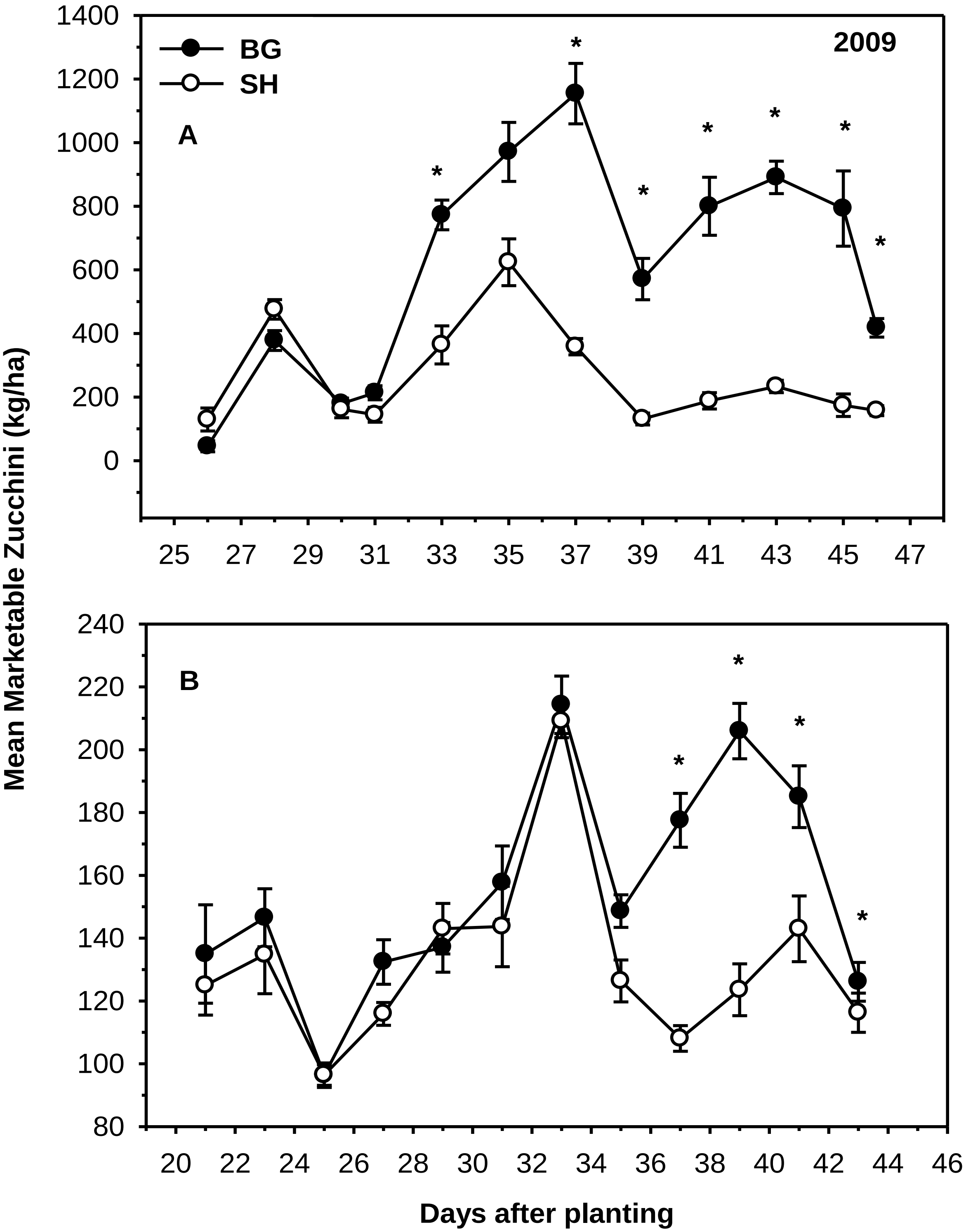

1400
1200
1000
800
600
400
200
0
BG
SH
2009
A
*
*
*
*
*
*
*
25
27
29
31
33
35
37
39
41
43
45
47
Mean Marketable Zucchini (kg/ha)
240
220
200
180
160
140
120
100
80
B
*
*
*
*
20
22
24
26
28
30
32
34
36
38
40
42
44
46
Days after planting

Figure II-8

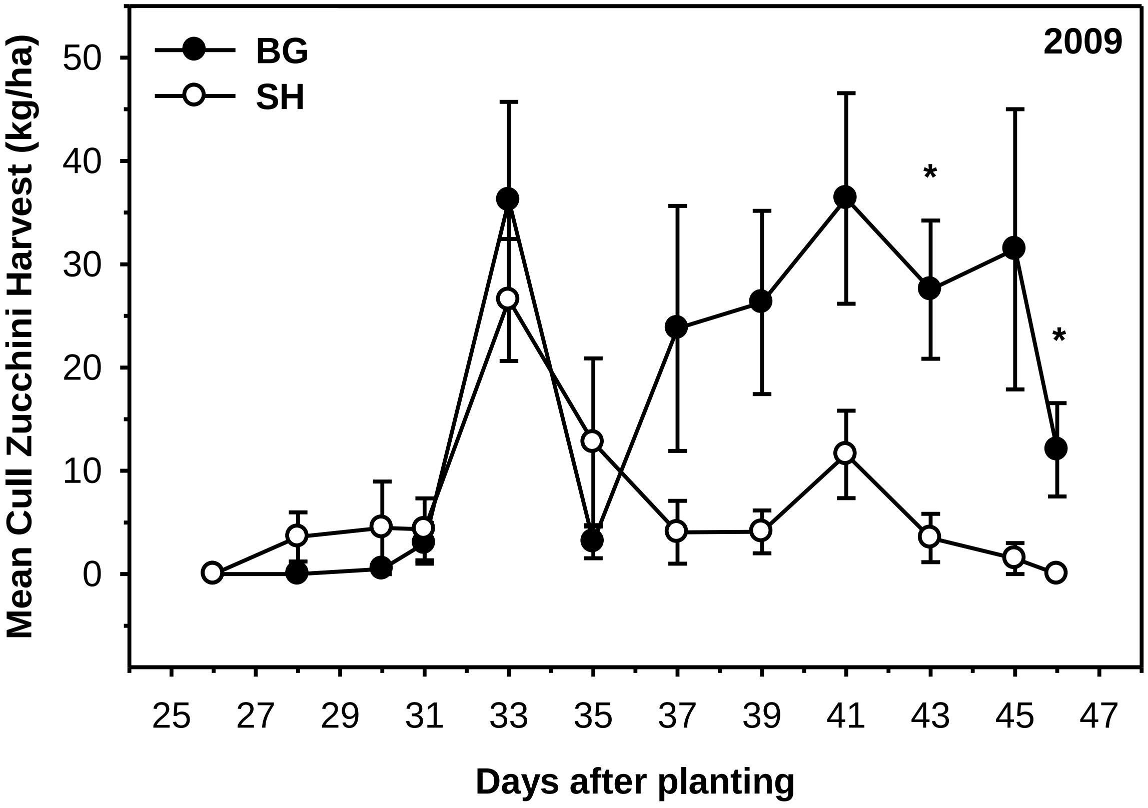
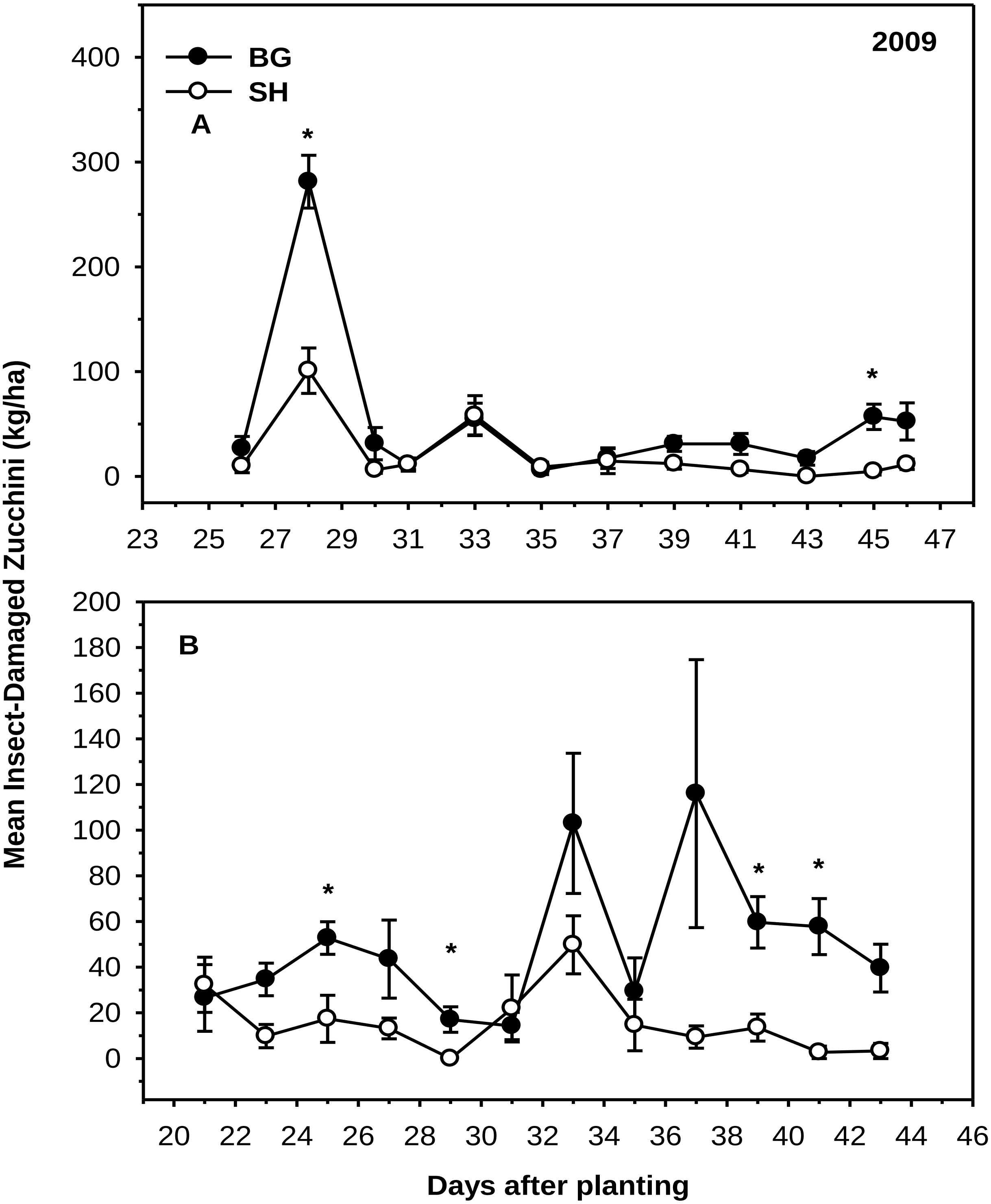


Figure II-9



2009
BG
SH
A
400
300
200
100
0
23
25
27
29
31
33
35
37
39
41
43
45
47
*
*
Mean Insect-Damaged Zucchini (kg/ha)
B
200
180
160
140
120
100
80
60
40
20
0
*
*
*
*
20
22
24
26
28
30
32
34
36
38
40
42
44
46
Days after planting

Figure II-10

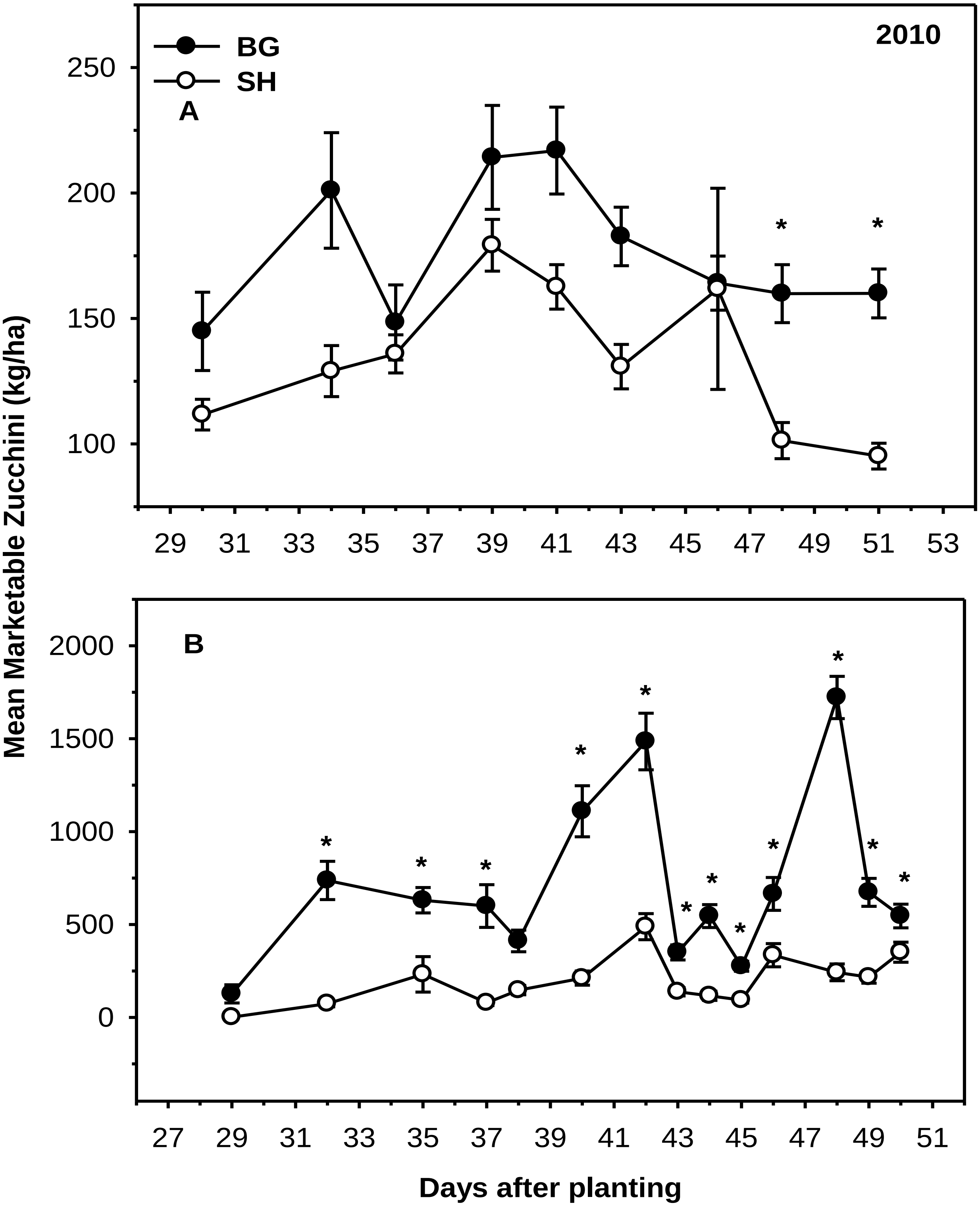

BG
SH
A
2010
250
200
150
100
29
31
33
35
37
39
41
43
45
47
49
51
53
*
*
B
2000
1500
1000
500
0
27
29
31
33
35
37
39
41
43
45
47
49
51
*
*
*
*
*
*
*
*
*
*
*
*
Mean Marketable Zucchini (kg/ha)
Days after planting

Figure II-11

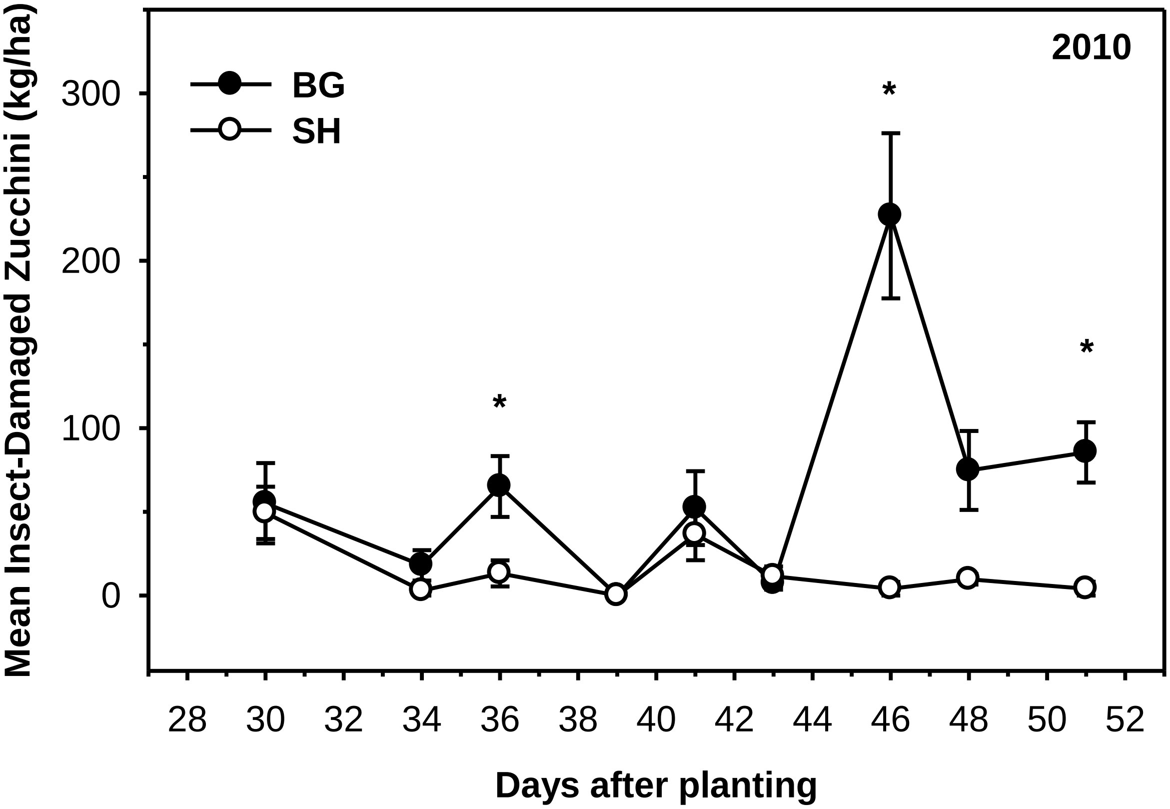

2010
BG
SH
Mean Insect-Damaged Zucchini (kg/ha)
0
100
200
300
*
*
*
28
30
32
34
36
38
40
42
44
46
48
50
52
Days after planting

Figure II-12

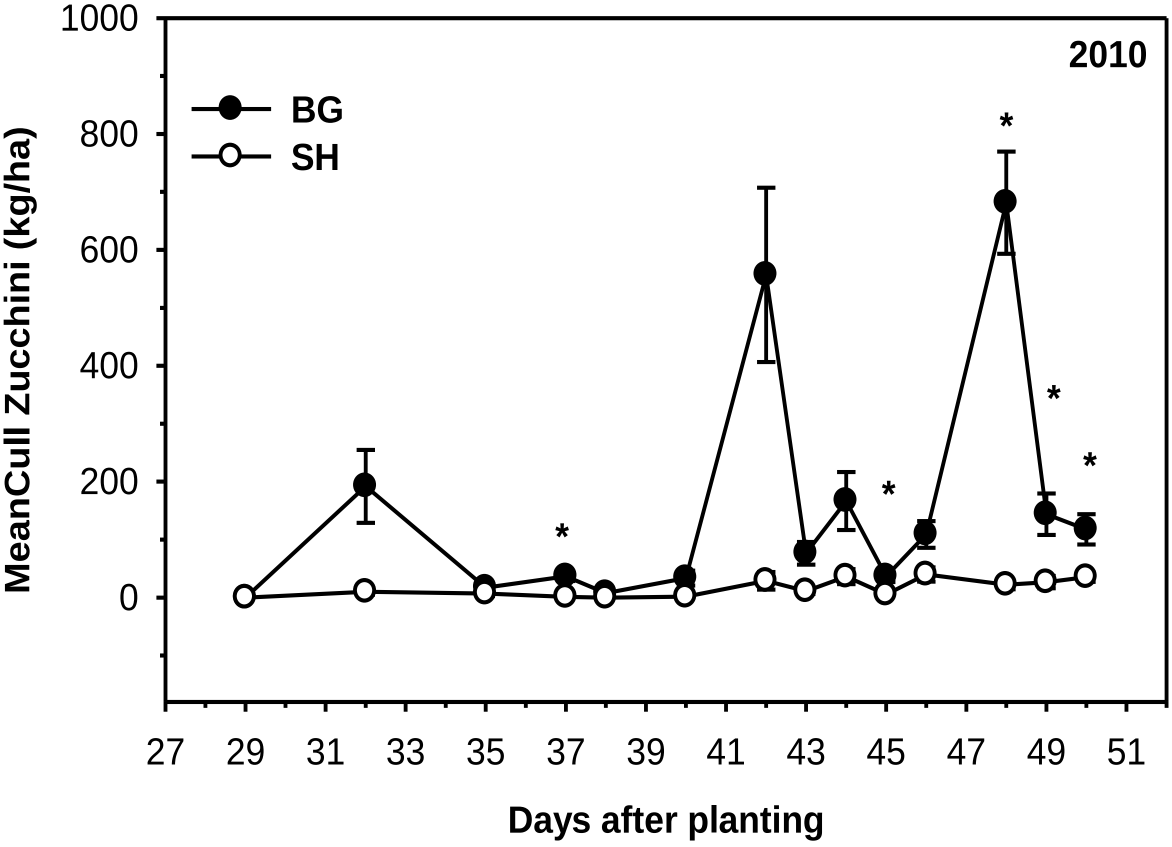
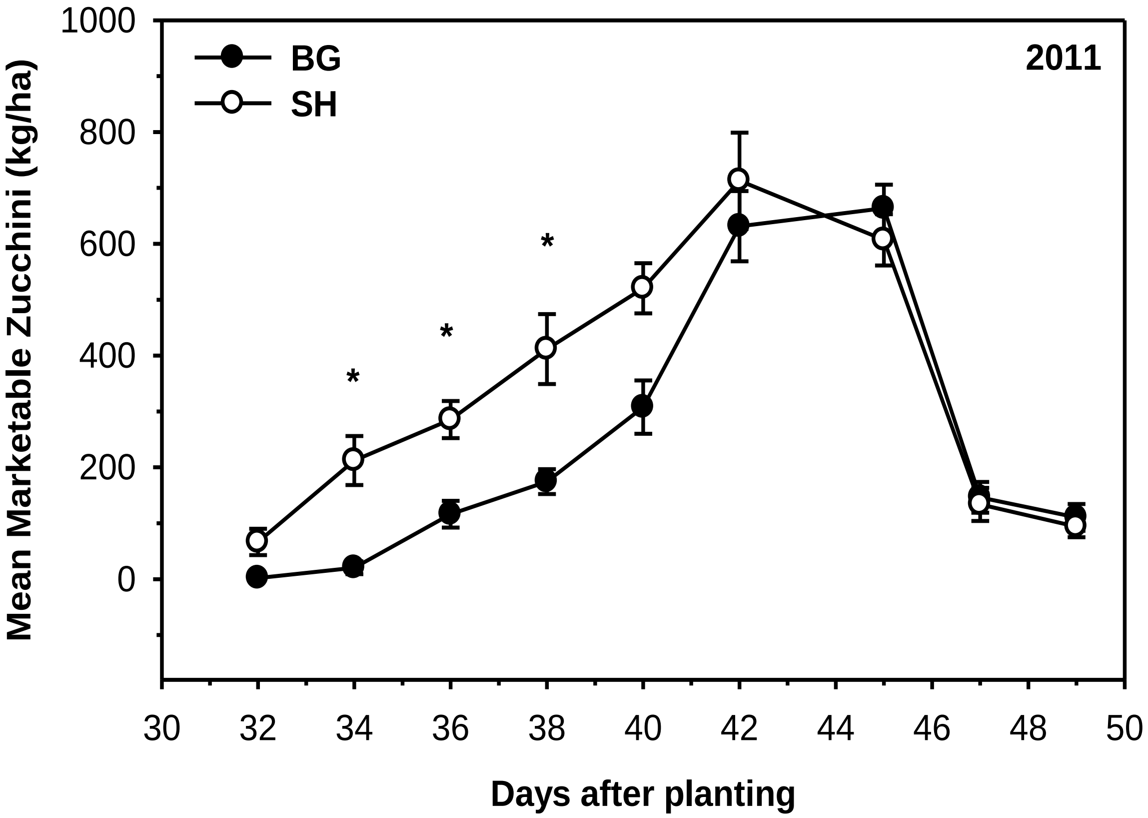

2010
BG
SH
MeanCull Zucchini (kg/ha)
1000
800
600
400
200
0
*
*
*
*
*
27
29
31
33
35
37
39
41
43
45
47
49
51
Days after planting

Figure II-13



1000
800
600
400
200
0
Mean Marketable Zucchini (kg/ha)
BG
SH
2011
*
*
*
30
32
34
36
38
40
42
44
46
48
50
Days after planting

Figure II-14

Tables.

Table II-1. Mean (± SE) dry biomass collected from Queenstown, MD and Upper Marlboro, MD during the 2009, 2010, and 2011 growing seasons. BG represents zucchini planted into bare soil and SH represents zucchini inter-planted into sunn hemp

	Queenstown, MD		Upper Marlboro, MD	
Parameter	Treatment			
	BG	SH	BG	SH
Dry Biomass (g)				
2009	N/A	N/A	476.0 ± 31.3	313.7 ± 24.5 *
2010	63.4 ± 5.1	36.0 ± 2.8 *	213.0 ± 43.8	79.2 ± 5.9 *
2011	143.8 ± 10.8	194.4 ± 12.3	128.5 ± 15.9	158.4 ± 13.6

* Denotes significant difference between treatments ($P< 0.05$)

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Chapter 4: Investigating the influence of sunn hemp (*Crotalaria juncea*) cover cropping and organic fertilizer on soil nematode community structure in a zucchini (*Cucurbita pepo* L.) cropping system

Abstract

Nematode abundance and community structure have long been used as soil health indicators. Previously sunn hemp, or *Crotalaria juncea*, has been shown to improve soil health, reduce plant-parasitic nematodes, and increase nematode-antagonistic organisms. However, these studies have been largely conducted in tropical and subtropical regions. To investigate the impacts of sunn hemp companion planting on nematode community structure and soil health in the Northeastern USA, experiments were conducted in Upper Marlboro, Maryland from 2009 to 2011. Field plots were established to investigate the effect of using sunn hemp concurrently as a living and surface mulch (SH) on the nematode community and compare it to bare-ground (BG) treatment plots. Additionally, organic and synthetic fertilizer treatments were added as subplot treatments to examine their impact on the nematode community. Results showed that sunn hemp, when used as a living and surface mulch did not significantly impact the nematode community. In 2011, when strip-tilled into the soil prior to planting the zucchini, there was an early season increase in the number of bacterivore and fungivore nematodes as well as species richness. However, these effects did not persist towards final zucchini harvest. In contrast, application of organic fertilizer did not improve soil health initially. Toward the end of the zucchini crop organic fertilizer significantly improved all soil health

parameters as indicated by beneficial nematode abundances. Thus, integration of strip-till SH cover cropping and organic fertilizer sustained a healthy soil conditions from early planting towards the end of a zucchini cropping system.

Introduction

Nematodes are widely recognized as nutrient recyclers making up an important component of soil micro-fauna (Ferris et al. 2001). Because free-living nematodes are highly engaged in nutrient cycling and interactions with other soil organisms, their community composition is often indicative of soil health (Ferris et al. 2001, Ferris et al. 2004). Free-living nematodes encompass a variety of functional groups and perform vital ecosystem services within the soil ecosystem. Succession of bactivorous and fungivorous nematodes is important for nutrient cycling of nitrogen and carbon rich organic matter (Ferris et al. 2004). However, plant-parasitic nematodes such as root-knot (*Meloidogyne spp.*) and lesion (*Pratylenchus spp.*) nematodes are economically important pests in agriculture, resulting in retardation of plant growth and yield reductions of 15-35% in wide variety of vegetable crops (Vawdrey and Stirling 1996).

Crop diversification strategies such as cover cropping are often utilized to investigate their impacts on the soil microfaunal community, namely nematodes. Cover crops have been reported to reduce populations of plant-parasitic nematode populations by functioning as poor or non-hosts and producing allelopathic chemicals toxic to nematodes (Wang et al. 2002, Hooks et al. 2010). Integration of cover crops into crop production practices have been shown to improve soil health, cash crop growth, and crop production by enhancing nutrient cycling organisms, reducing plant-parasitic nematodes, and enhancing nematode antagonistic activity (Wang et al. 2001). Ferris et al. (2004) found that implementation of summer and winter cover crops and irrigation increased the abundances of bactivorous and fungivorous nematodes, thus enhancing mineral N available to tomato crop. Sipes and Arakaki (1997) found reduced numbers of the root knot nematode, *Meloidogyne javanica,* when marigold was implemented as a cover crop and incorporated into the soil prior to taro planting. Higher taro yield was also reported in marigold treatments (Sipes and Arakaki 1997). Similar studies suggested suppression of plant- parasitic nematodes by marigold in a variety of other crops (Alexander and Waldenmaier 2002, El-Hamawi et al. 2004, Evenhuis et al. 2004).Wang et al. (2003) found increased numbers of nematode trapping-fungi when pineapple was inter-planted with sunn hump (*Crotalaria juncea*), rapeseed (*Brassica napus*), or marigold (*Tagetes erecta*) compared to bare ground treatment.

The current study is focused on sunn hemp, a cover crop well known for its nematode management properties. Sunn hemp is a leguminous tropical cover crop native to India that is known for its rapid biomass production and ability to fix nitrogen (USDA-NCRS 1999, Marshall 2002, Wang and McSorley 2004). Sunn hemp has been reported to produce as much as 6,725 kg/ha dry biomass and 165 kg/ha nitrogen (Rotor and Joy 1983). Additionally, sunn hemp is often cited for its beneficial impacts on soil borne organisms and ability to suppress plant-parasitic nematodes when incorporated into the soil (Wang et al. 2004, Marahatta et al. 2010, Wang et al. 2011a). Sunn hemp, has been found to suppress plant-parasitic nematodes by functioning as poor or non-hosts, producing nematicidal secondary metabolites, and providing niches for nematode-antagonistic fungi (Wang et al. 2002).

One potential limitation of relying solely on leguminous cover crops to improve soil health is its limited contribution to N. Though cover crops could supply a portion of the N requirements, additional fertilizers are often needed. Since cover crops are well known to enhance soil biological activity, and synthetic fertilizers are known to have a negative impact on soil health organisms (Wang et al. 2006), current research is aimed at investigating the potential of integrating sunn hemp cover cropping with organic fertilizer to further improve soil health conditions.

As referenced above, several studies have demonstrated the plant-parasitic nematode suppressing characteristics of sunn hemp when used as a soil amendment. However, field experiments investigating the influence of sunn hemp on nematodes have been studied largely in tropical and subtropical climates such as Hawaii and Florida (Rich and Rahi 1995, Sipes and Arakaki 1997, McSorley 1999, Wang et al. 2001, Wang et al. 2002, Wang et al. 2004, Wang et al. 2006). Limited research has been conducted to investigate the impact of sunn hemp cover cropping on soil health in temperate regions (Stocking-Gruver 2007). Another goal of this study is to compare the impact of organic and synthetic fertilizer treatments on the nematode community. Thus, objectives of this study included investigating the impacts of using sunn hemp as a cover crop and organic fertilizer on nematode community structure and soil health in a zucchini, *Cucurbita pepo* L. agroecosystem. More specifically, the impact of sunn hemp living mulch on abundances of beneficial nutrient cycling bacterivore and fungivore nematodes, herbivorous plant parasitic nematodes, and omnivorous and predatory nematodes.

Materials and Methods

Field Experiments. To evaluate the impacts of sunn hemp as a living and surface mulch on nematode community structure, field experiments were conducted at the University of Maryland's Central Maryland Research and Education Center (CMREC) in Upper Marlboro, MD during the 2009, 2010, and 2011 growing seasons. *Cucurbita pepo L.*, zucchini (variety Fortune; Seedway; Hall, New York) was used as the cash crop during the 2009 and 2010 growing seasons and zucchini variety Gold Star (Syngenta Inc.) during the 2011 growing season. Sunn hemp cultivar 'Tropic Sun' was used as the intercropped living mulch for each study year. Sunn hemp plots were seeded approximately one month prior to zucchini transplant. Prior to zucchini planting, sunn hemp plants were clipped using hand shears and its foliage allowed to fall in the inter-row spacing between zucchini plants to function as a living and surface mulch.

Experimental Design. The study site consisted of eight plots arranged in a randomized complete block design. Each study year, main plots were replicated four times and consisted of two treatments; zucchini planted into a sunn hemp living mulch (SH) and zucchini planted into bare-ground (BG). Each plot measured 14.6 m x 14.6 m with an inter- and intra-row spacing of 1.2 m. Main plots were split into subplots to compare the potential effects of synthetic ammoniated fertilizer (5-10-10 Southern States) (SF) and organic pelletized chicken manure (7-2-2 Perdue AgriRecycle Seaford, DE) (OF) fertilizers on nematode community. Each year, sunn hemp seeds were seeded at a rate of 44.8 kg/ha and each SH treatment plot consisted of 12 sunn hemp rows so that each row of zucchini was surrounded on either side by a row of sunn hemp. Zucchini transplants were planted between sunn hemp rows. Each treatment plot contained 143 zucchini plants. Synthetic and organic fertilizers were applied to their respective sub plots approximately 28 days after transplanting at rates of 35 and 58 g for synthetic and organic fertilizer, respectively.

Plot Establishment. During the 2009 growing season, SH seeds were planted in SH treatment plots on June 15. SH plants were allowed 6 weeks to accumulate biomass prior to being cut to a height of approximately 45 cm. On July 23, two-week old greenhouse grown zucchini seedlings were transplanted into each treatment plot. In 2010, sunn hemp seeds were sown on June 2 and due to competition with the main crop experienced during 2009, biomass accumulation period of sunn hemp was reduced to 3 weeks during the 2010 growing season. Two-week old zucchini seedlings were transplanted into each treatment plot on June 24.

To examine the cumulative effect of sunn hemp as living mulch over consecutive years, at the end of the 2010 growing season, plots were flagged and re-established in the same locations in 2011. The number of sunn hemp rows established during the 2011 field trial differed from the preceding years in that 24 rows of sunn hemp were planted with an inter-row spacing of 0.61 m. Sunn hemp plants were allowed to grow for 6 weeks before zucchini were transplanted into the treatment plots. Prior to zucchini transplanting, sunn hemp plants were flail mowed to a height of ~ 20 cm. Alternating sunn hemp rows were then strip-tilled and zucchini seedlings were transplanted into tilled strips on July 27. All plots were drip irrigated to mitigate periods of low rainfall. When necessary, plots and border areas were weeded via hoeing, hand weeding and spot applications of glyphosate (Roundup; Monsanto, St. Louis, MO, USA) applied with a backpack sprayer.

Soil Sampling. Nematode collection via soil sampling consisted of 10 randomly gathered soil samples using 2.68 cm diameter soil probes penetrated to a depth of 20 cm in each subplot. Collected samples from subplots were then combined and a subsample of 100 cm^3 was taken. In 2009 soil samples were taken on June 22, August 27, and September 15 corresponding to pre-plot establishment, first zucchini harvest, and final zucchini harvest. In 2010 soil samples were collected on June 25 and August 18, corresponding to date of zucchini transplanting and date of final zucchini harvest. During the 2011 growing season, soil samples were gathered on August 13, September 1, and September 21 corresponding to post-mow, first harvest, and final harvest, respectively.

Nematode Assays. Assays were conducted similar to that described by Wang et al. (2011a). Nematodes were extracted via centrifugal flotation from 100-cm^3 soil (Jenkins 1964). Nematodes were then identified to the genus level, counted and categorized to six trophic groups including bacterivore, fungivore, herbivore, omnivore, algivore and predator (Yeates et al. 1993). Colonizer-persister groups (c-p) were assigned based on (Bongers and Bongers 1998). Soil food web indices were calculated as proposed by Ferris et al. (2001). The maturity index (MI) calculated, is a measure of soil disturbance. Smaller values are indicative of a more disturbed environment while larger values represent less disturbed environments. Enrichment index (EI), based on the responses of opportunistic bacterivore and fungivore colonizers, is a measure of nutrient enrichment of the environment. Higher numbers are indicative of a more nutrient enriched soil. Structure index (SI) is based on the proportion of nematodes belonging to higher trophic groups. Structure index values are indicative of the overall connectedness and stability of soil food webs. Channel index (CI), which is the proportion of fungal feeding nematodes to total opportunistic bacterivores and fungivores describes the dominant decomposition pathway. Low channel index values suggest nitrogen mineralization while high CI values suggest carbon mineralization.

Statistical Analyses. Nematode data were analyzed via an analysis of variance (ANOVA) using (SAS Institute 2002). Data from final harvesting in 2010 and 2011 were subjected to 2 × 2 ANOVA. Prior to analysis, all data were checked for normality using the UNIVARIATE procedure. Parameters that were abnormally distributed were log(x+1) transformed prior to analysis. Means reported are from non-transformed data

Results

During the 2009 growing season, no significant differences existed between SH and BG treatments in terms of nematode abundance in each trophic group except for herbivorous nematodes (Table III-1). During the first zucchini harvest, numbers of herbivores were higher in SH ($P < 0.05$) than in BG, but this effect did not persist to the final harvest.

Among the nematode community indices, SH only increased genera richness compared to BG ($P < 0.10$) by first harvest but this did not persist until final harvest (Table III-2). At final harvest, SH resulted in lower diversity ($P < 0.01$), EI ($P < 0.10$), and higher CI ($P < 0.01$), all of which indicates less healthy soil quality in SH compared to BG.

During 2010, SH plots supported more total nematodes on the day of transplanting. By final harvest, SH plots supported significantly higher abundance of total nematodes. (*P* < 0.01) (Table III-3). This was mainly attributed to higher numbers of bacterivores and fungivores in SH than BG plots at final harvest (*P* < 0.01) (Table III-3). On both sampling occasions, transplant day and final harvest, omnivorous and predatory nematodes were similar in SH and BG plots. However, by the final zucchini harvest, omnivorous nematode abundances were significantly higher in SH than in BG (*P* < 0.10) (Table III-3). At zucchini planting, algivorous nematodes were not significantly different across treatments. However, by final harvest, algivorous nematodes were significantly higher in SH plots (*P* < 0.10) (Table III-3). There were few significant differences between organic and synthetic fertilizer treatments on the nematode community. However, synthetic fertilizer resulted in higher abundances of bactivorous nematodes than organic fertilizer by final zucchini harvest (*P* < 0.10) (Table III-3).

Although at zucchini planting, no differences were detected between SH and BG treatments among all indices (Table III-4), SH treatment resulted in lower diversity and SI at harvest (*P* < 0.05). Fertilizer type did not significantly affect community composition, F/(F+B), genera richness, diversity, EI, SI, or CI.

In 2011, during the post-mow and strip-till sampling occasion, SH supported more total nematodes than BG plots (*P* < 0.01) (Table III-5), mainly due to higher bacterivorous and fungivorous nematodes in SH than BG (*P* < 0.05). The effect of SH on bacterivores was not significant on the subsequent sampling occasion, but fungivorous and omnivorous nematodes were both higher (*P* < 0.05) in SH than BG at final harvest. Although at SH mowing and strip till, synthetic fertilizer plots sustained more nematodes than organic fertilizer plots (*P* < 0.1), numbers of bacterivorous, omnivorous and predatory nematodes were higher in organic fertilizer than synthetic fertilizer plots at final harvest ($P < 0.10$) (Table III-5).

In terms of nematode community indices, SH reduced % omnivores and SI immediately after mowing of sunn hemp, but it increased diversity, and reduced CI at first harvest, while increasing richness at final harvest ($P < 0.10$)(Table III-6). Unlike 2010, fertilizer affected nematode community indices more than cropping type in 2011. Organic fertilizer slightly reduced % bacterivores, but increased % fungivores and F/(F+B) immediately after mowing ($P < 0.10$) (Table III-6). At first harvest, although organic fertilizer decreased diversity ($P < 0.10$), it resulted in decreased % fungivores and F/ (F+B), increased richness, EI, SI, and reduced in CI at final harvest ($P < 0.05$) (Table III-6). Generally, there were no significant interactions between cropping type and fertilizer type except for species richness. Adding organic fertilizer only increased ($P < 0.05$) species richness in BG plots but not in SH plots.

Discussion

The goal of the current study was to investigate the impact of sunn hemp as a living and surface mulch on nematode abundances and community indices. Previous work has demonstrated that sunn hemp, when used as an organic surface mulch and/or incorporated into the soil, can have beneficial impacts on nematode community composition and soil health indices (Sipes and Arakaki 1997, Wang et al. 2001, Wang et al. 2002, Wang et al. 2003, Wang and McSorley 2004, Wang et al. 2004, Wang et al. 2006, Wang et al. 2011a). These impacts include improved nutrient cycling, improved soil health condition, reduction of plant-parasitic nematodes, and increased abundances of nematode-antagonistic organisms. Results from the current study suggest that when sunn hemp is used as surface mulch it has minimum impact on soil health, but when incorporated into the soil it improves nutrient cycling and soil health.

During the 2009 and 2010 field trials, sunn hemp foliage was clipped and allowed to fall onto the soil surface to function as organic surface mulch. Community indices calculated from the 2009 soil samples showed lower diversity and enrichment index, and higher channel index in SH than in BG treatments, which are indicative of a simple, nutrient depleted, and fungal dominated decomposition pathways. This data supported the theory that SH as surface mulch could rob N from fertilizer, resulting in lower nematode diversity, more stressed, and less structured soil characterized by fungal dominated decomposition pathway (Wang et al. 2011a). In 2010, although SH resulted in significantly higher bacterivore, fungivore, algivore and omnivore abundances, which are often indicative of improved nutrient mineralization, this surface mulching did not result in greater soil health according to nematode community indices. Diversity and SI were significantly lower in SH than in BG treatment at harvest, which suggested lower trophic diversity and more disturbed soil food web.

In 2011, all sunn hemp rows were flail mowed and alternating sunn hemp rows were strip-tilled. This practice resulted in an improvement in richness and diversity by final zucchini harvest. When SH foliage is incorporated into the soil, leaf tissue residues may have undergone rapid bacterial decomposition as indicated by higher bacterivore abundance in SH plots by mid-August. Wang et al.(2004) found that nutrient mineralization of sunn hemp foliage peaked two weeks after incorporation. SH stem tissue with a higher C:N ratio stimulated fungivore abundance as indicated by higher fungivore abundances in SH plots. Although there was early season stimulation of bacterivores and fungivores, the effect slowly dissipated and was no longer significant by final harvest. Recently, Wang et al.(2011a) demonstrated that in addition to SH foliage incorporation, routine addition of SH surface mulch resulted in prolonged nutrient enrichment of the soil in a cucurbit cropping system. Improved soil health by SH in 2011 could also be partly due to the consecutive planting of SH in field plots. The organic mulch protocol utilized in 2010 could have built up biological activities, which was indicated by higher abundances of bacterivores, fungivores, omnivores, and algivores found at final harvest in 2010 similarly observed by Wang et al. (2011a).

Current results also suggest that the use of organic fertilizer did not increase soil health initially, but increased nematode diversity, enrichment and structure indices, and reduced channel index towards the end of 2011 trial. Each of which are indicative of a more enriched, structured, and bacteria dominated decomposition soil profile. The benefits associated with organic fertilizer were likely due to the additional organic matter contributed by the pelletized chicken manure fertilizer. Studies have indicated that application of organic matter can sustain higher abundances of bactivorous and fungivorous nematodes than synthetic fertilizer (Ferris et al. 1996, Wasilewska 1998, Wang et al. 2006, Okada and Harada 2007, Sanchez-Moreno et al. 2009). Toward the end of the 2011 study, organic fertilizer subplots were more nutrient enriched (higher EI), less disturbed (higher SI) and less stressful (lower CI) than synthetic subplots. Results of these indices suggest improved soil health resulting from organic fertilizer application similarly seen in Wang and McSorley (2005). Organic fertilizer improved most soil health parameters toward the end of the 2011 growing season whether the zucchini was grown in SH or BG plots except for nematode richness. Organic fertilizer only increased nematode genera richness in BG plots but not in SH plots as SH alone already tended to support higher nematode richness.

Plant-parasitic nematode populations were low at the study sites and there were no significant differences across zucchini habitat or fertilizer types during the 3 year study.

In conclusion, SH did not improve soil health when used as surface mulch in 2009 and 2010 according to the indices. However, when strip-tilled into the soil in 2011, SH provided a short-term improvement in soil health as indicated by the increased abundances of bacterivores and fungivores after strip-tilling, and by season end, nematode trophic diversity and genera richness was enhanced in SH treatment plots. While routine addition of fresh clipped surface mulch may prolong beneficial effects of SH towards the end of the cropping season as found by Wang et al. (2011a), current study indicated that subsequent application of organic fertilizer completes the soil health enhancement towards the end of the cropping season. Findings from this study suggest that strip tilling SH and applying an organic fertilizer may help sustain healthy soil conditions from early season to the end of a zucchini cropping system.

Future studies should be directed towards examining the long term impacts of using sunn hemp concurrently as a green manure and surface mulch with reduced applications of organic fertilizer on soil health qualities.

Appendix III

Table III-**1**. Effects of sunn hemp living mulch on nematode trophic groups at Upper Marlboro, MD during the 2009 growing season. BG represents plots where zucchini was planted into bare-ground. SH represents plots where zucchini was inter-planted in sunn hemp rows.

	Nematode number/100 cm^3 soil	
Trophic group	BG	SH
	On 06/22/2009	
Bacterivores	5808	770
Fungivores	474	$728^{@}$
Herbivores	616	552
Omnivores	42	20
Predators	18	24
Algivores	4	12
Total nematodes	6990	2164
	On 08/27/2009	
Bacterivores	305	321
Fungivores	232	356
Herbivores	73	123*
Omnivores	53	61
Predators	15	15
Algivores	1	6
Total nematodes	685	898
	On 09/15/2009	
Bacterivores	331	264
Fungivores	149	238
Herbivores	109	107
Omnivores	15	20
Predators	14	13
Total nematodes	629	655

Means followed by $^{@}$ and * indicate $P < 0.1$ and <0.05, respectively based on *F*-test results.

Table III-**2**. Effects of sunn hemp living mulch on nematode community indices at Upper Marlboro, MD during the 2009 growing season. BG represents plots where zucchini was planted into bare-ground. SH represents plots where zucchini was inter-planted in sunn hemp rows.

	Nematode Community Indices/100 cm^3 soil	
Indices	BG	SH
	On 6/22/2009	
% Bacterivores	47.11	33.59
% Fungivores	21.46	36.12
% Herbivores	26.64	25.23
% Omnivores	2.21	0.91
% Predators	0.96	1.13
% Algivores	0.19	0.45
F/(F+B)	0.36	0.52
Richness	21	23
Diversity	8.00	7.70
Enrichment index	65.85	61.61
Structure index	49.37	41.47
Channel index	36.91	40.88
	On 08/27/2009	
% Bacterivores	42.33	37.78
% Fungivores	34.30	37.14
% Herbivores	13.26	31.81
% Omnivores	7.03	7.26
% Predators	2.07	1.75
% Algivores	0.22	0.52
F/(F+B)	0.45	0.49
Richness	20	23$^{@}$
Diversity	8.05	9.09
Enrichment index	51.31	55.48
Structure index	49.85	55.30
Channel index	50.34	49.02

Means followed by $^{@}$, * and ** indicate P<0.1, <0.05, and <0.01, respectively based on F-test results.

Table III-**2**. Cont'd.

	Nematode Community Indices/100 cm^3 soil	
Indices	BG	SH
	On 09/15/2009	
% Bacterivores	50.79	40.81
% Fungivores	24.33	35.88
% Herbivores	18.86	16.78
% Omnivores	2.24	2.99
% Predators	1.73	1.81
F/(F+B)	0.33	0.46
Richness	18	18
Diversity	9.12	5.61**
Enrichment index	53.33	41.23$^{@}$
Structure index	51.58	36.97
Channel index	33.71	73.16**

Means followed by $^{@}$, * and ** indicate P<0.1, <0.05, and <0.01, respectively based on F-test results.

Table III-**3**. Effects of sunn hemp living mulch on nematode trophic groups at Upper Marlboro, MD during the 2010 growing season. BG represents plots where zucchini was planted into bare-ground. SH represents plots where zucchini was inter-planted in sunn hemp rows.

	Nematode number/100 cm^3 soil			
	Cover crop		Fertilizer	
Trophic Group	BG	SH	Organic	Synthetic
		On 6/25/2010		
Bacterivores	1047	1250	N/A	N/A
Fungivores	784	937	N/A	N/A
Herbivores	60	115	N/A	N/A
Omnivores	72	99	N/A	N/A
Predators	50	56	N/A	N/A
Algivores	24	21	N/A	N/A
Total	2039	2480	N/A	N/A
		On 8/19/2010		
Bacterivores	646	1094**	801	939$^{@}$
Fungivores	361	719**	512	568
Herbivores	55	57	60	52
Omnivores	48	74$^{@}$	63	59
Predators	31	25	22	34
Algivores	13	23$^{@}$	20	17
Total	1157	1996**	1480	1673

Means followed by $^{@}$ and ** indicate $P<0.1$ and <0.01, respectively based on F-test results.

Table III-**4**. Effects of sunn hemp living mulch on nematode community indices at Upper Marlboro, MD during the 2010 growing season. BG represents plots where zucchini was planted into bare-ground. SH represents plots where zucchini was inter-planted in sunn hemp rows.

	Nematode Community Indices/100 cm^3 soil			
	Cover crop		Fertilizer	
Indices	BG	SH	Organic	Synthetic
			On 6/25/2010	
% Bacterivores	53.38	50.85	N/A	N/A
% Fungivores	36.21	37.16	N/A	N/A
% Herbivores	3.03	4.80	N/A	N/A
% Omnivores	3.62	3.98	N/A	N/A
% Predators	2.39	2.26	N/A	N/A
%Algivores	1.22	0.86	N/A	N/A
F/(F+B)	0.40	0.41	N/A	N/A
Richness	37	36	N/A	N/A
Diversity	9.86	8.19	N/A	N/A
Enrichment index	59.54	60.40	N/A	N/A
Structure index	57.65	47.38	N/A	N/A
Channel index	39.71	38.71	N/A	N/A
			On 8/19/2010	
% Bacterivores	58.28	56.06	55.93	58.41
% Fungivores	29	34.51$^{@}$	32.22	31.29
% Herbivores	4.38	2.96	4.27	3.08
% Omnivores	4.09	3.71	4.40	3.40
% Predators	2.70	1.27	1.53	2.45
% Algivores	1.23	1.23	1.43	1.03
F/(F+B)	0.33	0.37	0.36	0.34
Richness	30	28	29	30
Diversity	10.02	7.56**	8.6	8.97
Enrichment index	43.41	46.35	45.53	44.24
Structure index	51.77	40.27*	49.13	42.91
Channel index	52.65	53.97	54.53	52.09

Means followed by $^{@}$, * and ** indicate significant differences between SH vs. BG or Organic vs. Synthetic fertilizer at $P<0.1$, <0.05 and <0.01, respectively based on *F*-test results.

Table III-**5**. Effects of sunn hemp living mulch on nematode trophic groups at Upper Marlboro, MD during the 2011 growing season. BG represents plots where zucchini was planted into bare-ground. SH represents plots where zucchini was inter-planted in sunn hemp rows.

	Nematode number/100 cm^3 soil			
	Cover crop		Fertilizer	
Trophic group	BG	SH	Organic	Synthetic
	On 8/13/2011			
Bacterivores	427	669*	456	640$^{@}$
Fungivores	222	357*	334	245
Herbivores	20	21	25	16
Omnivores	103	78	81	100
Predators	22	24	18	28
Total	797	1153**	916	1034$^{@}$
	On 9/1/2011			
Bacterivores	361	561	499	423
Fungivores	188	242	225	205
Herbivores	10	8	10	8
Omnivores	34	93	84	43
Predators	17	31	21	27
Total	614	937	843	708
	On 9/21/2011			
Bacterivores	1231	922	1532	621$^{@}$
Fungivores	229	339*	311	257
Herbivores	18	29.5	23	24.5
Omnivores	78	126*	132	72**
Predators	36	59.5	64	31.5$^{@}$
Total	1604	1486	2070	1020$^{@}$

Means followed by $^{@}$, * and ** indicate significant differences between SH vs. BG or Organic vs. Synthetic fertilizer at $P<0.1$, <0.05 and <0.01, respectively based on *F*-test results.

Table III-**6**. Effects of sunn hemp living mulch on nematode community indices at Upper Marlboro, MD during the 2011 growing season. BG represents plots where zucchini was planted into bare-ground. SH represents plots where zucchini was inter-planted in sunn hemp rows.

	Nematode Community Indices/100 cm^3 soil			
	Cover crop		Fertilizer	
Indices	BG	SH	Organic	Synthetic
		On 8/13/2011		
% Bacterivores	52.34	57.64	49.78	60.19$^{@}$
% Fungivores	29.49	31.12	35.92	24.69$^{@}$
% Herbivores	2.33	20.98	2.84	1.58
% Omnivores	12.6	6.53*	9.08	10.05
% Predators	2.85	2.31	2.07	3.1
F/(F+B)	0.36	0.35	0.42	0.29$^{@}$
Richness	18.13	17.63	18.13	17.63
Diversity	8.34	6.27	7.57	7.04
Enrichment index	63.8	65.8	64.08	65.49
Structure index	61.6	50.81*	53.1	59.28
Channel index	29.78	29.35	34.13	25.00
		On 9/1/2011		
% Bacterivores	57.2	61.04	60.11	58.11
% Fungivores	32.62	25.44	27.02	31.04
% Herbivores	1.92	0.90	1.16	1.67
% Omnivores	4.77	9.02	8.49	5.30
% Predators	2.86	3.32	2.64	3.54
F/(F+B)	0.36	0.30	0.31	0.35
Richness	15.5	17.13	16.63	16.00
Diversity	7.30	8.40$^{@}$	7.65	8.04$^{@}$
Enrichment index	58.13	65.93	62.30	61.75
Structure index	54.32	59.42	60.25	53.50
Channel index	41.24	23.04*	30.67	33.61

Means followed by $^{@}$, * and ** indicate significant differences between SH vs. BG or Organic vs. Synthetic fertilizer at *P*<0.1, <0.05 and <0.01, respectively based on *F*-test results.

Table III-**6** Cont'd

	Nematode Community Indices/100 cm^3 soil			
	Cover crop		Fertilizer	
Indices	BG	SH	Organic	Synthetic
			9/21/2011	
% Bacterivores	64.12	62.45	65.80	60.77
% Fungivores	22.16	21.91	19.35	24.73*
% Herbivores	2.167	2.33	1.56	2.94
% Omnivores	7.28	8.28	8.53	7.04
% Predators	3.087	4.37	4.31	3.16
F/(F+B)	0.26	0.26	0.23	0.29*
Richness	18.63	22.00*	21.63	19.00*
Diversity	7.00	8.06	7.10	8.00
Enrichment index	69.27	67.33	75.26	61.33*
Structure index	59.80	61.86	65.06	56.60*
Channel index	22.61	20.90	15.22	28.30*

Means followed by $^{@}$, * and ** indicate significant differences between SH vs. BG or Organic vs. Synthetic fertilizer at $P<0.1$, <0.05 and <0.01, respectively based on *F*-test results.

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