ARTHROPOD BIODIVERSITY IN A GEORGIA COTTON AGROECOSYSTEM: THE ROLE OF BT COTTON, TILLAGE, COVER CROPS AND RED IMPORTED FIRE ANTS

by

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(Under the Direction of John Ruberson)

Genetically modified (GM) crops and invasive species have been extensively studied in the field of agroecology, particularly for their effects on biodiversity. While studies examining GM crops have found very little evidence that these crops negatively affect arthropod communities, they have not taken into account the various agricultural management strategies within which they are nested.

Due to the frequent disturbance inherent in agricultural production, invasive species are common members of arthropod communities in agroecosystems. Invasives can have both positive and negative impacts on arthropod communities within these systems; however, there are many areas in which their influence has not been adequately assessed.

In the chapters that follow I first examine the effects of genetically modified, Bt cotton on non-target arthropod communities relative to and in combination with the effects of tillage and cover crop identity. While Bt cotton appeared to have subtle effects on a few taxa, they were minor and inconsistent compared to and in combination with the effects of tillage and cover crop type.

Next I assess the impact of the red imported fire ant *Solenopsis invicta* on arthropods both at and below the soil surface. The removal of fire ants significantly altered the abundance of certain arthropod families. Many of the same trophic groups were affected at two field sites, but the abundance of these groups did not always respond in the same direction. Potential mechanisms leading to the differences detected between field sites, including weed density and arthropod species-specific responses, are further discussed.

Finally, I examine the contribution of the red imported fire ant, *Solenopsis invicta* on egg predation rates in a cotton agroecosystem. Fire ants contributed greatly to predation of eggs of the beet armyworm, *Spodoptera exigua*, both on cotton foliage and at the soil surface. While fire ants also contributed significantly to predation of eggs of the southern green stink bug, *Nezara viridula*, in cotton foliage, they ignored eggs of the redbanded stink bug *Piezodorus guildinii.* This study also suggests that whitefly densities on cotton foliage may significantly influence egg removal rates of *N. viridula* eggs by fire ants.

KEY WORDS: *Bacillus thuringiensis,* tillage, cover crops, *Solenopsis invicta*, cotton, arthropods, soil ecology, egg predation

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Agricultural systems support diverse communities of arthropods whose populations are influenced by multiple factors. While variation in geography and climate influence the overall patterns of arthropod communities and dictate the range of crops that may be grown in a given region, subtle variations in agricultural management within each region can also have dramatic effects on these communities.

The most fundamental decision a grower makes is which crop to produce, and more specifically, which genotype to plant. Crop plants vary widely in their characteristics and the decision of which crop species and variety to plant determines in large measure the community of arthropods that will be present in a given area. The advent of genetically-engineered crops can further significantly modify community structure. Genetically-modified pesticide-incorporated plants, engineered to express a defense against herbivory, are preferentially planted in several major agricultural countries. Their remarkably rapid adoption has been facilitated by their capacity to increase crop yield while decreasing insecticide costs (Cattaneo *et al.* 2006; Morse *et al.* 2006; Russell & Deguine 2006); however, this novel technology has the potential to exert a variety of non-target ecological effects. While much is known about the impact of genetically-modified crops on non-pest arthropods, many questions remain (O'Callaghan *et al.* 2005).

The impact of specific crops on arthropod communities is further affected by the cropping practices of the overall production system in which the crop plants are embedded. For instance, within the cotton-growing region of the United States, a variety of soil management strategies occur, including conservation tillage and the use of specific cover crops. Fifty percent of agricultural land devoted to cotton production in the state of Georgia was under conservation tillage during 2007 (Karlen and Doran 2001; UGA-CAES 2007). Farmers in Georgia also utilize a variety of cool- and warm-season cover crops, including wheat, rye, hairy vetch and crimson clover (UGA-CAES 2007). Although each of these factors alone can play a significant role in shaping arthropod communities, the interactive importance of these variables is poorly understood. Co-occurring factors, such as tillage strategy and crop type, may interact with one another to produce effects different from those generated when the factors are studied in isolation.

Invasive species can also play a significant role in shaping arthropod communities in agricultural systems. While, by definition, their impact is generally negative (e.g. pests and intraguild predators such as the soybean aphid and Asian ladybeetle), successful invasive species can also have positive effects on biological communities (e.g. pest control by the red imported fire ant). In some cases, these taxa may even become such a part of the invaded habitat that they become keystone species (Nunez & Simberloff 2005). Often, however, the full extent to which invasive species interact with “native” communities is not known.

This chapter summarizes our current understanding of the impact of four factors common to agricultural systems of the Southeastern United States on beneficial and non-target arthropod communities. I first present what is known about the impact of Bt-transgenic cotton on non-target arthropods. Second, I consider the impact of tillage on arthropod communities both above- and below-ground. Third, I review the current understanding of the effects of various cover crops on arthropods. Fourth, I present what is known about the effects of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), on arthropod communities in agricultural systems both above- and below-ground. I conclude by presenting the objectives of the research conducted for the purpose of this dissertation and list the predictions and hypotheses accompanying each element of the project.

THE EFFECTS OF BT COTTON ON NON-TARGET ARTHROPODS

The effects of genetically modified crops on non-target organisms have been the topic of much recent research and a number of review papers on the subject, particularly in the case of crops modified to express toxins of the bacterium *Bacillus thuringiensis* Berliner (Bt) (O'Callaghan *et al.* 2005). These reviews span roughly 10 years of literature (1995, one year before the commercial release of Bt crops, to 2005) and report the results from experiments conducted on cotton, corn, potato, oil-seed rape and rice expressing an array of Bt endotoxins. The potential effects of these crops on non-target arthropods include toxicity of pest species to their natural enemies due to secondary ingestion of the Bt endotoxin, loss of prey/hosts for predators/parasitoids, and decreased prey/host suitability due to endotoxin effects. Although each review covers a very different body of research they come to the same general conclusions: first, the effects of Bt crops on non-target arthropods are generally insignificant but when present, the effects are both subtle and mixed; and second, in cases where Bt crops do exert an effect on non-target organisms this effect is most likely mediated by indirect factors, such as insecticide use in non-Bt fields or a decrease in prey or host suitability for predators or parasitoids, rather than directly via the Bt endotoxin (Head *et al.* 2005; Romeis *et al.* 2006).

Field Studies

Since 2005 there have been a number of field experiments examining the effects of Bt cotton on a wide variety of non-target arthropods. In agreement with previous work, these studies confirm that the effects of Bt cotton on non-target pests and predators are either insignificant or mixed (e.g., Torres & Ruberson 2005; Cattaneo *et al.* 2006). In cases where significant effects are noted they generally agree with past findings and attribute effects to insecticide use in non-Bt cotton plots (Torres & Ruberson 2005; Mellet & Schoeman 2007; Sharma *et al.* 2007). There are however, some exceptions to this trend. For example, Parajulee *et al.* (2006) found higher abundances of cotton fleahoppers, *Pseudatomoscelis seriatus* Reuter (Hemiptera: Miridae), in non-Bt than in Bt cotton. Whitehouse *et al.* (2005) also noted a decrease in the abundance of fruitflies (Diptera: Drosophilidae), grass flies (Diptera: Chloropidae), damsel bugs (Hemiptera: Nabidae) and jassids (Hemiptera: Cicadellidae) in Bt compared to conventional cotton. Naranjo (2005) also detected a decrease in the abundance of the western damsel bug, *Nabis alternatus* Parshley, as well as in the convergent lady beetle *Hippodamia convergens* Guérin-Méneville, and spiders (excluding the families Dictynidae, Thomisidae and Salticidae) in Bt compared to non-Bt cotton. In all of the above cases, the effects of Bt cotton were entirely independent of insecticide use, and mechanisms underlying this change are unknown.

Laboratory Studies

Similarly, there have been a handful of new laboratory studies investigating the impact of Bt cotton on parasitoids and predators. Although laboratory studies have revealed significant effects of the Bt entodoxin from cotton on parasitoids and predators, the effects appear to be mixed and highly taxon-specific. For example, the longevity, number of eggs parasitized, emergence rate and sex ratio of the parasitoid wasp *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) were not significantly affected when parasitoids were fed pollen from cotton plants genetically modified to express Cry1Ac protein (although the dosage of the toxin in the pollen was never verified) (Geng *et al.* 2006). The opposite was observed for the ichneumonid parasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae), as egg and larval growth stages were prolonged when *C. chlorideae* was reared on Bt-fed host larvae of *Helicoverpa armigera* (Sharma *et al.* 2007). Significant effects were also noted for the aphid predator *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae), as the percentage of mated to unmated female ladybeetles was lower when beetles were fed cotton aphids reared on Bt cotton than when fed aphids reared on non-Bt cotton (Zhang *et al.* 2006).

Non-target Effects in Soil Food Webs

The potential effects of Bt crops on soil food webs due to ingestion of either decomposing transgenic plant material or root-exuded endotoxins have also been summarized in recent reviews (O'Callaghan *et al* 2005). While the authors note that Bt crops have not been found to influence soil fauna -- including microbes, meso- and macro-invertebrates -- they suggest that failure to find any effect may be due largely to the inherent difficulty in studying soil systems.

A handful of recent studies continue to explore the impact of Bt crops on soil biotic communities with mixed results. Contrary to the general findings noted in the review by O'Callaghan *et al* (2005), Knox *et al.* (2007) report that the Bt endotoxin is exuded from cotton seedling roots (from 10 varieties) in the form of mucilage and sloughed border cells. Other cotton root exudates, such as amino acids and soluble sugars, have also been found to differ significantly between Bt and non-Bt cotton (Yan *et al.* 2007). Under such differences one might expect to find significant trophic shifts within the soil food web, but studies exploring this area have generated mixed results. For instance, Shen *et al.* (2006) found no effects of Bt cotton on soil enzyme activity or microbial functional group, while Sun *et al.* (2007) found that 4 out of 5 enzymes increased in the rhizosphere of Bt cotton. In the case of detritivores, the effects of Bt cotton remain poorly studied. There is evidence that decomposing plant material of many Bt crops degrades more slowly than that of conventional crops (Flores S. *et al.* 2005). The rhizosphere of Bt crops has also proven to differ significantly from that of conventional crops (Saxena *et al.* 2004). There is little evidence however that any differences in detrital or rhizosphere characteristics based on the Bt toxin lead to differences in non-target soil invertebrates (Oliveira *et al.* 2007).

In a review by Romeis *et al* (2006) the authors recommend a tiered approach to assessing the risk of Bt crops, involving a series of questions to ascertain the level of interaction between the Bt endotoxin and the organism (host/prey/predator/parasitoid). Although the authors were not examining studies of Bt crop effects in soil food webs, this step-wise approach may prove very appropriate in soil systems given their complex trophic and spatial structuring.

In summary, the most recent studies investigating the potential effects of Bt crops on the non-target biota generally agree with past research suggesting that in cases where Bt crops have a significant impact on non-targets, these effects are subtle and mixed. The mixed nature of the effects continues to be attributed to variation in management strategies among study sites, suggesting that it may be useful to investigate the impact of these crops in combination with, and relative to, a variety of management techniques.

THE IMPACT OF TILLAGE ON PEST AND BENEFICIAL ARTHROPODS

Soil tillage was widely adopted in the United States in the mid-1800's with the growing popularity of the iron plow and this shift facilitated agriculture on a massive commercial scale (Coughenour & Chamala 2000). Concern over the loss of topsoil due to erosion, and more recent interest in soil carbon sequestration have sparked interest in sustainable soil management practices that decrease the intensity of tillage (Lal R. 2002). Conservation tillage includes less intense forms of soil cultivation including strip and no-tillage, both of which have been intensively studied, most commonly for their impacts on surface and soil biota. A large body of literature, including a handful of review papers, highlights two main pathways by which arthropod biodiversity may be increased under reduced tillage strategies (House & Alzugaray 1989; Wardle D.A. 1995). The first is an increase in habitat complexity due to an accumulation of plant residue at the soil surface, and the second is maintenance of soil structure as well as intact rhizospheres, both of which allow for the development of more complex food webs at and below the soil surface (Finke & Denno 2002). However, reducing or eliminating tillage can have variable effects on both pest and beneficial arthropods (Chilcutt & Matocha 2007).

Soil surface effects

Increasing plant litter biomass at the soil surface has been shown to decrease antagonism among predators and subsequently increase their impact on prey populations (Finke & Denno 2002). Many studies have investigated the impact of conservation tillage at the soil surface and, in general, it has been found to increase the overall abundance of arthropods (Cederbaum *et al.* 2004). Specifically, the abundance of certain predators including carabid and staphylinid beetles as well as many families of spiders and parasitoids have been found to increase under no-till soil management (House & Alzugaray 1989; Stinner & House 1990; Marasas M.E. 2001). The abundance of predatory larvae has also been found to increase significantly under conservation tillage (Holland 2004). Richness and diversity of beneficial arthropods have also been found to increase under no-till management (Blumberg & Crossley 1983; House and Alzugaray 1989). Although Hatten *et al.* (2007) found similar increases in richness and diversity of carabid beetles under no-till management, the responses of individual carabid species were highly mixed.

A decrease in tillage intensity can also enhance populations of pest arthropods, including thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), cotton fleahoppers *Pseudatomoscelis seriatus* Reuter (Hemiptera: Miridae), crickets of the genus *Gryllus* (Orthoptera: Gryllidae), planthoppers and leafhoppers (Hemiptera: Delphacidae and Cicadellidae, respectively), as well as the aphid *Rhopalosiphum padi* L. (Hemiptera: Aphididae) (Veazey *et al.* 1976; Marasas *et al.* 2001; Ishijima *et al.* 2004). A review paper by Stinner & House (1990) noted highly mixed effects of tillage intensity on arthropod pests, with 43% exhibiting a decrease in abundance with decreasing tillage intensity, 28% showing an increase, and 28% exhibiting no effect. The authors pointed out that in the majority of cases in which decreased tillage intensity had a positive effect on arthropod pests; this effect was often indirectly mediated by a higher weed biomass in conservation tillage systems. For example, the plant bug *Leptopterna dolabrata* L. (Hemiptera: Miridae) was found to be more abundant in no-till plots with high weed density than in conventionally tilled plots (Andersen 1999). Stinner *et al.* (1984) demonstrated that both oviposition and damage by the stalk borer *Papaipema nebris* Gn. (Lepidoptera: Noctuidae) were positively correlated with grass density in Ohio corn systems. Both of these studies, however, also reported positive correlations between the abundance of predators, including spiders as well as carabid and staphylinid beetles, and weed density. Andersen (1999) further noted a significant variability in the response of different species of carabid beetles to tillage intensity, with some preferring weedy, no-till soils and others preferring tilled plots with bare soil. Many arthropods (e.g., crickets, ants and some carabid species) preferentially feed on seeds and have been found in higher abundances in no-till than in tilled fields and have been found to make a significant contribution to the destruction of weed seeds in these systems (Brust & House 1988). The net outcome of these interactions (high weed density, increased pests and predators, increased seed feeders) on pest management and crop productivity in no-till systems is not known.

Sub-surface effects

Tillage is known to have many effects on the quality of soil as a habitat. Along with the soil mixing that occurs during tillage, tillage also alters soil structure at a much finer scale, decreasing soil organic carbon and disrupting soil water-stable-aggregates (Kisselle *et al.* 2001; Six *et al.* 2004). Six *et al.* (2004) demonstrated that a no-till soil management strategy can lead to the protection and sequestration of carbon in such aggregates. Soil aggregates, bound together by various plant residues and fungal hyphae are considered one of the spheres of biological activity within soils and their disruption, following tillage, is likely one of many factors influencing below-ground arthropod communities in agricultural systems (Lavelle 2002)**.** Microbial populations are also hypothesized to shift between bacterially-dominated communities under conventional tillage to fungal-dominated communities under conservation tillage (Hendrix *et al.* 1987). Evidence suggests that overall microbial biomass does in fact increase under conservation tillage strategies and that increased crop residue at the soil surface can foster high fungal biomass; however, shifts between fungal and bacterial communities within the soil matrix are not commonly observed (Hendrix *et al*. 1987; Wardle 1995). Free-living nematodes, which serve as potential prey for many soil arthropods, have also been found to increase in abundance under no-till systems, possibly in response to increased microbial biomass (Fu *et al.* 2000; Fu *et al.* 2001).

Although studies have demonstrated increases in soil microarthropod abundance under conservation tillage, the impact of tillage on individual arthropod groups is highly taxon-specific (Stinner & House 1990; Garrett *et al.* 2001). While collembolan abundance commonly increases under conservation tillage, the response of mites is mixed. A review by Wardle (1995) revealed that while mites of the suborder Mesostigmata are often reduced by tillage, those of the suborder Prostigmata can be either inhibited or stimulated depending upon the species in question. Species richness of oribatid mites often decreases under cultivation and this change is attributed to the long generation times (often over 1 year) of many oribatid species (Crossley *et al.* 1992).

Many important arthropod pests inhabit soil during at least a portion of their lives. One of the main benefits provided by tillage is the disruption of pests by mechanical damage during plowing. Results, however, appear to be mixed on the ability of soil-dwelling pests to survive tillage events. For example, while the abundance of Japanese beetle larvae, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is known to be negatively impacted by tillage, other larval taxa such as the seedcorn maggot, *Delia platura* Meigen (Diptera: Anthomyiidae), appear to benefit from tillage (Szendrei *et al.* 2005).

In general a decrease in the intensity of soil tillage appears to increase the abundance, richness, and diversity of many arthropods including predators, parasitoids, detritivores and, in some cases, pests. The overall influence of tillage on pest control is less well understood. Although many pest species are known to decrease after soil cultivation, the co-occurring decrease in predators and parasitoids may outweigh any of the direct benefits gained by tillage. For example, conservation tillage can enhance pest egg predation rates in some systems (House and Alzugaray 1989). But as noted above, while the abundance of beneficial species typically increases with a decrease in tillage intensity, the effects on pests are mixed and both direct (due to soil cultivation alone) and indirect effects (mediated by shifts in predators and parasitoids) should be examined on a case-by-case basis.

The negative correlation between tillage intensity and weed density/diversity, mentioned above, is also worth noting in relation to below-ground systems. Numerous studies have determined that altering the diversity of primary producers subsequently alters the diversity of carbon substrate or plant residue entering the soil system (Hooper *et al.* 2000). Although research investigating the link between plant/detrital resource diversity and below-ground communities and processes has generated highly mixed results, there does appear to be a consistent effect of plant functional type (Diaz & Cabido 2001). Given that many functional types can co-occur within a single weed community the link between weed community composition and detrital food webs may be strong.

THE EFFECT OF COVER CROPS ON ARTHROPOD BIODIVERSITY

Cover crops are often used in combination with conservation tillage and they have been found to offer similar benefits. Bugg & Waddington (1994) cite multiple authors who have reported reduced soil erosion and increases in both soil moisture and nitrogen. In contrast to what is known about the effects of tillage on above- and below-ground arthropods, few generalizations can be made about the impact of cover crops. While the use of cover crops can lead to higher abundance of beneficial arthropods when compared to conventionally managed soils (Phatak 1998), there exists a great deal of variation in the effect of different cover crop types on both pest and beneficial species. House and Alzugaray (1989) detected significant effects of clover, vetch and wheat winter covers on every trophic group; however, these effects were temporary and interacted highly with sampling date. Similarly, Bugg & Dutcher (1989) detected significant but variable responses of many taxa, including aphids and aphidophagous predators, to cover crop type in pecan orchards of southern Georgia. There is evidence suggesting that much of this variation may be caused by changes in cover crop phenology as growing seasons progress. For instance, Tremelling *et al.* (2003) found that Hemiptera and Hymenoptera preferred a clover cover crop late in flowering. Variation aside, there are patterns recognized across a handful of studies suggesting that leguminous cover crops may foster more abundant arthropod communities than graminaceous covers (Tremelling *et al.* 2003; Cederbaum *et al.* 2004; Tillman *et al.* 2004). There is also evidence that cover crop type significantly affects detritivorous arthropods based on the quality of each crop once it becomes detrital residue. For example, House and Alzugaray (1989) found an increase in Collembola in clover residue but not under residues of wheat or vetch. Leguminous cover crops can also foster more abundant pest populations. For example, crimson clover can support both higher numbers and higher survival rates of the tarnished plant bug, *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae), a common pest of cotton (Bugg *et al.* 1990). Leguminous cover crops are also of particular interest for their role in soil N retention. This has sparked interest in subterranean clover, *Trifolium subterraneum* L., because it retains its ability to fix soil nitrogen but lacks features, such as extrafloral nectaries, that may foster higher pest populations (Bugg et al. 1990).

Cover crops are suggested to serve as an overlap/transition habitat in many systems, offering non-pest prey for predators and parasitoids when crop pests are not available (Bugg and Waddington 1994; Tillman et al. 2004). Studies have also determined that cover crop type can influence the efficiency of predators within crops. For instance, Bugg *et al.* (1991) found that a clover cover crop fostered higher abundance of and predation by the big-eyed bug *Geocoris punctipes* Say (Hemiptera: Geocoridae) within the cantaloupe crop that followed. This is not always the case as some research demonstrates that the arthropod communities within cover crops may differ greatly from those of the crop plants in which they are planted, suggesting that arthropods do not move between the two systems (Bugg and Dutcher 1989).

As a whole, cover crops alone can benefit arthropods but we are far from a clear understanding of all the pathways by which cover crops influence various taxa. This line of investigation has provided very little insight into the biological control of pests or the efficacy of specific predators (e.g., tarnished plant bug, big-eyed bug) and much work remains to be done.

THE ROLE OF THE RED IMPORTED FIRE ANT IN AGROECOSYSTEMS

Ants have been studied extensively for their roles as both pests and predators in agricultural systems (Vinson 1985; Morrison *et al.* 1997; Philpott *et al.* 2006). Since their arrival in the 1930's, red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), have spread across the Southeastern United States and quickly earned the status of agricultural pest. However, the status of fire ants as pests has proven difficult to gauge, with reports including everything from farm equipment damage to hospitalization of field workers and the death of newborn livestock (Lofgren 1986). The most consistent adverse impact of fire ants as agricultural pests deals with their tendency to damage a variety of crop seeds including wheat, corn, sorghum and soybean (Lofgren 1986; Morrison *et al.* 1997).

Fire ants are also known to be opportunistic predators in above-ground systems (Tschinkel 2006). While they have been found to directly increase predation in terrestrial systems by 20 to 30%, they are also known to decrease the abundance of beneficial predators (Lofgren 1986; Eubanks *et al.* 2002; Diaz *et al.* 2004). However, this impact typically varies at higher levels of taxonomic resolution. For example, while the abundance of coccinellid beetles and lacewings have been found to decrease, spiders often show no response to the presence of fire ants (Eubanks *et al.* 2002). The impact of fire ants on agricultural pests is similarly mixed. For instance, while lepidopteran abundance has been found to decrease in the presence of *S. invicta*, aphid abundance increases due to the mutualistic relationship involving aphid honeydew and protection by *S. invicta* (Eubanks 2001; Diaz *et al.* 2004). Further, the mutualism between *S. invicta* and aphids has been found to enhance the strength of the negative effect of fire ants on lepidopteran larvae (Kaplan & Eubanks 2002; 2005). This impact has been found to result in an indirect, positive effect on plant fitness in over 73% percent of studies examined (Styrsky & Eubanks 2007). These interactions, both positive and negative, have made it even more difficult to understand the overall impact of *S. invicta* on agroecosystems.

Below-ground, ants have primarily been studied for their role as ecosystem engineers and most studies reveal that mound building and maintenance activities increase soil moisture, phosphorus and potassium, while decreasing soil bulk density (Dostal *et al.* 2005; Boulton & Amberman 2006). These localized changes to the soil habitat are suspected to be a contributing factor to the increased diversity of biota that inhabit ant mounds (Hölldobler & Wilson 1990; Laakso & Setälä 1998); Boulton & Amberman 2006; Wagner *et al.* 1997).

Research on the red imported fire ant has generated similar results, as they have been found to alter soil aggregate structure and increase water infiltration (Green *et al.* 1999), alter soil nutrients, increase soil organic matter and decrease soil bulk density (Lafleur B. 2005). Fire ant mounds are also known to increase the abundance of multiple taxa, including fungi and many arthropods, within fire ant mounds when compared to adjacent soil (Wojcik 1990; Lafleur 2005).

There is also evidence that ants function as predators in soil systems. For example, Wilson (2005) observed ants in the genus *Pheidole* specializing on a selective range of mite species in the suborder Oribatida. Yet other studies have found ants to have little to no effect on soil fauna (Lenoir *et al.* 2003). The authors suggest that this may be due to the high degree of heterogeneity in the abundance and composition of soil fauna.

In North America, the red imported fire ant is an exotic arthropod. It is a highly efficient forager and functions largely as a scavenger and generalist predator. Accordingly, the most abundant prey available will often become the prey of choice (Tschinkel 2006). These traits, combined with their high abundance in disturbed agricultural soils, suggest that, unlike other ants, fire ants may have a significant predatory effect on the soil arthropod community in agricultural systems. However, trophic interactions between *S. invicta* and other soil fauna outside of ant mounds have rarely been studied. Resource heterogeneity and quality at the soil surface may influence the intensity with which fire ants defend that resource. For instance, Vinson (1991) assessed predation events and trophic interactions between detritivores and *S. invicta* using pieces of rotting fruit as bait stations and determined that in the presence of *S. invicta*, the abundance of fly larvae, sap beetles and rove beetles, and parasitic wasps decreased significantly. Vinson's findings suggest that fire ants aggressively defend high quality food sources. This bears a strong resemblance to the manner in which fire ants have been found to defend aphids on plant foliage in exchange for honeydew, another high quality resource. Such studies may be a poor indication of how fire ants interact within soil food webs where resources are often homogeneously distributed. Morrison & Porter (2003) surveyed soil surface arthropods over varying densities of fire ants in a Florida pasture and found a positive correlation between the density of *S. invicta* and morphospecies richness of non-ant arthropods. These findings are contradictory to those of (Porter & Savignano 1990) who observed a significant decrease in the abundance and richness in many arthropods (75 and 30% decrease, respectively) in a Texas mixed forest and grassland invaded by *S. invicta* compared to areas in which invasion had not yet occurred. In some cases, specific groups such as the predatory mite family Erythraeidae completely disappeared in invaded areas. In two studies, predation efficiencies have also been examined in systems invaded by *S. invicta* using insect egg masses (Lepidoptera and Diptera), and in both cases *S. invicta* contributed more to egg removal rates than any other predator in the system (Lee *et al.* 1994; Nuessly & Sterling 1994). These studies, however, relied on observations of predation events and were unable to determine whether or not fire ants significantly altered the activity of other predators in the system.

As a whole, conclusions on the impact of *Solenopsis invicta* in soil systems remains mixed. While it is well established that they significantly affect soil properties and increase local diversity within their mounds, the effects of fire ants on soil communities outside of these mounds, at the landscape-level, remain a mystery. It is obvious that they contribute greatly to egg predation at the soil surface and may contribute greatly to the control of pests; however, there does not appear to be a consensus on how fire ants interact with non-inquiline soil arthropods.

CURRENT STUDY SYSTEM

Cotton, peanuts, corn, soybeans and tobacco are the major crops grown in the state of Georgia, USA. Of these, cotton has the largest number of acres under agricultural production with 1,010,000 acres planted in 2007 (GA-Department-of-Agriculture 2007). 90% of this acreage was planted with Bt cotton while 50% of all cotton acreage was under conservation tillage, utilizing a variety of winter cover crops during this same year (Karlen and Doran 2001; UGA-CAES 2007). Each of the above factors can independently have significant effects on arthropod communities. The effects of Bt cotton, however, have rarely been examined under varying management strategies (e.g., conservation and conventional tillage with varying cover crop identity).

The first report of red imported fire ants found in Georgia dates back to the early 1950's and their populations can now be found across the entire state (Canerday 1988). While much is known about the above-ground impact of these generalist predators in agricultural systems, their effects have been understudied in soil food webs.

In the chapters that follow I explore the following hypotheses:

Chapter 2: The Effect of Transgenic Bt Cotton on Arthropod Communities Under Varying Tillage and Cover Cropping Practices

H_1 – tillage and cover crop type will exert much more influence on arthropod abundance, richness and diversity than will the use of Bt cotton

H_2 – tillage and cover crop strategy will not significantly alter the effect of cotton type on arthropod abundance, richness or diversity

RATIONALE

A small number of field studies have demonstrated significant direct effects of Bt cotton on non-target arthropods such as the cotton fleahopper (Parajulee *et al.* 2006). Research also suggests that Bt cotton has the potential to influence decomposer communities through changes in plant residue quality (Flores *et al.* 2005). Other agricultural management techniques within which the use of Bt cotton is nested (i.e., tillage and cover crop choice) can significantly affect arthropod communities both above- and below-ground. Conservation tillage can drastically alter arthropod communities by decreasing the frequency of soil disturbance and by increasing habitat complexity through the accumulation of crop residue at the soil surface as well as by fostering a more diverse weed community. These effects extend across multiple taxa including both pest and beneficial arthropods. Cover crops vary tremendously in structural and chemical composition and subsequently have been found to have a variety of effects on arthropod communities. These effects can be either direct (i.e., suitability as food for herbivores, flowering characteristics and structural/chemical traits of dead residue) or indirect (i.e., cover crop as either a simple or complex habitat) and in most cases, the functional differences between two cover crops (rye and clover in Chapter 2) are much greater than those between Bt and non-Bt cotton. Accordingly, I expect that the differences between conventional and no-till soil management strategies as well as those between clover and rye will lead to significant differences in many taxa and that the magnitude of these differences will be much greater than any differences based on cotton type.

Chapter 3: Impact of the Red Imported Fire Ant (*Solenopsis invicta*) on the Soil Arthropod Community of a Cotton Agroecosystem

H_1 – The overall abundance, richness and diversity of arthropods, both at and below the soil surface, will not be significantly affected by the removal of *Solenopsis invicta*.

H_2 – Individual families, both at and below the soil surface, will be significantly affected by the removal of *Solenopsis invicta*; however, while the abundance of some families will increase, that of others will decrease.

RATIONALE

While past research has demonstrated that fire ants can significantly alter arthropod communities on plant foliage and at the soil surface, each has examined this impact in habitats with localized resources (e.g., aphid honeydew in cotton foliage, rotting fruit masses at soil surface). Resource distribution within the soil matrix of cotton systems can vary based on the management strategies employed at a particular system (e.g., weed management, tillage strategy) but in general, the detrital resource base in these systems is considered to be fairly homogeneous and of low quality. Due to the uniformity in resources I do not expect fire ants to prey heavily upon all taxa within the soil food web. Instead I predict that while fire ants may prey specifically on some taxa, other taxa will benefit from the activities of fire ants.

Chapter 4: Egg Predation by *Solenopsis invicta*, the Red Imported Fire Ant, on Foliage and at the Soil Surface of a Cotton Agroecosystem

H_1 – eggs of the beet armyworm, *Spodoptera exigua*, will be removed at a significantly faster rate from both the crop canopies and the soil surface in the presence of *S. invicta* than in its absence

H_2 – eggs of both the redbanded stink bug, *Piezodorus guildinii* and southern green stink bug, *Nezara viridula* will also be removed at a significantly faster rate from cotton foliage in the presence of *S. invicta* than in its absence

H_3 – an increase in the abundance of honeydew-producing hemipterans -- cotton aphids, *Aphis gosypii*, Glover (Hemiptera: Aphididae) and sweetpotato whitefly, *Bemisia tabaci*, Gennadius (Hemiptera: Aleyrodidae) -- in cotton foliage will lead to:

a) a significant increase in egg removal rates from cotton foliage, and
b) a significant decrease in egg removal rates from the soil surface

RATIONALE

I predict that fire ants will make a significant contribution to predation of eggs of both beet armyworm and stink bugs in cotton foliage. While past research has shown that fire ants do significantly affect predation on eggs of *S. exigua*, this is the first attempt to examine their impact on predation of stink bug eggs (Diaz *et al.* 2004; Ehler 2007). Past research on stink bug egg predation has demonstrated that they are preyed upon infrequently; however, I expect stink bug egg predation to be significant in the presence of *S. invicta* (Ehler 2002). I also expect that due to their foraging activity at the soil surface, *S. invicta* will contribute significantly to egg predation in soil food webs. It has also been suggested that seasonal fluctuation in aphid populations in cotton systems may cause a shift in *S. invicta* feeding activity from predation at the soil surface to a diet of hemipteran honeydew in crop canopies (Kaplan & Eubanks 2005). If this is true, I predict that the intensity of predation by *S. invicta* at the soil surface will decrease during periods of high aphid abundance within the cotton canopy.

Chapter 5: Conclusions and Future Research

REFERENCES

Andersen A. (1999). Plant protection in spring cereal production with reduced tillage. II Pests and beneficial insects. . *Crop Protection*, 18, 651-657.

Blumberg A.Y. & Crossley D.A. (1983). Comparison of soil surface arthropod populations in conventional tillage, no-tillage and old field systems. *Agro-Ecosystems*, 8, 247-253.

Boulton A.M. & Amberman K.D. (2006). How ant nests increase soil biota richness and abundance: a field experiment. *Biodiversity and Conservation*, 15, 69-82.

Brust G.E. & House G.J. (1988). Weed seed destruction by arthropods and rodents in low-input soybean agroecosystems. *American Journal of Alternative Agriculture*, 3, 19-25.

Bugg R.L. & Dutcher J.D. (1989). Warm-season cover crops for pecan orchards - horticultural and entomological implications. *Biological Agriculture & Horticulture*, 6, 123-148.

Bugg R.L., Wackers F.L., Brunson K.E., Dutcher J.D. & Phatak S.C. (1991). Cool-season cover crops relay intercropped with cantaloupe - influence on a generalist predator, Geocoris punctipes (Hemiptera Lygaeidae). *Journal of Economic Entomology*, 84, 408-416.

Bugg R.L., Wackers F.L., Brunson K.E., Phatak S.C. & Dutcher J.D. (1990). Tarnished plant bug (Hemiptera: Miridae) on selected cool-season leguminous cover crops. *Journal of Entomological Science*, 25, 463-474.

Bugg R.L. & Waddington C. (1994). Using cover crops to manage arthropod pests of orchards - A review. *Agriculture Ecosystems & Environment*, 50, 11-28.

Canerday D.T. (1988). Fire Ants in Georgia. University of Georgia College of Agriculture and Environmental Sciences, Cooperative Extension Services, http://pubs.caes.uga.edu/caespubs/pubcd/l332-w.html.

Cattaneo M.G., Yafuso C., Schmidt C., Huang C.Y., Rahman M., Olson C., Ellers-Kirk C., Orr B.J., Marsh S.E., Antilla L., Dutilleu P. & Carriere Y. (2006). Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 7571-7576.

Cederbaum S.B., Carroll J.P. & Cooper R.J. (2004). Effects of alternative cotton agriculture on avian and arthropod populations. *Conservation Biology*, 18, 1272-1282.

Chilcutt C.F. & Matocha J.E. (2007). Effects of crop rotation, tillage, and fertilizer applications on sorghum head insects. *Journal of Economic Entomology*, 100, 88-94.

Coughenour M.C. & Chamala S. (2000). *Conservation Tillage and Cropping Innovation: Constructing the new culture of agriculture*. Iowa State University Press, Ames, IA.

Crossley D.A., Mueller B.R. & Perdue J.C. (1992). Biodiversity of Microarthropods in agricultural soils - relations to processes. *Agriculture Ecosystems & Environment*, 40, 37-46.

Diaz D., Knutson A. & Bernal J.S. (2004). Effect of the red imported fire ant on cotton aphid population density and predation of bollworm and beet armyworm eggs. *Journal of Economic Entomology*, 97, 222-229.

Diaz S. & Cabido M. (2001). Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*, 16, 646-655.

Dostal P., Breznova M., Kozlickova V., Herben T. & Kovar P. (2005). Ant-induced soil modification and its effect on plant below-ground biomass. *Pedobiologia*, 49, 127-137.

Ehler L.E. (2002). An evaluation of some natural enemies of Nezara viridula in northern California. *Biocontrol*, 47, 309-325.

Ehler L.E. (2007). Impact of native predators and parasites on Spodoptera exigua, an introduced pest of alfalfa hay in northern California. *Biocontrol*, 52, 323-338.

Eubanks M.D. (2001). Estimates of the direct and indirect effects of red imported fire ants on biological control in field crops. *Biological Control*, 21, 35-43.

Eubanks M.D., Blackwell S.A., Parrish C.J., Delamar Z.D. & Hull-Sanders H. (2002). Intraguild predation of beneficial arthropods by red imported fire ants in cotton. *Environmental Entomology*, 31, 1168-1174.

Finke D.L. & Denno R.F. (2002). Intraguild predation diminished in complex-structured vegetation: Implications for prey suppression. *Ecology*, 83, 643-652.

Flores S., Saxena D. & Stotzky G. (2005). Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry*, 37, 1073-1082.

Fu S., Kisselle K.W., Coleman D.C., Hendrix P.F. & Crossley D.A. (2001). Short-term impacts of aboveground herbivory (grasshopper) on the abundance and ^{14}C activity of soil nematodes in conventional tillage and no-till agroecosystems. *Soil Biology and Biochemistry*, 33, 1253 - 1258.

Fu S.L., Coleman D.C., Hendrix P.F. & Crossley D.A. (2000). Responses of trophic groups of soil nematodes to residue application under conventional tillage and no-till regimes. *Soil Biology & Biochemistry*, 32, 1731-1741.

GA-Department-of-Agriculture (2007). Statistics Service Releases: Forecast Of Yields of Major Georgia Crops, http://agr.georgia.gov

Garrett C.J., Crossley D.A., Coleman D.C., Hendrix P.F., Kisselle K.W. & Potter R.L. (2001). Impact of the rhizosphere on soil microarthropods in agroecosystems on the Georgia piedmont. *Applied Soil Ecology*, 16, 141-148.

Geng J.H., Shen Z.R., Song K. & Zheng L. (2006). Effect of pollen of regular cotton and transgenic Bt plus CpTI cotton on the survival and reproduction of the parasitoid wasp Trichogramma chilonis (Hymenoptera: Trichogrammatidae) in the laboratory. *Environmental Entomology*, 35, 1661-1668.

Green W.P., Pettry D.E. & Switzer R.E. (1999). Structure and hydrology of mounds of the imported fire ants in the southeastern United States. *Geoderma*, 93, 1-17.

Hatten T.D., Bosque-Perez N.A., Labonte J.R., Guy S.O. & Eigenbrode S.D. (2007). Effects of tillage on the activity density and biological diversity of carabid beetles in spring and winter crops. *Environmental Entomology*, 36, 356-368.

Head G., Moar M., Eubanks M., Freeman B., Ruberson J., Hagerty A. & Turnipseed S. (2005). A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. *Environmental Entomology*, 34, 1257-1266.

Hendrix P.F., Crossley D.A., Coleman D.C., Parmelee R.W. & Beare M.H. (1987). Carbon dynamics in soil microbes and fauna in conventional and no-tillage agroecosystems. *INTECOL Bulletin*, 15, 59-63.

Holland J.M. (2004). The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. *Agriculture Ecosystems & Environment*, 103, 1-25.

Hölldobler B. & Wilson E.O. (1990). *The Ants*. Belknap Press, Cambridge.

Hooper D.U., Bignell D.E., Brown V.K., Brussaard L., Dangerfield J.M., Wall D.H., Wardle D.A., Coleman D.C., Giller K.E., Lavelle P., Van Der Putten W.H., De Ruiter P.C., Rusek J., Silver W.L., Tiedje J.M. & Wolters V. (2000). Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. *BioScience*, 50, 1049-1061.

House G.J. & Alzugaray M.D. (1989). Influence of cover cropping and no-tillage practices on community composition of soil arthropods in a North Carolina agroecosystem. *Environmental Entomology*, 18, 302-307.

Ishijima C., Motobayashi T., Nakai M. & Kunimi Y. (2004). Impacts of tillage practices on hoppers and predatory wolf spiders (Araneae: Lycosidae) in rice paddies. *Applied Entomology and Zoology*, 39, 155-162.

Kaplan I. & Eubanks M.D. (2002). Disruption of cotton aphid (Homoptera: Aphididae) - Natural enemy dynamics by red imported fire ants (Hymenoptera: Formicidae). *Environmental Entomology*, 31, 1175-1183.

Kaplan I. & Eubanks M.D. (2005). Aphids alter the community-wide impact of fire ants. *Ecology*, 86, 1640-1649.

Karlen D.L. and Doran J.W. (2001). Soil Quality: current concepts and applications. *Advances in Agronomy*, 74.

Kisselle K.W., Garrett C.J., Fu S., Hendrix P.F., Crossley D.A., Coleman D.C. & Potter R.L. (2001). Budgets for root-derived C and litter-derived C: comparison between conventional tillage and no tillage soils. *Soil Biology & Biochemistry*, 33, 1067-1075.

Knox O.G.G., Gupta V., Nehl D.B. & Stiller W.N. (2007). Constitutive expression of Cry proteins in roots and border cells of transgenic cotton. *Euphytica*, 154, 83-90.

Laakso J. & Setälä H. (1998). Composition and trophic structure of detrital food web in ant nest mounds of Formica aquilonia and in the surrounding forest soil. *Oikos*, 81, 266-278.

Lafleur B. H.-B.L.M., Mumma E.P., Geaghan J.P. (2005). Soil fertility and plant growth in soils from pine forests and plantations: effect of invasive red imported fire ants *Solenopsis invicta* (Buren). *Pedobiologia*, 49, 415-423.

Lal R. (2002). Soil carbon dynamics in cropland and rangeland. *Environmental Pollution*, 116, 353-362.

Lavelle P. (2002). Functional domains in soils. *Ecological Research*, 17, 441-450.

Lee D.K., Bhatkar A.P., Vinson S.B. & Olson J.K. (1994). Impact of foraging red imported fire ants (Solenopsis invicta) (Hymenoptera: Formicidae) on Psorophora columbiae eggs. *Journal of the American Mosquito Control Association*, 10, 163-173.

Lenoir L., Bengtsson J. & Persson T. (2003). Effects of Formica ants on soil fauna-results from a short-term exclusion and a long-term natural experiment. *Oecologia*, 134, 423-430.

Lofgren C.S. (1986). The Economic Importance and Control of Imported Fire Ants in the United States. In: *Economic Impact and Control of Social Insects* (ed. Vinson BS). Praeger Publishers New York, pp. 227 - 256.

Marasas M.E., Sarandon S.J. & Cicchino A.C. (2001). Changes in soil arthropod functional group in a wheat crop under conventional and no tillage systems in Argentina. *Applied Soil Ecology*, 18, 61-68.

Mellet M.A. & Schoeman A.S. (2007). Effect of Bt-cotton on chrysopids, ladybird beetles and their prey: Aphids and whiteflies. *Indian Journal of Experimental Biology*, 45, 554-562.

Morrison J.E., Williams D.F., Oi D.H. & Potter K.N. (1997). Damage to dry crop seed by red imported fire ant (Hymenoptera: Formicidae). *Journal of Economic Entomology*, 90, 218-222.

Morrison L.W. & Porter S.D. (2003). Positive association between densities of the red imported fire ant, Solenopsis invicta (Hymenoptera: Formicidae), and generalized ant and arthropod diversity. *Environmental Entomology*, 32, 548-554.

Morse S., Bennett R. & Ismael Y. (2006). Environmental impact of genetically modified cotton in South Africa. *Agriculture Ecosystems & Environment*, 117, 277-289.

Naranjo S.E. (2005). Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. *Environmental Entomology*, 34, 1193-1210.

Nuessly G.S. & Sterling W.L. (1994). Mortality of Helicoverpa zea (Lepidoptera: Noctuidae) eggs in cotton as a function of oviposition sites, predator species, and desiccation. *Environmental Entomology*, 23, 1189-1202.

Nunez M.A. & Simberloff D. (2005). Invasive species and the cultural keystone species concept. *Ecology and Society*, 10.

O'Callaghan M., Glare T.R., Burgess E.P.J. & Malone L.A. (2005). Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology*, 50, 271-292.

Oliveira A.R., Castro T.R., Capalbo D.M.F. & Delalibera I. (2007). Toxicological evaluation of genetically modified cotton Bollgard (R) and Dipel (R) WP on the non-target soil mite Scheloribates praeincisus (Acari : Oribatida). *Experimental and Applied Acarology*, 41, 191-201.

Parajulee M.N., Shrestha R.B. & Leser J.F. (2006). Influence of tillage, planting date, and Bt cultivar on seasonal abundance and within-plant distribution patterns of thrips and cotton fleahoppers in cotton. *International Journal of Pest Management*, 52, 249-260.

Phatak S.C. (1998). Managing Pests With Cover Crops. In: *Managing Cover Crops Profitably* (eds. Bowman G, Shirley C & Cramer C). Sustainable Agricultural Network Beltsville, MD.

Philpott S.M., Perfecto I. & Vandermeer J. (2006). Effects of management intensity and season on arboreal ant diversity and abundance in coffee agroecosystems. *Biodiversity and Conservation*, 15, 139-155.

Porter S.D. & Savignano D.A. (1990). Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. *Ecology*, 71, 2095-2106.

Romeis J., Meissle M. & Bigler F. (2006). Transgenic crops expressing Bacillus thuringiensis toxins and biological control. *Nature Biotechnology*, 24, 63-71.

Russell D. & Deguine J.P. (2006). Sustainability of Bt cotton in China and India. *Cahiers Agricultures*, 15, 54-59.

Saxena D., Stewart C.N., Altosaar I., Shu Q. & Stotzky G. (2004). Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato and rice but not of *B. thuringiensis* canola, cotton, and tobacco. *Plant Physiology and Biochemistry*, 42, 383-387.

Sharma H.C., Arora R. & Pampapathy G. (2007). Influence of transgenic cottons with Bacillus thuringiensis cry1Ac gene on the natural enemies of Helicoverpa armigera. *Biocontrol*, 52, 469-489.

Shen R.F., Cai H. & Gong W.H. (2006). Transgenic Bt cotton has no apparent effect on enzymatic activities or functional diversity of microbial communities in rhizosphere soil. *Plant and Soil*, 285, 149-159.

Six J., Bossuyt H., Degryze S. & Denef K. (2004). A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research*, 79, 7-31.

Stinner B.R. & House G.J. (1990). Arthropods and other invertebrates in conservation tillage agriculture. *Annual Review of Entomology*, 35, 299-318.

Stinner B.R., McCartney D.A. & Rubink W.L. (1984). Some observations on ecology of the stalk borer (Papaipema nebris (Gn), Noctuidae) in no-tillage corn agroecosystems. *Journal of the Georgia Entomological Society*, 19, 229-234.

Styrsky J.D. & Eubanks M.D. (2007). Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B-Biological Sciences*, 274, 151-164.

Sun C.X., Chen L.J., Wu Z.J., Zhou L.K. & Shimizu H. (2007). Soil persistence of Bacillus thuringiensis (Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzyme activities. *Biology and Fertility of Soils*, 43, 617-620.

Szendrei Z., Mallampalli N. & Isaacs R. (2005). Effect of tillage on abundance of Japanese beetle, Popillia japonica Newman (Col., Scarabaeidae), larvae and adults in highbush blueberry fields. *Journal of Applied Entomology*, 129, 258-264.

Tillman G., Schomberg H., Phatak S., Mullinix B., Lachnicht S., Timper P. & Olson D. (2004). Influence of cover crops on insect pests and predators in conservation tillage cotton. *Journal of Economic Entomology*, 97, 1217-1232.

Torres J.B. & Ruberson J.R. (2005). Canopy- and ground-dwelling predatory arthropods in commercial Bt and non-Bt cotton fields: Patterns and mechanisms. *Environmental Entomology*, 34, 1242-1256.

Tremelling M.J., McSorley R. & Gallaher R.N. (2003). Effects of winter cover crops on the soil surface invertebrate community. *Soil and Crop Science Society of Florida Proceedings*, 62, 77-82.

Tschinkel W.R. (2006). *The fire ants*. Belknap Press, Cambridge.

UGA-CAES (2007). Cotton Guide: conservation tillage

Veazey J.N., Kay C.A.R., Walker T.J. & Whitcomb W.H. (1976). Seasonal abundance, sex-ratio, and macroptery of field crickets in northern Florida Orthoptera Gryllidae Gryllus. *Annals of the Entomological Society of America*, 69, 374-380.

Vinson B.S. (1985). *Economic Impact and Control of Social Insects*. Praeger Publishers, New York.

Vinson S.B. (1991). Effect of the Red Imported Fire Ant (Hymenoptera, Formicidae) on a Small Plant-Decomposing Arthropod Community. *Environmental Entomology*, 20, 98-103.

Wagner D., Brown M.J.F. & Gordon D.M. (1997). Harvester ant nests, soil biota and soil chemistry. *Oecologia*, 112, 232-236.

Wardle D.A. (1995). Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. *Advances in Ecological Research*, 26, 105-183.

Whitehouse M.E.A., Wilson L.J. & Fitt G.P. (2005). A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. *Environmental Entomology*, 34, 1224-1241.

Wilson E.O. (2005). Oribatid mite predation by small ants of the genus Pheidole. *Insectes Sociaux*, 52, 263-265.

Wojcik D.P. (1990). Behavioral Interactions of Fire Ants and Their Parasites, Predators and Inquilines. In: *Applied Myrmecology* (ed. Vander Meer R.K. JK, Cedeno A.). Westview Press Boulder, pp. 329 - 344.

Yan W.D., Shi W.M., Li B.H. & Zhang M. (2007). Overexpression of a foreign Bt gene in cotton affects the low-molecular-weight components in root exudates. *Pedosphere*, 17, 324-330.

Zhang G.F., Wan F.H., Lovei G.L., Liu W.X. & Guo J.Y. (2006). Transmission of Bt toxin to the predator Propylaea japonica (Coleoptera : Coccinellidae) through its aphid prey feeding on transgenic Bt cotton. *Environmental Entomology*, 35, 143-150.

CHAPTER 2

THE EFFECT OF TRANSGENIC BT COTTON ON ARTHROPOD COMMUNITIES UNDER VARYING TILLAGE AND COVER CROPPING PRACTICES[1]

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ABSTRACT

Many researchers have concluded that Bt crops have little impact on non-target arthropods while a few studies have shown deleterious effects. Most of these studies have looked solely at the effects of Bt crops on non-target species without considering other management practices taking place within the agroecosystem. This project examined effects of Bt cotton on non-target arthropod communities in combination with, and relative to, the effects of tillage and cover crop identity.

Plant- and ground-active arthropods were surveyed over two years from a two-acre cotton farm in Athens, GA, supporting whole plot treatments of conventional- (CT) and no-tillage (NT) with subplot treatments of Bt and non-Bt cotton and a winter cover crop of rye or clover. Although neither Bt cotton nor its residue affected total abundance, richness or diversity, three families were significantly affected. Conversely, richness and diversity of non-target arthropods were both significantly higher in NT than in CT plots and a clover cover crop supported higher levels of abundance, richness and diversity during 2005. An interaction was detected between cover crop and cotton residue type with higher abundance, richness and diversity occurring in clover plots with Bt cotton residue than in clover plots with non-Bt residue. Arthropods contributing to significant differences at the community level include plant- and soil surface-active predators, detritivores, pollen feeders, and non-target herbivores.

GM crops are a rapidly developing technology, and it is necessary to investigate each agricultural context under which they may be grown. While this study demonstrates slight variations in the impact of Bt cotton under various agricultural management schemes it also reveals that cotton type is the least important factor shaping non-target arthropod communities relative to the other management strategies employed at this particular site.

Keywords: biodiversity, conservation tillage, *Bacillus thuringiensis*

INTRODUCTION

The effects of genetically modified crops on non-target organisms have been the topic of much recent research, particularly crops modified to express toxins of the bacterium *Bacillus thuringiensis* Berliner (Bt) (O'Callaghan et al. 2005). The potential effects include toxicity of pest species to their natural enemies due to secondary ingestion of the Bt endotoxin, loss of prey/hosts for predators/parasitoids, decreased prey/host suitability due to endotoxin effects, and effects on soil meso- and macrofauna due to ingestion of either decomposing transgenic plant material or root-exuded endotoxins. O'Callaghan et al. (2005) report that results from both laboratory and field studies reveal mixed effects of Bt cotton on non-target arthropods. This suggests that if Bt cotton does affect non-target arthropods, the impact may be both subtle and context specific.

Most studies addressing the effects of Bt crops on non-target arthropods are limited to simple comparisons between Bt and non-Bt crops. Given the mixed nature of results from past experiments, an examination of the effects of Bt cotton relative to other important agronomic factors that shape arthropod communities may shed light on the true ecological impact of these crops in the context of the overall cropping system. Beyond the context of simple experiments, Bt crops are grown in a complex landscape matrix of agronomic management strategies that include variation in tillage regimes and in cover crops. To begin addressing potential interactions among crop choices and management strategies, we examined the relative contributions of tillage and cover crops to the foliar and ground-dwelling arthropod communities in Bt and non-Bt cotton fields.

Tillage is a powerful tool for reducing the competitive strength of many agricultural pests. However, tillage also reduces both soil moisture and structural complexity at the soil surface with potential consequences for non-target arthropod communities (Magdoff & van Es 2000). For example, abundant plant litter at the soil surface has been shown to decrease antagonism among predators and subsequently increase their impact on prey populations (Finke & Denno 2002). No-till strategies greatly increase structural complexity at the soil surface and have been shown to have variable effects on both pest and beneficial arthropods both above-, and below-ground (Chilcutt & Matocha 2007). At the same time, the use of cover crops can increase populations of many beneficial arthropods, reduce soil erosion and maintain inputs of soil organic matter into detrital food webs (House & Alzugaray 1989). Cover crops vary widely in terms of plant quality, which directly influences plant litter quality, microbial community structure, nematode and microarthropod population densities, as well as earthworms, termites, and ants (Yeates 1979; Tian *et al.* 1993; Wardle 1993; Badejo and Tian 1999).

Objectives

The purpose of this study is to compare the effects of Bt and non-Bt cotton on the arthropod communities that are active on plants and at the soil surface as a function of the tillage and cover crop practices in which the respective crops are embedded. By doing so, we can assess context-specific effects of the cotton types on the communities.

We tested the following hypotheses:

H_1 – tillage and cover crop type will exert much more influence on arthropod abundance, richness and diversity than will the use of Bt cotton

H_2 – tillage and cover crop strategy will not significantly alter the effect of cotton type on arthropod abundance, richness or diversity

MATERIALS AND METHODS

Site Description

All research was conducted at the Horseshoe Bend Long Term Research in Environmental Biology (LTREB) research site of the University of Georgia, Athens, GA. The 14-hectare site, surrounded by a bend in the North Oconee River, is comprised of eastern deciduous forest and a 0.8-ha agricultural plot. Agroecosystem research has been conducted at the site since 1978 when the site was divided into eight plots (28 m x 28 m) that were randomly assigned to either a conventional tillage (CT) or no-tillage (NT) management regime. The current study ran from January, 2004, through October, 2005, (Table 1). For the duration of our study, each of the eight main plots was subdivided into four subplots (14 x 14 m each) to support a fully-factorial experiment of summer cotton crop (Bt or non-Bt) crossed with winter cover crop (clover or winter wheat/rye) nested within the eight tillage plots (four conventional and four no-tillage). The experiment therefore consisted of a total of 32 subplots. Arthropod sampling was conducted each year in April (cover crop) and October (cotton crop) as described below (Table 1).

Sweep-net Sampling

During each sampling period, three sweep net samples were taken within each subplot with a net 40 cm in diameter. Each sample consisted of ten sweeps from the crop canopy taken over a five-meter transect. All samples were taken on the following dates, 21 April and 8 October 2004, 29 April and 13 October 2005. Three transects were run in each subplot for a total of 30 sweeps per subplot and all transects were located near the center of each subplot to avoid edge effect. Care was taken to not cross over unsampled transects. Arthropods were transferred to a kill bucket with 70% ethanol and then placed in plastic vials for storage and identification.

Pitfall Trapping

During each sampling period, four pitfall traps were placed in each subplot (128 total traps). Each trap consisted of a 470-ml. plastic drink cup with a 10 cm diameter opening. A plastic specimen cup containing 30 mL of 70% ethanol was inserted into each cup. The cups were buried at ground level before placement of specimen cups to avoid collection of soil and debris. Plastic funnels were then placed in each trap. Cardboard roofing was secured approximately seven cm above each trap to minimize desiccation and to keep traps from filling with rainwater. Traps were placed in the field on the following dates, 23 April and 13 October 2004, 20 April and 12 October 2005. Traps remained in the field for 24 hours, after which their contents were emptied into plastic vials and held until identified.

Statistical Analyses

Sampling data were analyzed separately for 2004 and 2005. Arthropods were identified to family where possible, and abundance, richness and diversity (H) were calculated using row and column summary analysis in PC-ORD (McCune & Grace 2002). To compare among treatments in the nested design, data were square root transformed and mixed model analysis was conducted using SAS software version 8 for Windows, with tillage, cover crop and cotton types as fixed variables and plot as a random variable (SAS 1999). After analysis of abundance, richness and diversity, Indicator Species Analysis was conducted (but at the family level) in order to determine which arthropod families contributed most heavily to significant differences found using the mixed model. The indicator species analysis uses a Monte-Carlo test for significance such that Indicator Values range from 0 to 100 and values greater than 25 with a *P*-

value less than 0.05 are considered significant (Dufrene & Legendre 1997). A significant value means that the occurrence and abundance of a given family is associated with a particular difference among treatments. If a family was assigned indicator status, the abundance of this family was further analyzed using mixed model analysis to confirm its significance.

RESULTS

Bt Cotton Effects

In isolation, neither Bt cotton (October sampling) nor its residue (April sampling) had any impact on total arthropod abundance, richness or diversity during either year of the study (Tables 2.2a & b). However, there were significant effects of both cotton and cotton residue type on specific arthropod families during both years of the study (Figs 2.1 & 2.2). During October of 2005 the mite suborder Prostigmata ($F_{1, 114}$=4.52; P=0.035) was significantly more abundant/active from pitfall traps in non-Bt cotton plots than from Bt cotton plots while the family Gryllidae was more abundant/active from Bt rather than non-Bt plots ($F_{1,12}$=6.06; P=0.029, Fig 2.1). Cotton residue type also had a significant effect on the abundance of tarnished plant bugs, *Lygus lineolaris* Palisot de Beauvois, and black fleahoppers *Halticus bractatus* Say, (Hemiptera: Miridae) during April of 2004 ($F_{1,12}$= 7.57; P=0.017, Fig 2.2).

Tillage Effects

Arthropod richness and diversity were significantly higher in No-till plots than in conventionally tilled plots in pitfall samples during October 2004 ($F_{1,6}$ =11.10, P=0.015, $F_{1,6}$ =13.18, P=0.011, Table 2.2a). Similarly, arthropod richness was higher in No-till plots than in conventionally tilled plots during October 2005 ($F_{1,6}$ =8.47; P=0.027, Table 2.2a).

During both years of the study, as well as from both cotton and cover crop seasons, many individual arthropod families were significantly more abundant/active in no-till than in conventional-till plots (Figs 2.3 & 2.4). The taxa included mites in the suborders Prostigmata (April 2004 pitfall traps $F_{1,6}$ =6.04; P=0.049, October 2005 pitfall traps $F_{1,6}$ =6.24; P=0.046) and Oribatida ($F_{1,12}$ =11.74; P=0.005), as well as the families Cicadellidae ($F_{1,6}$ =6.45; P=0.044), Delphacidae ($F_{1,24}$ =6.36; P=0.018), Miridae (October 2004: $F_{1,6}$ =11.46; P=0.014, April 2005: $F_{1,12}$ =34.38; P<.0001), Aphididae ($F_{1,6}$ =7.24; P=0.036), Syrphidae ($F_{1,24}$ =15.00; P=0.0007), Sminthuridae (October 2005: $F_{1,12}$ =17.06; P=0.001, October 2005: $F_{1,6}$ =6.06; P=0.049), Hypogastruridae ($F_{1,12}$ =16.35; P=0.001), Linyphiidae ($F_{1,6}$ =5.65; P=0.05) and Thomisidae ($F_{1,12}$ =8.41; P=0.013).

There were also five taxa significantly more abundant/active in conventional-till plots than in no-till plots (Figs 2.3 & 2.4). These taxa were primarily adult Dipterans (Tipulidae $F_{1,6}$ =7.34; P=0.035, Ceratopogonidae $F_{1,6}$ =5.94; P=0.05, Chironomidae $F_{1,12}$ =6.39; P=0.026), but also included thrips in the suborder Terebrantia ($F_{1,24}$ =18.05; P=0.0003) and the collembolan family Isotomidae ($F_{1,12}$ =15.53; P=0.002).

Cover Crop Effects

Independent effects of cover crop on arthropod communities were only detectable in the sweep-net samples of 2005. Arthropod abundance, richness and diversity were significantly higher in clover than in rye plots during April of 2005 ($F_{1,12}$ =12.47, P=0.004, $F_{1,12}$ =41.06, P<0.001, $F_{1,12}$ =47.49, P<0.001, Table 2.2b).

Indicator Species Analysis followed by mixed model analysis of the abundance of individual taxa for April of 2005 revealed that the following taxa were significantly more abundant/active in clover than in rye subplots: Diptera (Drosophilidae $F_{1,6}$ =7.16; P=0.036, Chironomidae $F_{1,88}$ =26.97; P<.0001, Sciaridae $F_{1,88}$ =5.07; P=0.026 and Dolichopodidae $F_{1,24}$ =5.26; P=0.03), Hemiptera (Miridae $F_{1,6}$ =15.53; P=0.007 and Cicadellidae $F_{1,24}$ =9.42; P=0.005), Hymenoptera (Apidae: *Apis mellifera*, L., $F_{1,6}$ =18.42; P=0.005 and Formicidae: chiefly *Solenopsis invicta*, Buren, $F_{1,6}$ =8.31; P=0.028), Coleoptera (Nitidulidae $F_{1,6}$ =10.97; P=0.016 and Tenebrionidae $F_{1,6}$ =11.95; P=0.013), and Orthoptera (Acrididae $F_{1,6}$ =9.35; P=0.009) (Figure 2.5). Aphids, however, were more abundant in rye than in clover subplots ($F_{1,6}$ =7.82; P=0.031, Fig 2.5).

Interactions

Interactions were detected in cover crop samples from both 2004 and 2005. The impact of Bt cotton as either a live crop or as plant residue varied based on the accompanying tillage and cover crop type. Arthropod abundance was significantly lower in clover subplots with non-Bt cotton residue than in clover subplots with Bt residue during both years of the study ($F_{1,82}$ =10.92, P=0.001; $F_{1,18}$ =5.48; P=0.031 Figs 2.6a & 2.7). During April of 2004 arthropod richness and diversity were also lowest in clover subplots with non-Bt cotton residue ($F_{1,24}$ =5.33, P=0.02; $F_{1,18}$ =7.44, P=0.01, Figs 2.6b & c).

Examination of the abundance of different arthropod families revealed a large number of significant interactions. However, these effects varied tremendously and rarely coincided with significant interactions at the whole community level (see Tables 2.3 through 2.10). In cases where family level interaction effects did coincide with whole community effects (April 2004), *L. lineolaris* and *H. bractatus* (Hemiptera: Miridae) were the primary contributors (Tables 2.3 and 2.7).

DISCUSSION

Bt cotton

Although there was no clear effect of Bt cotton or its residue alone on total arthropod abundance, richness or diversity, there were significant effects on specific taxa. Mites in the suborder Prostigmata were more abundant in non-Bt than in Bt cotton plots while the southern ground cricket, *Allonemobius socius* Scudder, (Orthoptera, Gryllidae) was more abundant in Bt cotton plots. Among the prostigmatid mites collected, the predatory family Eupodidae was the dominant taxon. The feeding ecology of eupodid mites is poorly understood, making it difficult to suggest any mechanism that may result in higher abundance of this family in non-Bt cotton. Similarly, *A. socius*, like many crickets, is omnivorous making it equally difficult to define a potential link between this species and Bt cotton (Howard & Harrison 1984). *L. lineolaris* and *H. bractatus* (Hemiptera: Miridae) were more abundant in subplots with Bt cotton residue. On living cotton plants, Parajulee *et al.* (2006) noted significantly higher abundance of the cotton fleahopper (*Pseudatomoscelis seriatus*) in Bt versus non-Bt cotton and Oliveira *et al.* (2007) found no difference in survival of the oribatid mite *Scheloribates praeinsicus* when reared on either Bt or non-Bt cotton. To our knowledge, no literature has examined the effects of Bt cotton on the families Eupodidae and Gryllidae. Likewise, this is the first study to report any effects of Bt crop residue on the family Miridae (O'Callaghan et al. 2005).

Tillage

Our data show that, during both cotton-growing seasons, no-till soil management increased arthropod richness (Table 2.2a). No-till management also increased arthropod diversity (H) in October 2004 (Table 2.2a). Analyses at the family level indicate that the taxa responsible for such changes are primarily members of the detrital food web (Figs 2.3 & 2.4), which would be appropriate given the enhanced organic matter present in no-till systems (Magdoff & van Es 2000). Other studies report an increase in the abundance of Collembola under no-till management compared to conventionally tilled soils (Wardle 1995). Although this was the case for the collembolan families Sminthuridae and Hypogastruridae in the current study, the family Isotomidae was more abundant in conventional tillage plots during April 2005 (Figs 2.3 & 2.4).

The effects of tillage on members of the Acari are highly taxon-specific. The increase in frequency and abundance of non-astigmatid, oribatid mites in no-till treatments in the current study is consistent with the findings of most research on this taxon and likely relates to their preference for soil with higher moisture and organic matter content (Wardle 1995; Behan-Pelletier 1999). The literature reports that the suborder Prostigmata, shows a mixed response to tillage, likely due to the variability in life history strategies within this suborder (Bedano et al. 2006). The prostigmatid family Eupodidae was an indicator of no-till plots in the present study suggesting that family-level analyses (or finer) may be useful in linking Prostigmata to given habitat types.

The spider families Linyphiidae and Thomisidae were also associated with no-tillage plots (Fig 2.4). In a previous study examining the impact of tillage on spider assemblages, Blumberg & Crossley (1983) determined that no-till systems support a higher diversity of spider species than do conventionally tilled systems. However, not all published studies report the same pattern. Motobayashi *et al.* (2006) found that populations of Linyphiidae did not differ significantly between conventional and conservation till rice paddies. Differences in agroecosystem type, including differences among crop species, may be responsible for the contrasting results of these studies.

The effects of tillage on the order Hemiptera have been poorly studied. However, in at least one case, the families Cicadellidae and Miridae appeared to prefer no-till over conventional tillage systems (Tonhasca 1994). The higher abundances of *L. lineolaris* and leafhoppers (Hemiptera: Cicadellidae) in the current study may reflect variation in weed community composition between conventional and no-till plots (Figs 2.3 & 2.4). It is well known that weeds serve as alternate hosts for the families Miridae and Cicadellidae (Lamp *et al*. 1984). No-till systems host a more complex weed community than do conventional till systems and this may be the reason for the higher abundance of mirids, cicadellids and delphacids in the present study (Lamp *et al*. 1984). Aphids were also more abundant in no-till plots during April 2005 (Fig 2.3). This finding partially supports the experiment by Hesler & Berg (2003) demonstrating higher abundances of cereal aphids (Hemiptera: Aphididae) in no-till systems. Adult syrphid flies (Diptera: Syrphidae) were also more abundant in no-till plots than in conventional till plots in April 2005 (Fig 2.3). Adult aphidophagous syrphids are known to lay their eggs near aphid colonies, upon which the emergent larvae will feed, suggesting a likely trophic link between aphids and syrphids in no-till plots of the current study (Kan 1988).

There were also a number of taxa that were more abundant in conventionally tilled than in no-till plots during the current study (Figs 2.3 & 2.4). The higher abundances of thrips during April 2004 support the findings of Parajulee et al. (2006). Most research on the response of Diptera to tillage has been conducted on larvae and pupae given their dependence upon soil for pupation and suggests that tillage has mixed results on fly emergence from soil (Chapin *et al*. 1992). The current study is, to our knowledge, the first to report higher abundances of ceratopogonid, chironomid and tipulid adults in conventional tillage systems than in no-till systems. The roles of these taxa in the cotton system are not known.

Cover Crops

Many families contributed to the higher abundance, richness and diversity of arthropods observed in clover plots over rye plots in the 2005 cover crop season (Fig 2.5). Similar to the indicator taxa from our analysis of tillage, the families serving as indicators of clover plots comprised multiple trophic groups including pollinators, herbivores, decomposers, and predators. For pollinators and nectar feeders, we should note that the cover crops were flowering during arthropod sampling. Insect-pollinated plants, such as clover, offer substantial resources for flower-foraging species when compared to wind-pollinated plants such as winter wheat and rye. As a general rule, insect-pollinated plants support higher abundances of many arthropods (Tremelling *et al.* 2003). Apidae, Chironomidae, Sciaridae, and Drosophilidae are primarily nectar feeders as adults, which would explain their status as indicator taxa in clover plots (Fig 2.5).

Key herbivores during the 2005 cover crop season were from the families Miridae (*L lineolaris* and *H. bractatus*), Cicadellidae, Acrididae and Aphididae (Fig 2.5). Similar to our findings, (Tillman *et al.* 2004) found a significantly higher abundance of the tarnished plant bug (*L. lineolaris*) in clover than in rye plots. Additionally, we found that the families Acrididae and Cicadellidae were indicators of clover plots. While, to our knowledge, this study presents the first record of cover crop effects on the family Acrididae, cicadellids have been found previously in higher numbers in clover than in rye plots (Tremelling et al. 2003). Unlike other families, Aphididae was a significant indicator of rye plots (Fig 2.5). This may have been due to either the large number of grain aphids that can infest rye or to the weed composition of rye plots during the current study.

Among the detritivores, beetles in the family Nitidulidae were indicators of clover plots during the 2005 season. These beetles often prefer moist environments and many species inhabit flowers (Triplehorn & Johnson 2005)**.** House and Alzugaray (1989) found no significant difference in soil arthropod diversity between clover and rye plots although the authors did not report the phenological stage of either crop during arthropod sampling. Although nitidulid beetles are not strictly detritivorous they do function largely in the soil litter layer and feed on fungi and fluids from decomposing plant tissue.

Among the predators, ants (chiefly *S. invicta*) and long-legged flies (Dolichopodidae) were also indicators of clover plots. In a study by (Tillman *et al.* 2004) *S. invicta* was also found to be significantly more abundant in clover than in rye plots. Although the authors suggested that both clover and rye offer stable over-wintering habitat for fire ants, they offer no suggestion as to why fire ant abundance may be higher in clover. Perhaps the accompanying increase in arthropod abundance in clover plots, along with changes in microclimate, allowed for more prey for *S. invicta*.

Dolichopodid flies are all known to be predators as adults (Clausen 1940). Bahrmann (1993) reported a high degree of species turnover (beta diversity) among different habitat types, perhaps reflecting variation in moisture and light conditions. Crimson clover and rye have very different growth patterns and probably create very different microclimates at the soil surface. While crimson clover grows close to the ground and likely causes higher relative humidity and more shade, rye allows sun to reach the soil surface leading to higher temperature and lower humidity. These differences may be the cause of the preference of the representative species in the family Dolichopodidae for clover over rye plots in the current study.

SYNTHESIS

Relative and Context-Specific Effects

Although the relative importance of tillage, crop and residue type varied based on year and sample type (pitfall traps or sweep net samples) tillage and cover crop appeared to have the largest impact on arthropods, both at the whole-community and individual family level. Tillage strategy had significant effects on arthropods during both years (and seasons) of the study, affecting 16 different arthropod families. Although cover crop effects were limited to one sample of the study (April 2005), 12 different arthropod families were significantly affected within the sampling period. The effects of Bt cotton and its residue were, however, limited to three taxa.

The interaction reported in the present study between cotton litter and cover crop was not contingent upon tillage treatment, suggesting that the location of litter biomass above or belowground is not key to the interaction. Flores *et al.* (2005) demonstrated that Bt cotton litter decomposes more slowly than does the litter of non-Bt cotton. The authors, having accounted for multiple factors affecting decomposition (i.e., lignin, C:N), concluded that the presence of the Bt endotoxin was the only factor that could have influenced the rate of decomposition. Moreover, the interaction detected at the whole-community level was not consistent across individual families suggesting that the mechanisms involved may be taxon- as well as context-specific. Although we do not understand the mechanism underlying the interaction at present, it will be the focus of future work.

The deployment of transgenic crops remains controversial and like all types of agricultural production, pose potential environmental risks. This study demonstrates that Bt cotton may have some context specific impact on non-target arthropod communities when examined under varying cover crop and tillage practices. However, the effect of Bt was negligible relative to the role of tillage and cover crop choice in shaping the non-target arthropod community of this particular system.

REFERENCES

Badejo M.A., and Tian G., (1999) Abundance of soil mites under four agroforestry tree species with contrasting litter quality. *Biology and Fertility of Soils* **30**, 107-112.

Bahrmann R. (1993). Niche utilization of native dolichopodid species (Diptera, Dolichopodidae). *Deutsche Entomologische Zeitschrift*, 40, 221-243.

Bedano J.C., Cantu M.P. & Doucet M.E. (2006). Influence of three different land management practices on soil mite (Arachnida : Acari) densities in relation to a natural soil. *Applied Soil Ecology*, 32, 293-304.

Behan-Pelletier V.M. (1999). Oribatid mite biodiversity in agroecosystems: role for bioindication. *Agriculture Ecosystems & Environment*, 74, 411-423.

Blumberg A.Y. & Crossley D.A. (1983). Comparison of soil surface arthropod populations in conventional tillage, no-tillage and old field systems. *Agro-Ecosystems*, 8, 247-253.

Chapin J.W., Thomas J.S. & Sullivan M.J. (1992). Spring tillage and fall tillage system effects on hessian fly (Diptera, Cecidomyiidae) emergence from a coastal plain soil. *Journal of Entomological Science*, 27, 293-300.

Chilcutt C.F. & Matocha J.E. (2007). Effects of crop rotation, tillage, and fertilizer applications on sorghum head insects. *Journal of Economic Entomology*, 100, 88-94.

Clausen C.P. (1940). *Entomophagous Insects*. 1 edn. McGraw Hill Book Company, New York.

Dufrene M. & Legendre P. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecological Monographs*, 67, 345-366.

Finke D.L. & Denno R.F. (2002). Intraguild predation diminished in complex-structured vegetation: Implications for prey suppression. *Ecology*, 83, 643-652.

Flores S., Saxena D. & Stotzky G. (2005). Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry*, 37, 1073-1082.

Hesler L.S. & Berg R.K. (2003). Tillage impacts cereal-aphid (Homoptera : Aphididae) infestations in spring small grains. *Journal of Economic Entomology*, 96, 1792-1797.

House G.J. & Alzugaray M.D. (1989). Influence of cover cropping and no-tillage practices on community composition of soil arthropods in a North Carolina agroecosystem. *Environmental Entomology*, 18, 302-307.

Howard D.J. & Harrison R.G. (1984). Habitat segregation in ground crickets - The role of interspecific competition and habitat selection. *Ecology*, 65, 69-76.

Kan E. (1988). Assessment of aphid colonies by hoverflies .1. Maple aphids and Ephisyrphus balteatus (De Geer) (Diptera, Syrphidae). *Journal of Ethology*, 6, 39-48.

Lamp W.O., Barney R.J., Armbrust E.J. & Kapusta G. (1984). Selective weed control in spring planted alfalfa - Effects on leafhoppers and Planthoppers (Homoptera, Auchenorrhyncha), with emphasis on potato leafhopper. *Environmental Entomology*, 13, 207-213.

Magdoff F. & van Es H. (2000). *Building Soils for Better Crops*. 2 edn. Sustainable Agriculture Publications, Burlington, VT.

McCune B. & Grace J.B. (2002). *Analysis of Ecological Communities*. 1 edn. MjM Software Design, Gleneden Beach, OR.

Motobayashi T., Ishijima C., Takagi M., Murakami M., Taguchi A., Hidaka K. & Kunimi Y. (2006). Effects of tillage practices on spider assemblage in rice paddy fields. *Applied Entomology and Zoology*, 41, 371-381.

O'Callaghan M., Glare T.R., Burgess E.P.J. & Malone L.A. (2005). Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology*, 50, 271-292.

Oliveira A.R., Castro T.R., Capalbo D.M.F. & Delalibera I. (2007). Toxicological evaluation of genetically modified cotton (Bollgard (R) and Dipel (R) WP on the non-target soil mite Scheloribates praeincisus (Acari: Oribatida). *Experimental and Applied Acarology*, 41, 191-201.

Parajulee M.N., Shrestha R.B. & Leser J.F. (2006). Influence of tillage, planting date, and Bt cultivar on seasonal abundance and within-plant distribution patterns of thrips and cotton fleahoppers in cotton. *International Journal of Pest Management*, 52, 249-260.

SAS (1999-2001). SAS System for Windows. In. SAS Institute inc. Cary, NC.

Tian G., Brussaard L. & Kang B.T. (1993). Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions - Effects on soil fauna. *Soil Biology & Biochemistry*, 25, 731-737.

Tillman G., Schomberg H., Phatak S., Mullinix B., Lachnicht S., Timper P. & Olson D. (2004). Influence of cover crops on insect pests and predators in conservation tillage cotton. *Journal of Economic Entomology*, 97, 1217-1232.

Tonhasca A. (1994). Response of soybean herbivores to 2 agronomic practices increasing agroecosystem diversity. *Agriculture Ecosystems & Environment*, 48, 57-65.

Tremelling M.J., McSorley R. & Gallaher R.N. (2003). Effects of winter cover crops on the soil surface invertebrate community. *Soil and Crop Science Society of Florida Proceedings,* 62, 77-82.

Triplehorn C.A. & Johnson N.F. (2005). *Borror and Delong's Introduction to the Study of Insects*. 7 edn. Thomson Brooks/Cole, Belmont, CA.

Wardle D.A., Nicholson K.S., Yeates G.W. (1993) Effects of weed management strategies on some soil associated arthropods in maize and asparagus ecosystems. *Pedobiologia* 37, 257-269.

Wardle D.A. (1995) *Impacts of disturbance on detritus food webs in agroecosystems of contrasting tillage and weed management practices.* In: Begon M., Fitter A.H. (Eds.) Advances in Ecological Research 26. Academic Press, New York, p. 105-185.

Yeates G.W. (1979) Soil nematodes in terrestrial ecosystems. *Journal of Nematology* **11**: 213-229.

Table 2.1: Sampling schedule and planting dates. Crop variety information includes seeding rate in kg/ha. Cover crops were seeded using broadcast seed spreader while cotton was seeded with a direct drill no-till planter. (RR) – Roundup Ready glyphosate resistance.

crop	date	activity	crop variety	crop stage at time of sampling
cover crops	01/06/04 04/21/04 04/23/04	planted sweep net pitfall	Dixie Reseeding Crimson Clover @ 9 kg/ha, and Saluda Wheat @ 113 kg/ha	clover flowering, wheat approx. 0.75m tall
cotton	05/11/04 10/08/04 10/13/04	planted sweep net pitfall	Delta Pine 458 RR Bt @ 28kg/ha, Delta Pine 5415 RR @ 28kg/ha	both varieties late in boll development before boll maturation
cover crops	12/05/04 04/20/05 04/29/05	planted pitfall sweep net	Dixie Reseeding Crimson Clover @ 9 kg/ha, and Wrens Abruzzi Rye @ 113 kg/ha	clover flowering, rye approx. 1.3m tall
cotton	06/17/05 10/12/05 10/13/05	planted pitfall sweep net	Delta Pine 458 RR Bt @ 28kg/ha, Delta Pine 5415 RR @ 28kg/ha	both varieties late in boll development before boll maturation

Table 2.2a: Means ±SE of single factor effects for October 2004 and October 2005. Asterisks denote significant differences between treatment factors based on mixed model analysis (*P<0.05). Abundance – average number of arthropods per per trap (pitfall) and transect (sweep net), Richness – total number of families per trap (pitfall) and transect (sweep net) and Diversity – Shannon (H) diversity index.

				2004			2005	
Season	Sample Type	Factor	abundance	richness	diversity	abundance	richness	diversity
cotton	Pitfall	Bt cotton	17.23+2.40	5.79±0.38	1.36±0.06	129.05±22.32	10.65±0.52	1.45±0.09
		non-Bt cotton	16.03+2.41	5.43±0.38	1.30±0.06	119.30±22.32	10.42±0.52	1.46±0.09
		clover residue	14.59+2.40	5.64±0.38	1.37±0.06	133.91±25.35	10.53±0.54	0.48±0.09
		rye residue	18.67+2.40	5.59±0.38	1.28±0.06	114.44±25.35	10.54±0.54	1.43±0.09
		NT	20.10±2.53	6.68±0.45	1.51±0.07	131.33±28.44	11.90±0.66	1.60±0.13
		CT	13.15+2.53	4.54±0.45	1.15±0.07	117.02±28.44	9.17±0.66	1.31±0.13
	Sweep Net	Bt cotton	88.87±21.39	14.47±1.65	2.29±0.14	199.15±44.54	12.10±0.61	1.44±0.10
		non-Bt cotton	83.83±21.39	15.45±1.65	2.33±0.14	198.12±44.54	11.91±0.61	1.41±0.10
		clover residue	86.04±21.39	14.75±1.65	2.31±0.14	205.83±48.46	12.37±0.63	1.45±0.10
		rye residue	86.66±21.39	15.18±1.65	2.31±0.14	191.44±48.46	11.64±0.63	1.40±0.10
		NT	98.79±29.66	17.39±2.27	2.51±0.19	213.19±59.08	12.20±0.71	1.46±0.14
		CT	73.91±29.66	12.54±2.27	2.11±0.19	184.08±59.08	11.81±0.71	1.40±0.14

Table 2.2b: Means ±SE of single factor effects for April 2004 and April 2005. Asterisks denote significant differences between treatment factors based on mixed model analysis (*P<0.05). Abundance – mean number of arthropods per trap (pitfall) and transect (sweep net), Richness – total number of families per trap (pitfall) and transect (sweep net) and Diversity – Shannon (H) diversity index.

			2004			2005		
Season	Sample Type	Factor	abundance	richness	diversity	abundance	richness	diversity
cover crop	Pitfall	clover	60.25±23.63	12.62±0.84	2.12±0.08	69.67±12.88	12.31±0.66	2.13±0.06
		rye	71.93±16.42	11.80±0.83	2.00±0.08	66.64±12.88	12.85±0.66	2.16±0.06
		Bt cotton residue	71.79±16.42	12.00±0.84	2.05±0.08	71.40±12.88	12.64±0.60	2.16±0.05
		non-Bt cotton residue	72.79±16.27	12.43±0.83	2.07±0.08	64.90±12.88	12.53±0.60	2.15±0.05
		NT	85.49±21.31	13.38±1.02	2.19±0.10	63.51±16.87	13.79±0.77	2.22±0.67
		CT	59.09±22.41	11.04±1.06	1.93±0.10	72.79±16.87	11.37±0.77	2.07±0.67
	Sweep Net	clover	82.68±7.37	10.93±0.51	1.97±0.05	80.72±5.86	16.27±0.62	2.54±0.04
		rye	71.37±7.37	12.08±0.51	2.07±0.05	51.45±5.86	10.64±0.62	2.06±0.04
		Bt cotton residue	84.64±7.37	11.72±0.51	2.05±0.05	65.93±4.71	13.47±0.53	2.30±0.04
		non-Bt cotton residue	69.41±7.37	11.29±0.51	2.00±0.05	66.25±4.71	13.43±0.53	2.31±0.04
		NT	71.37±7.37	12.14±0.51	2.04±0.06	72.12±5.86	13.83±0.62	2.36±0.04
		CT	82.68±7.37	10.87±0.51	2.00±0.06	60.06±5.86	13.08±0.62	2.24±0.04

Table 2.3: Two-way interactions for individual families between tillage and cover crop (April 04, 05). Values are least square mean differences between interaction means for arthropod abundance (±SE). Values in bold are significant and letter (A or B) denotes the interaction column for which the abundance of a particular taxon is highest.

		April Tillage x Cover Crop Interactions		
		2004		2005
A	B	Scelionidae	Miridae	Sminthuridae
CT x clover	CT x rye	0.46 ± 0.54 B	**24.04 ± 5.92A**	1.93 ± 1.11 A
CT x clover	NT x clover	**3.75 ± 1.33B**	**24.25 ± 8.96 A**	0.25 ± 1.59 A
CT x clover	NT x rye	2.14 ± 1.33 B	18.41 ± 8.96 A	2.12 ± 1.59 B
CT x rye	NT x clover	**3.28 ± 1.33 B**	0.20 ± 8.96 A	1.68 ± 1.59 B
CT x rye	NT x rye	1.67 ± 1.33 B	5.62 ± 8.96 B	**4.06 ± 1.59 B**
NT x clover	NT x rye	**1.60 ± 0.55 A**	5.83 ± 5.92 B	2.37 ± 1.11 B

Table 2.4: Two-way interactions for individual families between tillage and cotton type (October 2004, 2005). Values are least square mean differences between interaction means for arthropod abundance (±SE). Values in bold are significant (P<0.05) and letter (A or B) denotes the interaction column for which the abundance of a particular taxon is highest.

		October Tillage x Cotton Interactions			
		2004		2005	
A	B	Ceratopogonidae	Sminthuridae	Baeus	Gryllidae
CT x Bt cotton	CT x non Bt cotton	**2.37 ± 0.8 B**	0.16 ± 0.41 A	0.03 ± 0.14 B	0.00 ± 4.66 B
CT x Bt cotton	NT x Bt cotton	0.58 ± 1.85 A	0.20 ± 0.62 B	**0.5 ± 0.16 B**	26.71 ± 14.03 B
CT x Bt cotton	NT x non Bt cotton	1.75 ± 1.85 A	1.375 ± 0.62 B	0.03 ± 0.16 B	10.46 ± 14.03 B
CT x non Bt cotton	NT x Bt cotton	2.95 ± 1.85 A	0.37 ± 0.62 B	**0.46 ± 0.16 B**	26.71 ± 14.03 B
CT x non Bt cotton	NT x non Bt cotton	4.12 ± 185 A	**1.54 ± 0.62 B**	0.00 ± 0.16 A	10.46 ± 14.03 B
NT x Bt cotton	NT x non Bt cotton	1.16 ± 0.8 A	**1.16 ± 0.41 B**	**0.46 ± 0.14**	**16.25 ± 4.66 A**

Table 2.5: Two-way interactions for individual taxa between tillage and cotton residue type (April 2004, 2005). Values are least square mean differences between interaction means for arthropod abundance (±SE). Values in bold are significant ($P<0.05$) and letter (A or B) denotes the interaction column for which the abundance of a particular taxa is highest.

		April Tillage x Residue Interactions		
		2004		2005
A	B	Terebrantia	Aphididae	Formicidae
CT x Bt cotton	CT x non Bt cotton	8.95 ± 4.33 A	2.41 ± 2.14 B	**3.66 ± 1.36 B**
CT x Bt cotton	NT x Bt cotton	**20.12 ± 4.33 A**	**9.5 ± 2.76 B**	4.16 ± 2.19 B
CT x Bt cotton	NT x non Bt cotton	**14.87 ± 4.33 A**	5.37 ± 2.76 B	3.00 ± 2.19 B
CT x non Bt cotton	NT x Bt cotton	**11.16 ± 4.33 A**	**7.08 ± 2.76 B**	0.5 ± 2.19 B
CT x non Bt cotton	NT x non Bt cotton	5.91 ± 4.33 A	2.95 ± 2.76 B	0.66 ± 2.19 A
NT x Bt cotton	NT x non Bt cotton	5.25 ± 4.33 B	4.12 ± 2.14 A	1.16 ± 1.36 A

Table 2.6: Two-way interactions for individual families between tillage and cover crop residue (October 2005). Values are least square mean differences between interaction means for arthropod abundance (±SE). Values in bold are significant (P<0.05) and letter (A or B) denotes the interaction column for which the abundance of a particular taxon is highest.

October 2005 Tillage x Residue Interaction

A	B	Entomobryidae
CT x clover	CT x rye	7.46 ± 5.33 A
CT x clover	NT x clover	2.96 ± 6.67 A
CT x clover	NT x rye	11.06 ± 6.67 B
CT x rye	NT x clover	4.5 ± 6.67 B
CT x rye	NT x rye	**18.53 ± 6.67 B**
NT x clover	NT x rye	**14.03 ± 5.33 B**

Table 2.7: Two-way interactions for the family Miridae between cover crop and cotton residue type (April 2004). Values are least square mean differences between interaction means for mirid abundance (±SE). Values in bold are significant (P<0.05) and letter (A or B) denotes the interaction column for which the abundance of mirids is highest.

April 2004 Crop x Residue Interaction

A	B	Miridae
clover x Bt cotton residue	clover x nonBt cotton residue	**26.91 ± 5.72 A**
clover x Bt cotton residue	rye x Bt cotton residue	**24.87 ± 5.82 A**
clover x Bt cotton residue	rye x nonBt cotton residue	**20.25 ± 5.82 A**
clover x nonBt cotton residue	rye x Bt cotton residue	2.04 ± 5.82 B
clover x nonBt cotton residue	rye x nonBt cotton residue	6.66 ± 5.82 B
rye x Bt cotton residue	rye x nonBt cotton residue	4.62 ± 5.72 B

Table 2.8: Two-way interactions for individual families between cotton type and cover crop residue (October 2005). Values are least square mean differences between interaction means for arthropod abundance (±SE). Values in bold are significant (P<0.05) and letter (A or B) denotes the interaction column for which the abundance of a particular taxa is highest.

		October 2005 Crop x Residue Interactions		
A	B	RIFA	Cicadellidae	nonRIFA
Bt cotton x clover residue	nonBt cotton x clover residue	42.59 ± 24.11 A	0.41 ± 0.39 A	5.25 ± 5.5 B
Bt cotton x clover residue	Bt cotton x rye residue	**52.90 ±29.46 A**	0.83 ± 0.45 A	**12.29 ± 5.5 B**
Bt cotton x clover residue	nonBt cotton x rye residue	13.46 ± 29.46 A	0.04 ± 0.45 A	0.20 ± 5.50 A
nonBt cotton x clover residue	Bt cotton x rye residue	10.31 ± 29.46 A	0.41 ± 0.45 A	7.04 ± 5.5 B
nonBt cotton x clover residue	nonBt cotton x rye residue	29.12 ± 29.46 B	0.37 ± 0.45 B	5.45 ± 5.50 A
Bt cotton x rye residue	nonBt cotton x rye residue	39.43 ± 24.11 B	**0.79 ± 0.39 B**	**12.5 ± 5.5 A**

Table 2.9: Three-way interactions for individual families (April 2004, 2005). Values are least square mean differences between interaction means for arthropod abundance, standard error and P-value respectively. Values in bold are significant ($P<0.05$) and symbol (positive or negative) denotes the interaction column for which the abundance of a particular taxa is highest.

April 3-Way Interactions

		2004						2005		
Interactions		Cicadellidae			Acrididae			Aphididae		
(+)	**(-)**	estimate	error	p	estimate	error	p	estimate	error	p
CTxcloverxBt rez	CTxcloverxnonBtrez	-0.4375	0.6705	0.5155	-0.25	0.2477	0.3158	-4.9167	3.0391	0.1099
CTxcloverxBt rez	CTxryexBtrez	0.625	0.7902	0.4419	-0.1667	0.2477	0.5029	-5.9167	3.5068	0.1123
CTxcloverxBt rez	CTxryexnonBtrez	0.5	0.7902	0.5369	0.08333	0.2477	0.7374	-5.8333	3.5068	0.117
CTxcloverxBt rez	NTxcloverxBtrez	**-2.1875**	**0.848**	**0.0165**	0.08333	0.2703	0.7596	**-11.3333**	**3.7171**	**0.0053**
CTxcloverxBt rez	NTxcloverxnonBtrez	-0.75	0.848	0.3854	0.4167	0.2703	0.1319	-1.9167	3.7171	0.6106
CTxcloverxBt rez	NTxryexBtrez	-0.1875	0.848	0.8269	0.4167	0.2703	0.1319	**-13.5833**	**3.7171**	**0.0012**
CTxcloverxBt rez	NTxryexnonBtrez	-1.72	0.8691	0.0587	0.1667	0.2703	0.5414	**-14.75**	**3.7171**	**0.0005**
CTxcloverxBt rez	CTxryexBtrez	1.0625	0.7902	0.1997	0.08333	0.2477	0.7374	-1	3.5068	0.7794
CtxcloverxnonBtrez	CTxryexnonBtrez	0.9375	0.7902	0.2548	0.3333	0.2477	0.1821	-0.9167	3.5068	0.7973
CtxcloverxnonBtrez	NTxcloverxBtrez	-1.75	0.848	0.0502	0.3333	0.2703	0.2255	-6.4167	3.7171	0.0965
CtxcloverxnonBtrez	NTxcloverxnonBtrez	-0.3125	0.848	0.7158	**0.6667**	**0.2703**	**0.0185**	3	3.7171	0.4271
CtxcloverxnonBtrez	NTxryexBtrez	0.25	0.848	0.7707	**0.6667**	**0.2703**	**0.0185**	**-8.6667**	**3.7171**	**0.0279**
CtxcloverxnonBtrez	NTxryexnonBtrez	-1.2825	0.8691	0.1522	0.4167	0.2703	0.1319	**-9.8333**	**3.7171**	**0.0138**
CTxryexBtrez	CTxryexnonBtrez	-0.125	0.6705	0.8525	0.25	0.2477	0.3158	0.08333	3.0391	0.9782
CTxryexBtrez	NTxcloverxBtrez	**-2.8125**	**0.848**	**0.0029**	0.25	0.2703	0.3612	-5.4167	3.7171	0.1573
CTxryexBtrez	NTxcloverxnonBtrez	-1.375	0.848	0.1181	**0.5833**	**0.2703**	**0.0376**	4	3.7171	0.292
CTxryexBtrez	NTxryexBtrez	-0.8125	0.848	0.3477	**0.5833**	**0.2703**	**0.0376**	**-7.6667**	**3.7171**	**0.0495**
CTxryexBtrez	NTxryexnonBtrez	**-2.345**	**0.8691**	**0.0122**	0.3333	0.2703	0.2255	**-8.8333**	**3.7171**	**0.0253**
CTxryexnonBtrez	NTxcloverxBtrez	**-2.6875**	**0.848**	**0.0042**	1.39E-17	0.2703	1	-5.5	3.7171	0.1513
CTxryexnonBtrez	NTxcloverxnonBtrez	-1.25	0.848	0.1536	0.3333	0.2703	0.2255	3.9167	3.7171	0.302
CTxryexnonBtrez	NTxryexBtrez	-0.6875	0.848	0.4256	0.3333	0.2703	0.2255	**-7.75**	**3.7171**	**0.0473**
CTxryexnonBtrez	NTxryexnonBtrez	**-2.22**	**0.8691**	**0.0169**	0.08333	0.2703	0.7596	**-8.9167**	**3.7171**	**0.0241**
NTxcloverxBtrez	NTxcloverxnonBtrez	**1.4375**	**0.6705**	**0.0343**	0.3333	0.2477	0.1821	**9.4167**	**3.0391**	**0.0027**
NTxcloverxBtrez	NTxryexBtrez	**2**	**0.7902**	**0.0237**	0.3333	0.2477	0.1821	-2.25	3.5068	0.5308
NTxcloverxBtrez	NTxryexnonBtrez	0.4675	0.8128	0.5733	0.08333	0.2477	0.7374	-3.4167	3.5068	0.3454
NTxcloverxnonBtrez	NTxryexBtrez	0.5625	0.7902	0.488	0	0.2477	1	**-11.6667**	**3.5068**	**0.0046**
NTxcloverxnonBtrez	NTxryexnonBtrez	-0.97	0.8128	0.2504	-0.25	0.2477	0.3158	**-12.8333**	**3.5068**	**0.0023**
NTxryexBtrez	NTxryexnonBtrez	**-1.5325**	**0.697**	**0.03**	-0.25	0.2477	0.3158	-1.1667	3.0391	0.7021

Table 2.10: Three-way interactions for individual families (October 2005). Values are least square mean differences between interaction means for arthropod abundance, standard error and P-value respectively. Values in bold are significant ($P<0.05$) and symbol (positive or negative) denotes the interaction column for which the abundance of a particular taxa is highest.

		October 2005 3-Way Interactions					
Interactions		Isotomidae			Formicidae (non-*S. invicta*)		
(+)	**(-)**	estimate	error	p	estimate	error	p
CTxBtxcloverrez	CTxnonBtxcloverrez	0.25	0.6765	0.716	5.5	7.78	0.4887
CTxBtxcloverrez	CTxBtxryerez	0.4375	0.6765	0.526	4.9167	7.78	0.5354
CTxBtxcloverrez	CTxnonBtxryerez	-0.5	0.6765	0.4693	1.9167	7.78	0.8082
CTxBtxcloverrez	NTxBtxcloverrez	-0.5	0.8489	0.5635	-13.3333	16.1531	0.4313
CTxBtxcloverrez	NTxnonBtxcloverrez	**-2.25**	**0.8489**	**0.0167**	-29.3333	16.1531	0.104
CTxBtxcloverrez	NTxBtxryerez	-1.1875	0.8489	0.1797	**-42.8333**	**16.1531**	**0.0273**
CTxBtxcloverrez	NTxnonBtxryerez	-0.625	0.8489	0.4715	-14.8333	16.1531	0.3833
CTxnonBtxcloverrez	CTxBtxryerez	0.1875	0.6765	0.7848	-0.5833	7.78	0.9411
CTxnonBtxcloverrez	CTxnonBtxryerez	-0.75	0.6765	0.2821	-3.5833	7.78	0.6506
CTxnonBtxcloverrez	NTxBtxcloverrez	-0.75	0.8489	0.3892	-18.8333	16.1531	0.2747
CTxnonBtxcloverrez	NTxnonBtxcloverrez	**-2.5**	**0.8489**	**0.009**	-34.8333	16.1531	0.0606
CTxnonBtxcloverrez	NTxBtxryerez	-1.4375	0.8489	0.1085	**-48.3333**	**16.1531**	**0.0158**
CTxnonBtxcloverrez	NTxnonBtxryerez	-0.875	0.8489	0.317	-20.3333	16.1531	0.241
CTxBtxryerez	CTxnonBtxryerez	-0.9375	0.6765	0.1827	-3	7.78	0.7043
CTxBtxryerez	NTxBtxcloverrez	-0.9375	0.8489	0.2847	-18.25	16.1531	0.2889
CTxBtxryerez	NTxnonBtxcloverrez	**-2.6875**	**0.8489**	**0.0056**	-34.25	16.1531	0.0642
CTxBtxryerez	NTxBtxryerez	-1.625	0.8489	0.0724	**-47.75**	**16.1531**	**0.0167**
CTxBtxryerez	NTxnonBtxryerez	-1.0625	0.8489	0.2275	-19.75	16.1531	0.2537
CTxnonBtxryerez	NTxBtxcloverrez	-7.49E-16	0.8489	1	-15.25	16.1531	0.3707
CTxnonBtxryerez	NTxnonBtxcloverrez	-1.75	0.8489	0.0547	-31.25	16.1531	0.0863
CTxnonBtxryerez	NTxBtxryerez	-0.6875	0.8489	0.4291	**-44.75**	**16.1531**	**0.0225**
CTxnonBtxryerez	NTxnonBtxryerez	-0.125	0.8489	0.8846	-16.75	16.1531	0.3278
NTxBtxcloverrez	NTxnonBtxcloverrez	**-1.75**	**0.6765**	**0.0186**	-16	7.78	0.0545
NTxBtxcloverrez	NTxBtxryerez	-0.6875	0.6765	0.3229	**-29.5**	**7.78**	**0.0013**
NTxBtxcloverrez	NTxnonBtxryerez	-0.125	0.6765	0.8555	-1.5	7.78	0.8493
NTxnonBtxcloverrez	NTxBtxryerez	1.0625	0.6765	0.1337	-13.5	7.78	0.0998
NTxnonBtxcloverrez	NTxnonBtxryerez	**1.625**	**0.6765**	**0.0273**	14.5	7.78	0.0788
NTxBtxryerez	NTxnonBtxryerez	0.5625	0.6765	0.4166	**28**	**7.78**	**0.0021**

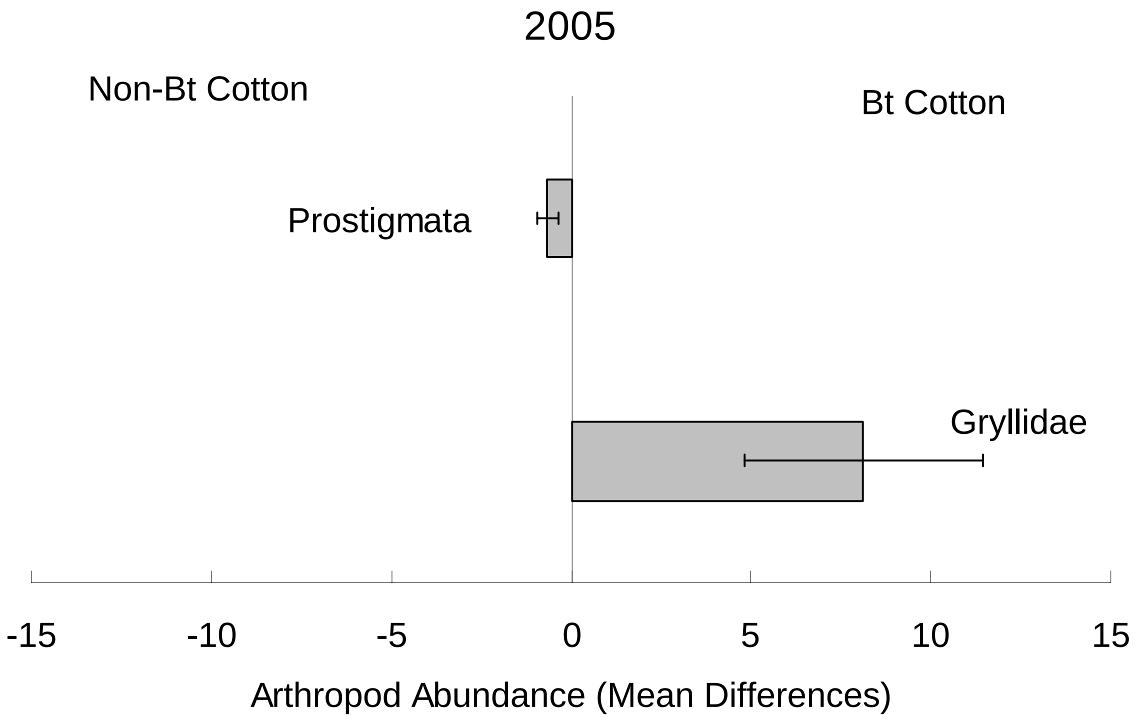


Figure 2.1: Least square mean differences (±SE) between cotton type effects on arthropod abundance during October 2005. All taxa shown represent groups whose abundances were significantly different between the respective treatments based on mixed model analysis ($P < 0.05$).

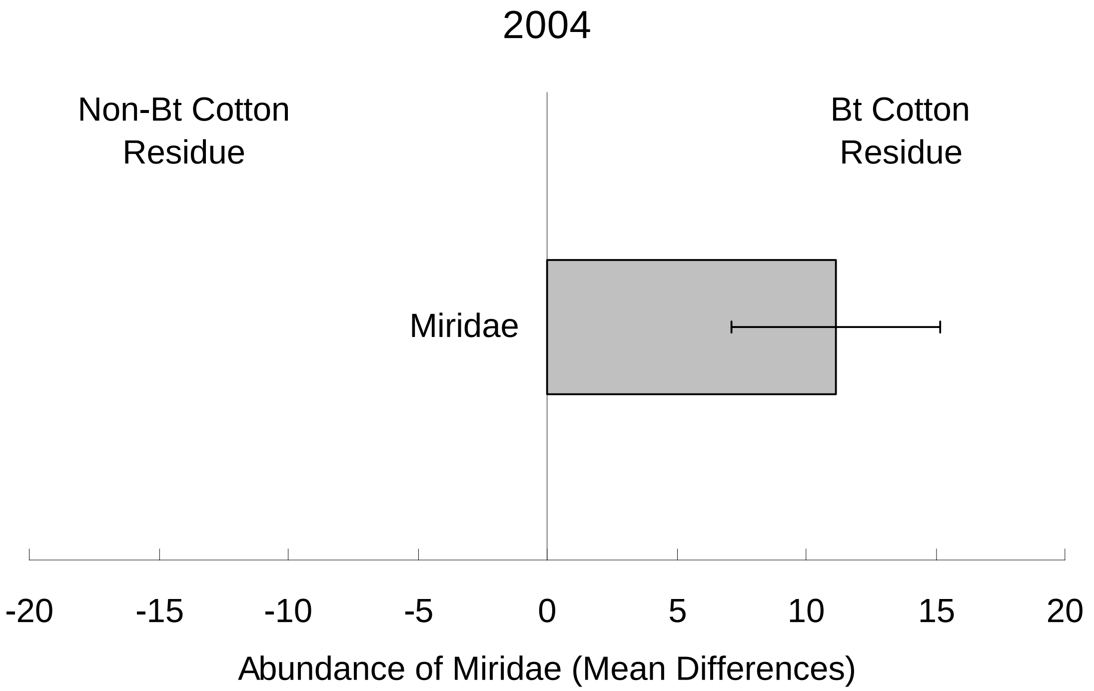


Figure 2.2: Least square mean differences (±SE) between cotton residue type effects on abundance of the family Miridae during April 2004. All taxa shown represent groups whose abundances were significantly different between the respective residue types based on mixed model analysis and regardless of tillage and cover crop type ($P < 0.05$).

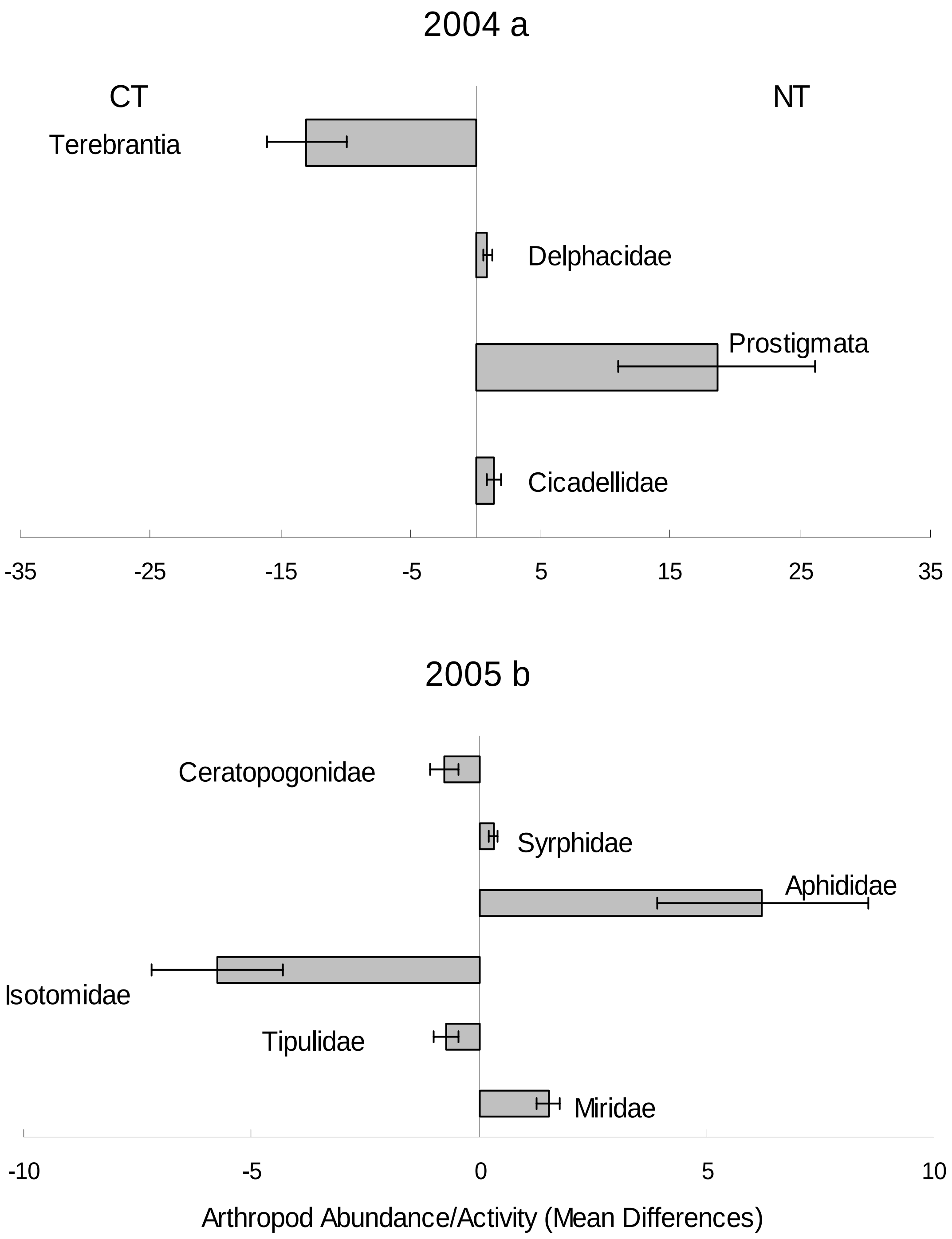
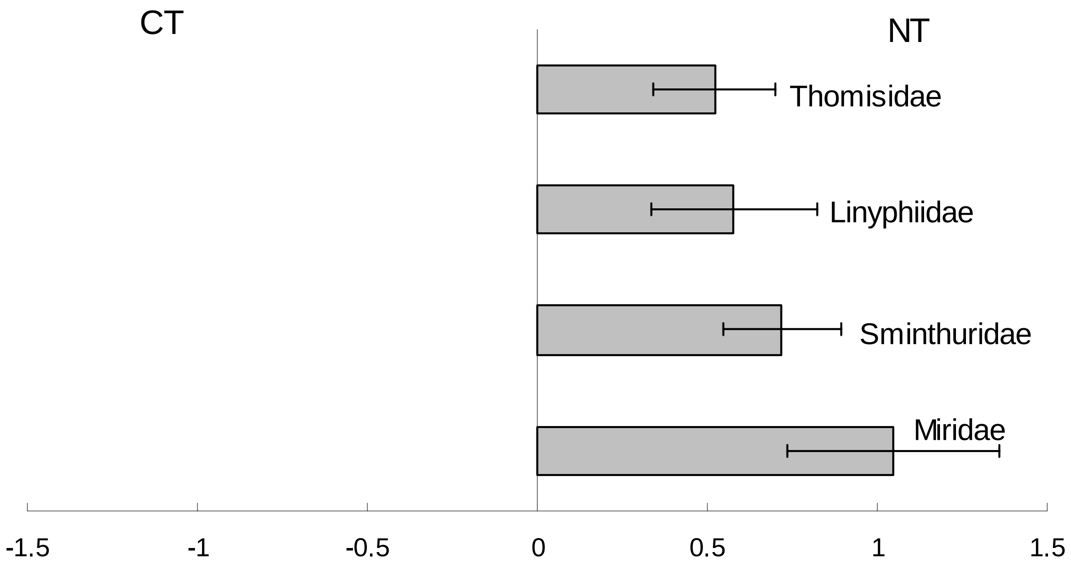
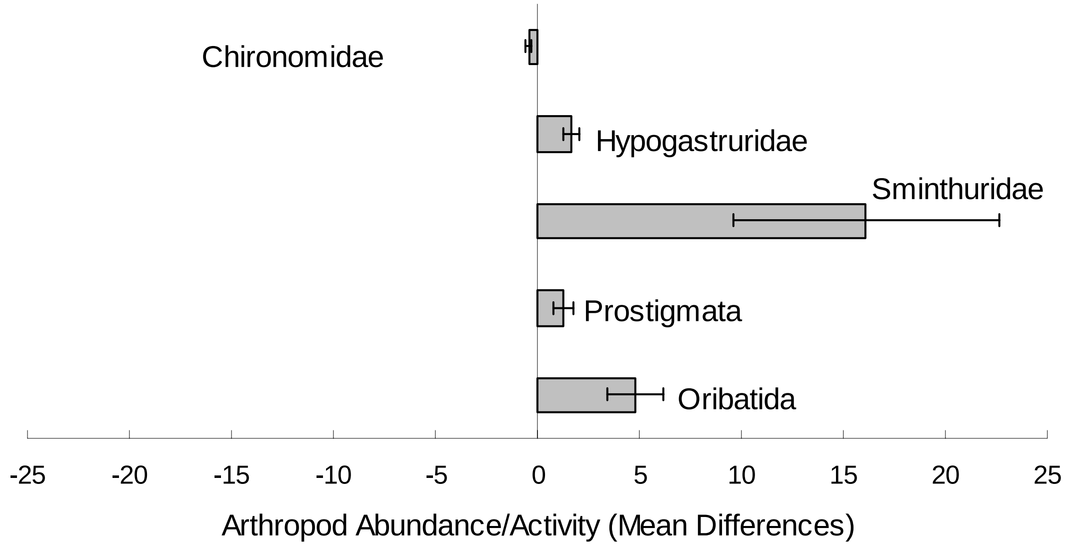

2004 a
CT
NT
Terebrantia
Delphacidae
Prostigmata
Cicadellidae
-35
-25
-15
-5
5
15
25
35
2005 b
Ceratopogonidae
Syrphidae
Aphididae
Isotomidae
Tipulidae
Miridae
-10
-5
0
5
10
Arthropod Abundance/Activity (Mean Differences)

Figure 2.3: Least square mean differences (±SE) between conventional (CT) and no-till (NT) effects on abundance of arthropod taxa during April 2004(a) and 2005(b). All taxa shown represent groups whose abundances were significantly different between the respective treatments based on mixed model analysis ($P < 0.05$).



2004 a

CT
NT
Thomisidae
Linyphiidae
Sminthuridae
Miridae
-1.5
-1
-0.5
0
0.5
1
1.5



2005 b

Chironomidae
Hypogastruridae
Sminthuridae
Prostigmata
Oribatida
-25
-20
-15
-10
-5
0
5
10
15
20
25
Arthropod Abundance/Activity (Mean Differences)

Figure 2.4: Least square mean differences (±SE) between conventional (CT) and no-till (NT) effects on abundance of arthropod taxa during October 2004(a) and 2005(b). All taxa shown represent groups whose abundances were significantly different between the respective treatments based on mixed model analysis ($P < 0.05$).

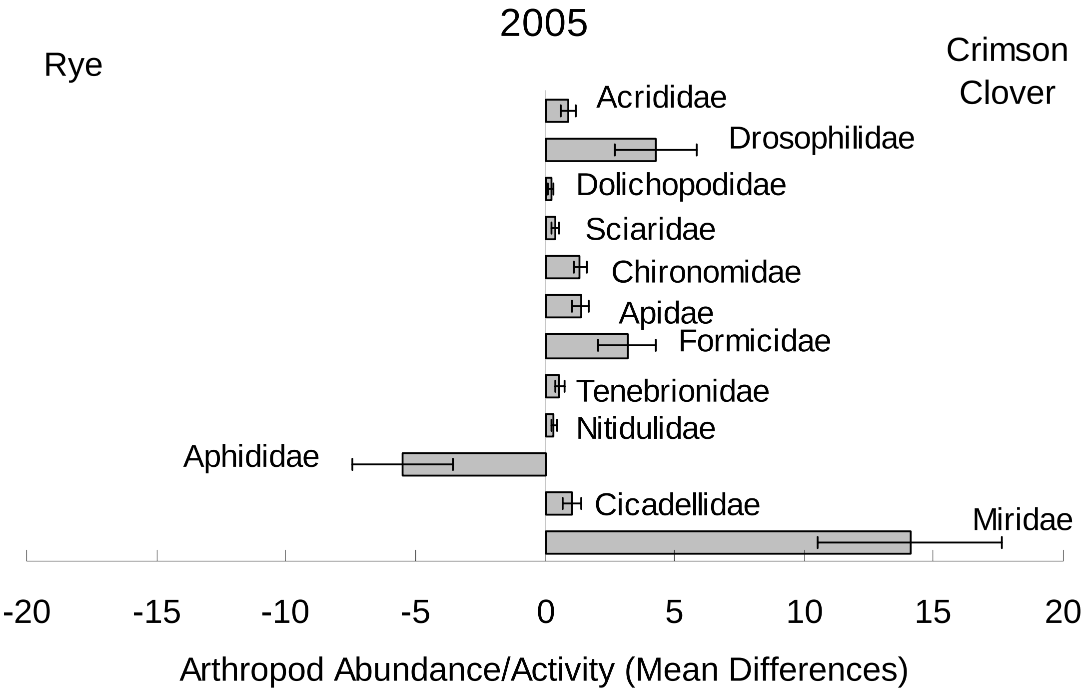


Figure 2.5: Least square mean differences (±SE) between cover crop effects on arthropod abundance during April 2005. All taxa shown represent groups whose abundances were significantly different between the respective treatments based on mixed model analysis (P <0.05).

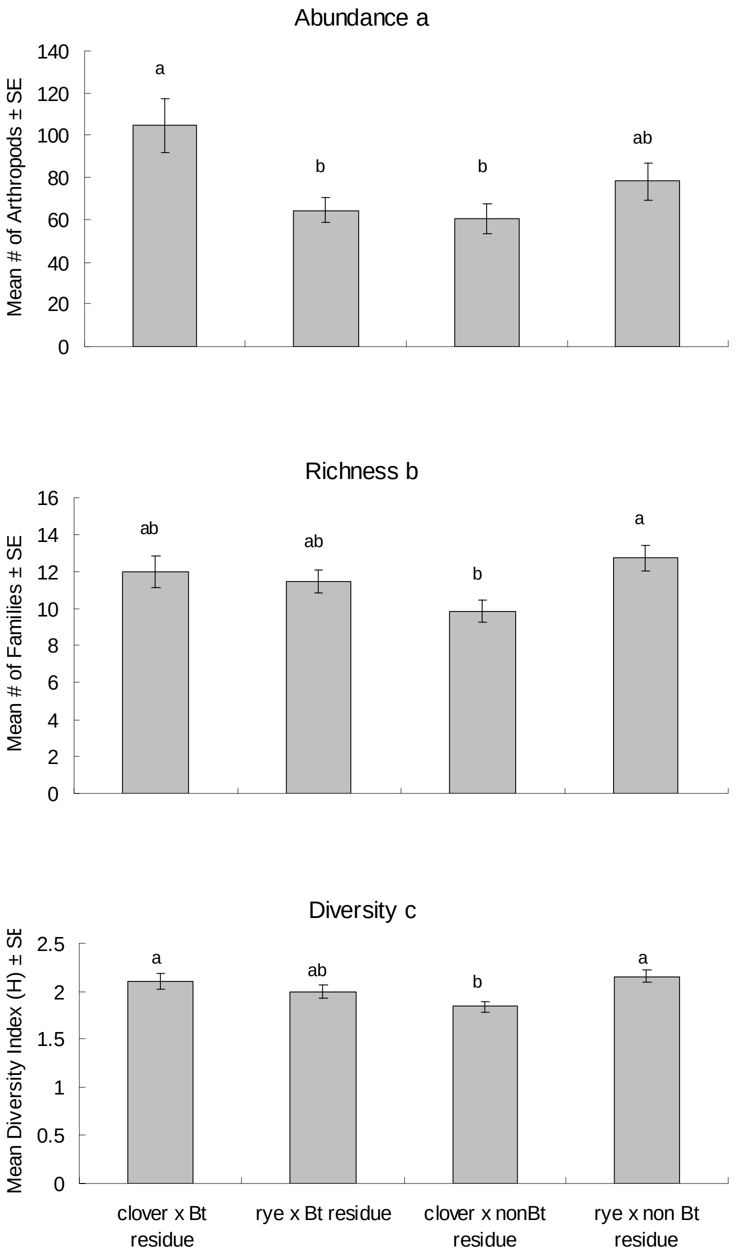


Figure 2.6: Cover crop by cotton residue type interaction effects (±SE) during April 2004. Data represent mean number of individuals(a), mean number of families(b) and mean diversity index using the Shannon index (H) (c). Significant differences were calculated by mixed model analysis and are marked by letters ($P<0.05$).

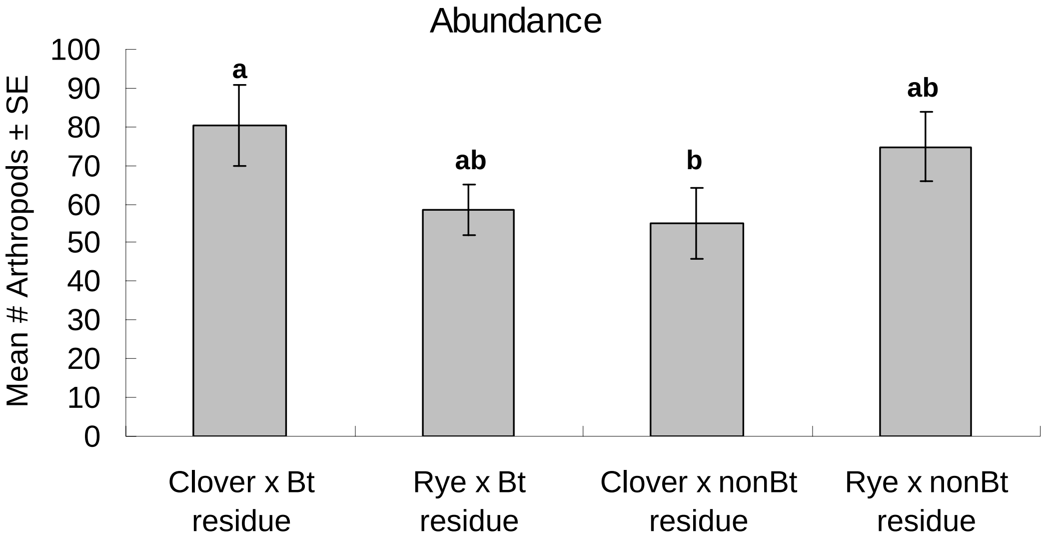


Figure 2.7: Cover crop by cotton residue type interaction effects (±SE) on arthropod abundance during April 2005. Data represent mean number of individuals. Significant differences were calculated by mixed model analysis and are marked by letters (P<0.05).

CHAPTER 3

IMPACT OF THE RED IMPORTED FIRE ANT (*SOLENOPSIS INVICTA*) ON SOIL ARTHROPOD COMMUNITIES OF COTTON AGROECOSYSTEMS[2]

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[2] Wickings, K. G. and J. Ruberson. To be submitted to *Pedobiologia*.

ABSTRACT

The Red Imported Fire Ant (*Solenopsis invicta*) is a common arthropod in most, if not all, disturbed soils of the southern United States. Aside from their well-understood role in aboveground food webs, there is also a limited body of evidence suggesting that *S. invicta* may have a significant impact on soil fauna. This study examined the influence of fire ants on the arthropod community both at and below the soil surface of cotton fields over the course of two growing seasons at two field sites operated by the University of Georgia in Athens and Tifton, GA. Arthropods were collected at the soil surface using week-long pitfall trap trials while soil arthropods were collected using heat extraction from soil cores once during each sampling month. Sampling was conducted from June through September, 2006, in Athens, GA, and from July through September, 2007, in Tifton, GA and was designed to test the following hypothesis: H_1 – Individual families, both at and below the soil surface, will be significantly affected by the removal of *Solenopsis invicta*; however, while the abundance of some families will increase, that of others will decrease.

Results indicate that although *S. invicta* did not significantly affect total arthropod abundance, richness or diversity at either field site, it did significantly alter the abundance of specific taxonomic groups. Specifically, the abundance of predators and parasitoids (carabid beetles, erythraeid and cunaxid mites, linyphiid spiders and scelionid wasps) increased in the presence of *S. invicta* at the Athens, GA field site. At the Tifton, GA field site the striped earwig, *Labidura riparia* dominated the predator community and, unlike the response of predators at the Athens site, was significantly less abundant in the presence of *S. invicta*. Staphylinid beetles were significantly influenced by fire ants but in opposite directions at the two field sites. This may reflect a species level response that we were unable to detect at the family level. Although they were composed of different taxonomic groups, detritivorous mites and collembola responded similarly to the removal of *S. invicta* at both field sites with an increase in abundance. The abundance of thrips also increased in the absence of *S. invicta* at both field sites.

This study demonstrates that *Solenopsis invicta* is capable of significantly influencing soil arthropod communities both at the soil surface and within the soil matrix. Variation between sites may be attributable to factors such as weed biomass and methodological differences between our field sites and is further discussed. The results from this experiment have implications for both above- and below-ground community dynamics and processes and the specific trophic interactions causing the effects noted should be further explored.

Keywords: *Solenopsis invicta*, soil ecology, agroecosystem, cotton, biodiversity

INTRODUCTION

From seed harvesters to scavengers and top predators, ants can occupy many trophic positions within ecosystems. Mutualism is also quite common between ants and plants, as well as with other arthropods, including honeydew-producing hemipterans (Hölldobler and Wilson 1990). Below-ground, ants are known to fill an equally diverse set of ecological functions. As ecosystem engineers, ants have been found to increase soil moisture, phosphorus and potassium, while decreasing soil bulk density (Dostal *et al.* 2005; Boulton & Amberman 2006). This, combined with their tendency to create localized patches of organic material through refuse piles in nests, can lead to increased root biomass in nest soils (Boulton & Amberman 2006). Ants are also known to engage in mutualistic and facilitative relationships below-ground. The tending of root aphids by *Lasius neoniger* Emery (Hymenoptera: Formicidae) in corn fields is noted in Hölldobler and Wilson (1990). The nests of ants are also known to host a diverse biota including microbes, micro-and macro-arthropods and earthworms (Hölldobler 1990; Laakso & Setala 1998). There is also evidence that ants function as predators in soil systems. For example, Wilson (2005) observed ants in the genus *Pheidole* specializing on a selective range of mite species in the suborder Oribatida. In contrast, other studies have found ants to have little to no effect on soil fauna (Lenoir *et al.* 2003). The authors suggest that this may be due to the high degree of heterogeneity in the abundance and composition of soil fauna.

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), has been found to directly increase predation in terrestrial systems by 20 to 30%, but is also known to decrease the abundance of many beneficial predators including multiple species from the orders Coleoptera and Neuroptera, as well as from the class Arachnida (Eubanks *et al.* 2002; Diaz *et al.* 2004). *Solenopsis invicta* commonly enters into mutualistic exchanges with aphids. Kaplan &

Eubanks (2002) discovered a positive correlation between fire ant density and aphid survival. Below-ground, fire ants have been studied primarily for their role as ecosystem engineers, and have been found to alter soil aggregate structure and increase water infiltration (Green *et al.* 1999), alter soil nutrients, increase soil organic matter and decrease soil bulk density (Lafleur *et al.* 2005). Trophic interactions between *S. invicta* and other soil fauna, however, remain largely overlooked. Vinson (1991) assessed predation events and trophic interactions among detritivores in the presence and absence of *S. invicta* using pieces of rotting fruit as bait stations and determined that in the presence of *S. invicta,* the abundance of fly larvae, sap beetles, rove beetles, and parasitic wasps was significantly decreased. Vinson's study provides insight into the aggressive nature of *S. invicta* when defending large, high quality food sources, and although this study assessed trophic interactions between *S. invicta* and an array of detritivorous arthropods it may be a poor estimate of the impact of these ants in soil foodwebs, especially in systems without such resources. A number of myrmecophiles including mites, millipedes, beetles, flies, hemipterans, parasitic wasps, butterflies, crickets and silverfish, as well as other ants, are also common inhabitants of fire ant nests (Wojcik 1990).

In North America, the red imported fire ant is an exotic arthropod. It is a highly efficient forager and functions largely as a scavenger and generalist predator. Accordingly, the most abundant prey available will often become the prey of choice (Tschinkel 2006). These traits, combined with their high abundance in disturbed agricultural soils, suggest that unlike other ants, fire ants may have a significant effect on soil arthropod communities in invaded habitats. The objective of the current study was to examine the impact of the removal of *Solenopsis invicta* from a typical cotton agroecosystem on the extant soil arthropod community in 2006 and 2007 (one season from each of two field sites).

We tested the following hypothesis:

> H_1 – Individual families, both at and below the soil surface, will be significantly affected by the removal of *Solenopsis invicta*; however, while the abundance of some families will increase, that of others will decrease.

RATIONALE

While past research has demonstrated that fire ants can significantly alter arthropod communities on plant foliage and at the soil surface, each has examined this impact in habitats with localized resources (e.g., aphid honeydew in cotton foliage, rotting fruit masses at soil surface). Resource distribution within the soil matrix of cotton systems can vary based on the management strategies employed in a particular system (e.g., weed management, tillage strategy) but in general, we consider the detrital resource base in these systems to be fairly homogeneous and of low quality. Due to the uniformity in resources we do not expect fire ants to prey heavily upon all taxa within the soil food web. Instead we predict that while fire ants may prey specifically on some taxa, other taxa will benefit from the activities of fire ants.

MATERIALS AND METHODS

Field Sites and Fire Ant Exclusion

This study was conducted at two University of Georgia field stations: the Horseshoe Bend Research Station in Athens, GA, and the Coastal Plain Experiment Station in Tifton, GA, which are approximately 320 km apart. Horseshoe Bend is a 0.8 hectare farm composed of eight main plots (four no-till, four conventional till) and thirty two subplots planted with a Bt cotton summer crop and either rye or clover as a winter cover crop. Four conventionally tilled plots measuring 28x28 m each were chosen for our study. All plots were planted with Bt cotton (variety DPL 555BR, which is genetically modified to express Cry1Ac toxin and for tolerance to the herbicide glyphosate). Between 15 and 18 May, 2006, a barrier was constructed out of aluminum flashing and buried to a depth of 13 cm to minimize lateral tunneling by ants under the barrier (Stiles & Jones 2001). The placement of this barrier ran evenly between the plots such that two entire plots were on each side. All plots on one side of the barrier were treated on 10 June and 23 July, 2006, with hydramethylnon fire ant bait (Amdro®, Ambrands) at a rate of 1.1 kg of formulated bait per ha to eliminate fire ants. Vegetation was regularly trimmed along the barrier wall to minimize ant crossing. Observations were conducted on a daily basis to assess the effectiveness of the exclusion. Due to the development of a significant weed community during the growing season, weed biomass estimates were also taken at the end of the growing season (27 October). All aboveground weed biomass was collected from eight randomly-selected $0.5m^2$ areas within each of the four treatment plots. Biomass was oven-dried at 50°C and subsequently weighed. Data are presented as grams of above-ground weed biomass per m^2.

The Tifton, GA, site is 1.62 ha also composed of 8 main plots (0.2 ha each). However, at the Tifton location all plots were managed under conventional tillage for the duration of the current study. All plots were planted with Bt cotton (DPL 555BR) on 4 June, 2007. Plots were separated from one another by open gaps of 3 m of bare soil tilled at regular intervals, rather than an aluminum flashing barrier as was used at Horseshoe Bend. The plots were arranged in 4 blocks, each containing one fire ant inclusion plot and one fire ant exclusion plot. Plots were approximately square, and a 10x10m area in the center of each plot was designated for sampling. Fire ant exclusion plots were treated with hydramethylnon ant bait at the rate noted above on 28 June, 16 July, 4 August, and 22 August 2007 to eliminate fire ants. To assess the exclusion treatment, ant detection tests were conducted on 6 August and 2 September. This test consisted of placing three 33-ml test tubes containing a small piece (5 gm) of hotdog in each plot. After 1 hour all tubes were recovered and sealed, and transported back to the lab where the tubes were emptied and the number of ants was recorded (Table 3.1).

Soil Arthropod Community Assessment - Pitfall Trapping

At the Horseshoe Bend site, pitfall trapping was conducted using 35ml glass test tubes. This size was chosen based on the results from Work *et al.* (2002) suggesting that higher numbers of small traps are better suited for assessing litter dwelling arthropod communities than are lower numbers of large traps. PVC tubes (15cm x 2cm internal diameter) were hammered into the ground to serve as trap sleeves. This was done to reduce the "digging in" effects that often influence pitfall trap catches (Greensla 1973). Ten traps were placed in each plot and were allowed to collect for 5 days over the following periods: 1 – 5 June, 15 – 19 June, 28 July – 1 August, 17 – 21 August and 14 – 18 September, 2006. Trap contents were regularly emptied at 24-hour intervals and replaced with fresh ethanol. The contents of each trap were bulked into specimen cups so each sample cup represented a full five days of trapping. In the lab, trap contents were sorted to eliminate organic debris and soil. All arthropods were then transferred to plastic scintillation vials with 70% ethanol for storage and identification.

At the Tifton, GA, site, pitfall trapping was conducted using 360-ml plastic cups containing a solution of 1% Tween20, 99% de-ionized water and approximately 3-5 pellets of NaCl (water softener tablets). Tween20 serves as a surfactant to break surface tension while NaCl was used as a preservative. In total, the traps contained 50 ml of solution. Trapping was conducted over a 3-week period during 2007 (20 – 27 July, 28 July – 3 August, and 4 – 10 August) and during each week five pitfall traps were set in each sampling plot. During this period, traps remained in the field and were not emptied on a daily basis. On the last day of each sample week trap contents were removed and transported to the lab, where the contents were sorted to eliminate organic debris and soil. All specimens were then transferred to 70% ethanol and stored in plastic scintillation vials for identification.

Soil Arthropod Community Assessment - Soil Sampling

At the Horseshoe Bend site all sampling occurred from early June until mid-September of 2006 (3 June, 30 June, 1 August, 28 August and 18 September). At each sampling event ten soil samples, were taken from the top 0-5cm of soil in each plot. Each core, 5cm in diameter, was placed on a Tullgren-type funnel for heat extraction of soil arthropods, the extraction procedure lasting 7 days (Crossley & Blair 1991). Soil arthropods were collected from the funnels into plastic scintillation vials containing 70% ethanol.

At the Tifton site sampling occurred on 17 August and 13 September, 2007. On both sampling dates five soil cores, approximately 5 cm in diameter were taken from the top 0-5 cm of soil from each of the 8 plots. Each core was placed on a Tullgren-type funnel for heat extraction of soil arthropods, the extraction procedure lasting 7 days, again following the procedure outlined by Crossley and Blair (1991). Arthropods were placed into 70% ethanol in plastic scintillation vials.

Arthropod Identification

Taxa initially were identified to the family level where possible following Triplehorn & Johnson (2005), and were further partitioned into morpho-species. Soil mite identification was conducted using keys provided by the Ohio State Soil Acarology Summer Program along with an interactive computer key to Mesostigmata (Lucid Player Standard v2.2).

Statistical Analysis

Total abundance, richness and diversity (Shannon - H) were calculated using row and column summary analysis in PC-ORD (McCune & Grace 2002). To compare among treatments in the nested design, data were square root transformed and mixed model analysis for repeated measures was conducted using SAS software version 8 for Windows, with ant status (present or absent) as a fixed effect and with plot as a random variable (SAS 1999). After analysis of abundance, richness and diversity the abundances of individual families were analyzed using mixed model analysis, again with ant status and plot as fixed and random effects respectively in order to detect significant differences between ant inclusion and exclusion plots.

RESULTS

Soil Surface Arthropods – Horseshoe Bend

Table 3.2 presents a complete list of all taxa collected during the course of the experiments from pitfall traps at both field sites. Mixed model analyses revealed no significant differences in total arthropod abundance, richness or diversity between fire ant inclusion and exclusion plot pitfall traps during any of the five sampling weeks ($P > 0.05$, Table 3.4). Upon analysis of major groups/feeding groups, however, a number of significant differences were detected demonstrating higher relative abundances of many groups in fire ant inclusion plots (Table 3.5). 79% of all Coleoptera were found in inclusion plots during sampling week four. This group was comprised of the families Carabidae, Staphylinidae and Nitidulidae (26, 25 and 49% of total). Additionally, mixed model analysis revealed that the abundance of both Carabidae and Staphylinidae were significantly higher in inclusion plots during this same week ($F_{1, 38}=7.80$, $P=0.008$; $F_{1, 38}=9.27$, $P=0.004$, Figure 3.1). During sampling weeks four and five, 71 and 80% of all hymenopteran parasitoids were collected from fire ant inclusion plots (Table 3.5). Parasitoids included the families Scelionidae, Diapriidae, Cynipidae, Mymaridae, Trichogrammatidae and Eurytomidae (week four, Scelionidae 93%, Diapriidae 7%; week five Scelionidae 65%, Cynipidae 6%, Diapriidae 24% and Eurytomidae 6%). At the family level

Scelionidae were significantly more abundant in inclusion plots during this sampling week ($F_{1, 38}$=8.25, P=0.006, Figure 3.2). Mites of the suborder Mesostigmata were commonly collected in pitfall traps during all five sampling weeks and included the families Pachylaelapidae, Macrochelidae and Uropodidae, however, none were significantly affected by fire ant removal. The suborder Oribatida, composed, in this study, of the families Tectocepheidae, Galumnidae, Oribatulidae, Camisiidae and Euphthiracaridae, was similarly not affected by fire ant exclusion. The mite suborder Prostigmata responded dramatically to the exclusion of fire ants during sampling weeks two, three and four (Table 3.5). The families Eupodidae, Cunaxidae and Erythraeidae were represented during each sampling week and mixed model analysis revealed that the abundance of Erythraeidae was significantly higher in fire ant inclusion plots during week two ($F_{1, 38}$=13.13, P=0.0008, Figure 3.3). Although Collembola, as a group were not affected by fire ant exclusion, the family Hypogastruridae was significantly more abundant in the absence of fire ants during week three ($F_{1, 38}$=17.24, P=0.0002, Figure 3.4). Of the spider families collected (Lycosidae, Linyphiidae and Salticidae) the family Linyphiidae was significantly more abundant in fire ant inclusion plots during week four ($F_{1, 38}$=6.66, P=0.01, Figure 3.5). Finally, thrips in the suborder Terebrantia were also more abundant in the absence of fire ants during sampling week two ($F_{1, 2}$=22.83, P=0.04, Figure 3.6).

Coastal Plain Experiment Station

Mixed model analysis revealed no significant differences in total arthropod abundance, richness or diversity using the Shannon index (P>0.05, Table 3.6). Through analysis of individual families we detected significant differences in the orders Dermaptera, Coleoptera, Collembola, and Thysanoptera. The striped earwig, *Labidura riparia* Pallas (Dermaptera: Labiduridae), was significantly more abundant in the absence of *S. invicta* during sampling weeks two and three ($F_{1, 6}$=8.45, P=0.02; $F_{1, 6}$=10.23, P=0.01, Figure 3.7). It is also worth noting that the abundance of *L. riparia* was marginally greater in the absence of *S. invicta* during sampling week one ($F_{1, 6}$=4.50, P=0.07, Figure 3.7). Staphylinid beetle abundance was highly variable during sampling week one, but during week two staphylinids were significantly more abundant in the absence of *S. invicta* ($F_{1, 6}$=9.68, P=0.02, Figure 3.7). Similarly, Collembola in the family Sminthuridae were significantly more abundant in the absence of *S. invicta* during sampling week three ($F_{1, 6}$=120.54, P<0.0001, Figure 3.7). Lastly, the abundance of the thysanopteran family Thripidae was significantly higher in fire ant exclusion plots during sampling week two ($F_{1, 6}$=5.43, P=0.05, Figure 3.8).

Sub-surface Arthropods – Horseshoe Bend

Table 3.3 presents a complete list of all taxa collected during the course of the experiments from soil cores at both field sites. Similar to the whole-community results from pitfall traps there were no significant differences in total abundance, richness or diversity from soil core extracted arthropods based on mixed model analysis (Table 3.7). There were significant effects of fire ant exclusion on soil arthropods at higher levels of taxonomic resolution, although the effects were less dramatic than those observed for pitfall traps. Major groups collected included predatory Coleoptera, hymenopteran parasitoids, Collembola, Protura, Diplura, Pauropoda, Symphyla, Thysanoptera and mites of the suborders Oribatida, Prostigmata and Mesostigmata (Table 3.2). Of these major groups the suborder Prostigmata was significantly affected by fire ant exclusion during sampling week three, with 67 percent of all individuals having been collected from fire ant inclusion plots (Table 3.8). The oribatid mite family Oribatulidae was significantly more abundant in fire ant exclusion plots based on mixed model analysis during sampling week four ($F_{1, 38}$=4.20, P=0.05, Figure 3.9), whereas the family

Cunaxidae (Prostigmata) was significantly more abundant in fire ant inclusion plots during sampling week three ($F_{1,2}$=16.12, P=0.05, Figure 3.9).

Coastal Plain Experiment Station

Again, no significant differences were found in total arthropod abundance, richness or diversity between fire ant inclusion and exclusion soils at the Tifton field site (Table 3.9). Although 16 families were collected, only Onychiuridae, Laelapidae and carabid larvae were common enough for statistical analysis (Table 3.3). Even among the common families collected, however, there were no significant differences in abundance based on mixed model analysis during any of the three sampling periods between fire ant inclusion and exclusion plots.

DISCUSSION

Most studies examining the impact of ants on soil arthropod communities have been restricted to comparisons between the biota from nests and adjacent soil. These studies demonstrate that across many ant taxa, nests often become nuclei of activity for a variety of soil organisms including fungi, protozoa and many arthropods (Boulton and Amberman 2006; Wagner *et al.* 1997; Zettler *et al.* 2002; Wojcik 1990). The trophic positions assumed by most ant-nest associated taxa include ecto- and endoparasites, predators, trophobionts and scavengers (Wojcik, 1990). Many ants, however, spend a large percentage of their time outside of the mound foraging for food.

In the case of fire ants, trophic interactions that ensue outside of the nest have been well studied in crop canopies. In general, both pest and beneficial arthropods have been found in significantly lower numbers on foliage in the presence of *S. invicta*. However, this impact typically varies at higher levels of taxonomic resolution. For example, while lepidopteran abundance has been found to decrease in the presence of *S. invicta* (Eubanks 2001), aphid abundance increases due to the mutualistic relationship involving aphid honeydew and protection by *S. invicta* (Eubanks 2001; Diaz *et al.* 2004). Further, the mutualism between *S. invicta* and aphids has been found to enhance the strength of the negative effect of fire ants on lepidopteran larvae (Kaplan & Eubanks 2005, 2002). This impact has been found to result in an indirect, positive effect on plant fitness in over 73% percent of studies examined (Styrsky & Eubanks 2007). The impact of fire ants on canopy dwelling predators is similarly mixed. While coccinellid beetles and lacewings have been found to decrease, spiders showed no response to the presence of fire ants (Eubanks *et al.* 2002).

At the soil surface the interactions between ants and other arthropods have been infrequently studied. This is the first study to assess the impact of *Solenopsis invicta* on both soil and surface-active arthropods. At the Horseshoe Bend field site, soil surface predators and parasitoids responding significantly to our ant exclusion treatment included the cicindelline beetle *Megacephala carolina* L. (Coleoptera: Carabidae), the mite family Erythraeidae, and the spider family Linyphiidae. An increase in cidindellines in the presence of fire ants has been observed in previous research (Stimac & Alves 1994); however, this is the first record of an increase in members of the predatory mite family Erythraeidae as well as linyphiid spiders. Interestingly, Porter & Savignano (1990) found that, while erythraeid mites were present in areas where *S. invicta* had not yet invaded, the family completely disappeared in invaded areas. Although the authors did not attempt to interpret the relationship between erythraeids and fire ants, the conflicting results between this and the current study may reflect behavioral differences between fire ants during invasion and in post-invasion circumstances. Cicindellines (chiefly *M. carolina*) as well as other carabids, erythraeids and linyphiids were also collected at the Coastal Plain Experiment Station, but their abundances were much lower at this site and did not differ between fire ant inclusion and exclusion plots. The predator community at the Coastal Plains field site was largely dominated by one species of earwig, *L. riparia*, the abundance of which was significantly higher in the absence of fire ants. This result could not be confirmed at the Horseshoe Bend field site as the order Dermaptera was poorly represented in trap catches. Calixto *et al.* (2006) detected the same pattern upon exclusion of *S. invicta* from a Texas pecan orchard using s-methopren (Extinguish). The authors suggested that the reduction in the abundance of *L. riparia* in the presence of fire ants was caused by either predation or competitive exclusion by *S. invicta.*

Abundance of the hymenopteran parasitoid family Scelionidae was also found to increase at the Horseshoe Bend field site. The members of this family were generally comprised of two wingless species, one of which was a species of the genus *Baeus*, which are known to be spider egg parasitoids, and the other was unidentified. Wojcik (2001, 1990) has reported increases in the abundance of the family Eucharitidae in fire ant mounds and decreases in that of two braconid species but this is the first study to detect a significant interaction between *S. invicta* and the family Scelionidae. Wingless scelionids were also collected at the Coastal Plain field site, but their abundances were low and highly variable across samples resulting in no significant differences between ant inclusions and exclusions.

Compared to the response of predators and parasitoids, the response of surface-active detritivores to fire ant exclusion was generally positive. Although different families were involved at each field site, the abundance of Collembola significantly decreased in the presence of fire ants. Specifically, hypogastrurids and sminthurids were less abundant in fire ant inclusion plots at Horseshoe Bend and at the Coastal Plain field sites, respectively. Sminthurids are highly mobile jumpers while hypogastrurids have short furculae and tend to be restricted to localized patches. Previous work demonstrates that at the ordinal level Collembola can make up a significant portion of the total prey captured by fire ants (Dindal 1990).

Only a handful of studies have documented predation on oribatid mites by ants. For example, Wilson (2005) recently discovered that ants of the genus *Pheidole* prey upon mites in the suborder Oribatida and discovered a strong correlation between ant head width and the body size of the mites that they fed upon. Our study detected a significant decrease in the abundance of oribatulid mites in the presence of fire ants but only at the Horseshoe Bend field site. Although our results may be explained by predation by *S. invicta* on mites in the family Oribatulidae, direct evidence is lacking.

Staphylinid beetles responded significantly to fire ant removal at both field sites during the course of the current study. At Horseshoe Bend, seven morphospecies of staphylinids were collected and, at the family level, their abundance increased in the presence of *S. invicta.* Only two morphospecies were commonly collected at the Coastal Plain field site and, contrary to our findings at Horseshoe Bend, staphylinid abundance decreased in the presence of fire ants. This difference may represent a species level response between *S. invicta* and members of the Staphylinidae; however, our inability to identify staphylinids beyond morphospecies prevented such an analysis. Morrison & Porter (2003) demonstrated a positive correlation between the density of *S. invicta* and species richness of non-ant arthropods, which included staphylinid beetles, providing some support for our findings at the Horseshoe Bend site, but the authors did not report specific results from analyses at the family level nor did they list the species collected.

Until now, interactions between fire ants and thrips have not been investigated. Although thrips are not commonly active at the soil surface as adults they are active both below and at the soil surface during pupation and adult emergence. Thrips were found to be significantly more abundant in the absence of fire ants during individual sampling weeks at both the Horseshoe Bend and Coastal Plain sites. These sampling weeks coincided with early and pre-flowering cotton development at the Horseshoe Bend and Coastal Plain sites, respectively. Although we were unaware of the developmental stages of thrips populations at this time, individuals collected included both immatures and adults. The potential interaction between fire ants and thrips during soil emergence should be investigated further.

Within the soil matrix the response of arthropods to the removal of fire ants was less pronounced than from pitfall trap collections at the soil surface. Nonetheless, at the Horseshoe Bend site there were significant differences found among soil mites in the families Oribatulidae (Acari: Oribatida) and Cunaxidae (Acari: Prostigmata). Oribatulids responded positively to the removal of fire ants, but cunaxids responded negatively. While oribatulids are assumed to function as secondary decomposers (Schneider *et al.* 2004), and cunaxids are predators and their increased abundance in the presence of fire ants may stem from the same mechanism that caused increases in predatory prostigmatids at the soil surface. The soil arthropod community at the Tifton field site was not as abundant as that from the Horseshoe Bend field site, likely due to the aggressive tillage practices. Many of the same families were collected at both sites including, Onychiuridae, Sminthuridae, Isotomidae, Hypogastruridae, Laelapidae, Scheloribatidae, Blattisociidae, Erythraeidae, Eupodidae and larvae of Carabidae. Nevertheless, fire ants did not appear to affect these taxa within the Tifton soils. As noted by Tschinkel (2006), prey items in high abundance often become the prey of choice for fire ants. This may be the primary reason for the lack of a significant effect of fire ants on the soil fauna at the Coastal Plain site as abundances were generally lower in these soils than in those at Horseshoe Bend.

Another important distinction that may help to explain the conflicting results from both soil surface and subsurface taxa between our two field sites was the difference in weed communities. During 2006, Horseshoe Bend had a fairly diverse weed community composed of palmer amaranth (pigweed), *Amaranthus palmeri* L., and sicklepod, *Senna obtusifolia* L., as well as multiple nightshades and grasses. This amounted to an average of 16.43±3.00 grams of dry biomass per meter squared (Figure 3.10). Although the amount of weed biomass varied among treatment plots this variation was not significant and the weed community was fairly consistent across the site. The Tifton site on the other hand, had virtually no weed biomass due to frequent tillage. These striking differences suggest that a diverse weed community may permit certain taxa such as staphylinids to benefit from the activity of fire ants by providing more refuge. In the absence of this weed complex these taxa may simply be more readily encountered by fire ants and therefore more susceptible to predation. There were also a number of methodological differences between the two sites/sampling years that may have led to some of the differences observed. For instance, the difference in pitfall trap size as well as the number of traps per area may have had significant effects on trap catch results (Work *et al.* 2002). Soil from Horseshoe Bend is considered to be a sandy clay loam (64% sand, 12% silt, and 22% clay), while those from the Coastal Plain site are considered a loamy sand (83% sand, 13% silt, and 4% clay) (Parker *et al.* 1988; Bossuyt *et al.* 2002). Research on Tullgren funnel extraction efficiency suggests that there is a great deal of variation in the arthropod extraction efficiencies among different soil types, which may also help explain the differences in soil arthropod communities between our sites (Andre *et al.* 2002).

In conclusion, this study demonstrated that *Solenopsis invicta* is capable of significantly influencing soil arthropod communities both at the soil surface and within the soil matrix, however, these effects may vary based on factors such as weed biomass. Methodological differences aside, the significant differences detected in the current study have potential implications for communities of beneficial arthropods involved in both pest management and soil processes warranting further study.

REFERENCES

Andre H.M., Ducarme X. & Lebrun P. (2002). Soil biodiversity: myth, reality or conning? *Oikos*, 96, 3-24.

Bossuyt H., Six J. & Hendrix P.F. (2002). Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. *Soil Science Society of America Journal*, 66, 1965-1973.

Boulton A.M. & Amberman K.D. (2006). How ant nests increase soil biota richness and abundance: a field experiment. *Biodiversity and Conservation*, 15, 69-82.

Calixto A., Dean A., Knutson A. & Harris M. (2006). Density changes of two earwigs, Labidura ripade (Pallas) and Euborellia annulipes (Lucas) following fire ant reduction in Mumford, Texas. *Southwestern Entomologist*, 31, 97-101.

Crossley D.A. & Blair J.M. (1991). A High-Efficiency, Low-Technology Tullgren-Type Extractor for Soil Microarthropods. *Agriculture Ecosystems & Environment*, 34, 187-192.

Diaz D., Knutson A. & Bernal J.S. (2004). Effect of the red imported fire ant on cotton aphid population density and predation of bollworm and beet armyworm eggs. *Journal of Economic Entomology*, 97, 222-229.

Dindal D.L. (1990). *Soil Biology Guide*. John Wiley & Sons, New York.

Dostal P., Breznova M., Kozlickova V., Herben T. & Kovar P. (2005). Ant-induced soil modification and its effect on plant below-ground biomass. *Pedobiologia*, 49, 127-137.

Eubanks M.D. (2001). Estimates of the direct and indirect effects of red imported fire ants on biological control in field crops. *Biological Control*, 21, 35-43.

Eubanks M.D., Blackwell S.A., Parrish C.J., Delamar Z.D. & Hull-Sanders H. (2002). Intraguild predation of beneficial arthropods by red imported fire ants in cotton. *Environmental Entomology*, 31, 1168-1174.

Green W.P., Pettry D.E. & Switzer R.E. (1999). Structure and hydrology of mounds of the imported fire ants in the southeastern United States. *Geoderma*, 93, 1-17.

Greensla P.J. (1973). Sampling ants with pitfall traps - Digging in effects. *Insectes Sociaux*, 20, 343-353.

Hölldobler B. & Wilson E.O. (1990). *The Ants*. Belknap Press, Cambridge.

Kaplan I. & Eubanks M.D. (2002). Disruption of cotton aphid (Homoptera : Aphididae) - Natural enemy dynamics by red imported fire ants (Hymenoptera : Formicidae). *Environmental Entomology*, 31, 1175-1183.

Kaplan I. & Eubanks M.D. (2005). Aphids alter the community-wide impact of fire ants. *Ecology*, 86, 1640-1649.

Laakso J. & Setälä H. (1998). Composition and trophic structure of detrital food web in ant nest mounds of Formica aquilonia and in the surrounding forest soil. *Oikos*, 81, 266-278.

Lafleur B., Hooper-Bui L.M., Mumma E.P. & Geaghan J.P. (2005). Soil fertility and plant growth in soils from pine forests and plantations: effect of invasive red imported fire ants *Solenopsis invicta* (Buren). *Pedobiologia*, 49, 415-423.

Lenoir L., Bengtsson J. & Persson T. (2003). Effects of Formica ants on soil fauna-results from a short-term exclusion and a long-term natural experiment. *Oecologia*, 134, 423-430.

McCune B. & Grace J.B. (2002). *Analysis of Ecological Communities*. 1 edn. MjM Software Design, Gleneden Beach, OR.

Morrison L.W. & Porter S.D. (2003). Positive association between densities of the red imported fire ant, Solenopsis invicta (Hymenoptera: Formicidae), and generalized ant and arthropod diversity. *Environmental Entomology*, 32, 548-554.

Parker M.B., Gaines T.P., Plank C.O., Jones J.B., Walker M.E., Boswell F.C. & Dowler C.C. (1988). The effects of limestone on corn, soybeans and peanuts grown on a Tifton soil at various for seventeen years. . *Georgia Agricultural Experiment Station Research Bulletin*.

Porter S.D. & Savignano D.A. (1990). Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. *Ecology*, 71, 2095-2106.

SAS (1999-2001). SAS System for Windows. In. SAS Institute inc. Cary, NC.

Schneider K., Migge S., Norton R.A., Scheu S., Langel R., Reineking A. & Maraun M. (2004). Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios (^{15}N/^{14}N). *Soil Biology and Biochemistry*, 36, 1769-1774.

Stiles J.H. & Jones R.H. (2001). Top-down control by the red imported fire ant (Solenopsis invicta). *American Midland Naturalist*, 146, 171-185.

Stimac J.L. & Alves S.B. (1994). Ecology and biological control of fire ants. In: *Pest Management in the Subtropics: Biological control - a Florida perspective* (ed. Rosen D, Bennett, F.D., Capinera, J.L.). Intercept Limited Andover, U.K., pp. 353-380.

Styrsky J.D. & Eubanks M.D. (2007). Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B-Biological Sciences*, 274, 151-164.

Triplehorn C.A. & Johnson N.F. (2005). *Borror and Delong's Introduction to the Study of Insects*. 7 edn. Thomson Brooks/Cole, Belmont, CA.

Tschinkel W.R. (2006). *The fire ants*. Belknap Press, Cambridge.

Vinson S.B. (1991). Effect of the Red Imported Fire Ant (Hymenoptera, Formicidae) on a Small Plant-Decomposing Arthropod Community. *Environmental Entomology*, 20, 98-103.

Wagner D., Brown M.J.F. & Gordon D.M. (1997). Harvester ant nests, soil biota and soil chemistry. *Oecologia*, 112, 232-236.

Wilson E.O. (2005). Oribatid mite predation by small ants of the genus Pheidole. *Insectes Sociaux*, 52, 263-265.

Wojcik D.P. (1990). Behavioral Interactions of Fire Ants and Their Parasites, Predators and Inquilines. In: *Applied Myrmecology* (ed. Vander Meer R.K. JK, Cedeno A.). Westview Press Boulder, pp. 329 - 344.

Wojcik D.P., Allen, C.R., Brenner R.J., Forys E.A., Jouvenaz D.P., Lutz R.S. (2001). Red Imported Fire Ants: Impact on Biodiversity. *American Entomologist*, 47, 16-23.

Work T.T., Buddle C.M., Korinus L.M. & Spence J.R. (2002). Pitfall trap size and capture of three taxa of litter-dwelling arthropods: Implications for biodiversity studies. *Environmental Entomology*, 31, 438-448.

Zettler J.A., McInnis T.M., Allen C.R. & Spira T.P. (2002). Biodiversity of fungi in red imported fire ant (Hymenoptera: Formicidae) mounds. *Annals of the Entomological Society of America*, 95, 487-491.

Table 3.1 Fire ant exclusion efficacy test. Data represent numbers of fire ants collected per glass test tube at the Coastal Plains Experiment Station on 6 August and 21 September, 2007. Test tubes remained in the field for a one-hour period.

		total # ants collected in test tubes			
		soil surface		cotton foliage	
	block	inclusion	exclusion	inclusion	exclusion
6-Aug-07	one	18 (1)	0	0	0
	two	13 (1)	0	0	0
	three	2 (1)	0	0	0
	four	66 (3)	0	34 (1)	0
2-Sep-07	one	87 (1)	0	*na*	*na*
	two	103 (1)	0	*na*	*na*
	three	164 (1)	0	*na*	*na*
	four	52 (2)	0	*na*	*na*

Table 3.2 List of all taxa collected from pitfall traps over the duration of 2006 and 2007 at both the Horseshoe Bend Research Station, Athens, GA and Coastal Plain Experiment Station, Tifton, GA.

			# of morpho-species	
Order	**common name**	**family/group**	**Horseshoe Bend**	**Coastal Plains**
Coleoptera	beetles	Staphylinidae	7	2
		Carabidae	3	3
		Chrysomelidae	3	0
		Scarabaeidae	3	2
		Scydmaenidae	2	0
		Coccinellidae	0	2
		Tenebrionidae	1	0
		Nitidulidae	1	2
		Anobiidae	1	0
		Anthicidae	0	1
		Ptiliidae	1	0
		Corylophidae	1	0
		Elateridae	1	1
Hymenoptera	ants	Formicidae	6	5
	parasitic wasps	Scelionidae	4	3
		Ceraphronidae	2	1
		Cynipidae	1	0
		Mymaridae	1	1
		Diapriidae	1	0
		Trichogrammatidae	1	1
		Eurytomidae	1	0
Collembola	springtails	Entomobryidae	3	1
		Isotomidae	2	1
		Sminthuridae	1	1
		Onychiuridae	1	1
		Hypogastruridae	1	1
Araneae	spiders	Linyphiidae	1	1
		Lycosidae	1	1
		Salticidae	1	1
		Thomisidae	1	1
		Gnaphosidae	0	1
Orthoptera	crickets	Gryllidae	1	1
		Gryllotalpidae	0	1
Dermaptera	earwigs	Labiidae	1	0
		Labiduridae	0	1
		Forficulidae	0	1
Hemiptera	bugs	Geocoridae	1	1
		Cicadellidae	0	1
		Miridae	0	1
Diptera	flies	Dolichopodidae	0	1
		Phoridae	1	1
Acari	mesostigmatid mites	Macrochelidae	1	0
		Pachylaelapidae	1	0
		Laelapidae	1	1
		Uropodidae	1	0
	oribatid mites	Tectocepheidae	1	0
		Scheloribatidae	1	0
		Galumnidae	2	0
		Oribatulidae	1	1
		Oppiidae	1	1
		Euphthiracaridae	1	0
	prostigmatid mites	Eupodidae	1	1
		Erythraeidae	1	1
		Cunaxidae	1	0
		Bdellidae	0	1
Thysanoptera	thrips	Thripidae	1	1

Table 3.3 List of all taxa collected from soil cores over the duration of 2006 and 2007 at both the Horseshoe Bend Research Station, Athens, GA and Coastal Plains Experiment Station, Tifton, GA. Columns for which orders are empty represent taxa for which only family or common name were known.

			# of morpho-species	
order	**common name**	**family/group**	**Horseshoe Bend**	**Coastal Plains**
Coleoptera	beetles	Staphylinidae	3	0
		Carabidae	1	1
		Scarabaeidae	1	1
		Nitidulidae	0	1
		Scydmaenidae	1	0
		Curculionidae	1	0
Hymenoptera	parasitic wasps	Scelionidae	2	0
		Cynipidae	1	0
Collembola	springtails	Entomobryidae	3	1
		Isotomidae	1	1
		Sminthuridae	1	1
		Onychiuridae	1	1
		Hypogastruridae	1	1
Araneae	spiders	Linyphiidae	1	0
Acari	mesostigmatid mites	Laelapidae	1	1
		Uropodidae	1	2
		Melicharidae	1	0
		Blattisociidae	0	1
		Ascidae	0	1
		Phytoseiidae	1	0
	oribatid mites	Galumnidae	2	0
		Tectocepheidae	1	0
		Scheloribatidae	1	0
		Oribatulidae	1	0
		Oppiidae	1	1
		Euphthiracaridae	1	0
	prostigmatid mites	Eupodidae	1	1
		Cunaxidae	1	0
		Bdellidae	0	1
		Epilomanniidae	1	0
		Lohmanniidae	1	0
		Hermanniellidae	1	0
		Platynothridae	1	0
Protura		proturan	1	0
		pauropod	1	0
		symphylan	1	0
Geophilomorpha	centipede	geophilomorph	1	0
Diplura		Japygidae	1	0
Oligochaeta	potworms	Echytraeidae	0	1

Table 3.4 Mean abundance, richness (number of families) and diversity (Shannon H) (±SE) from pitfall traps at each sampling date from both exclusion and inclusion plots during the 2006 field season at Horseshoe Bend. None of the differences were statistically significant based on mixed model analysis.

	abundance		richness		shannon	
	exclusion	inclusion	exclusion	inclusion	exclusion	inclusion
5-Jun-06	16.31±1.32	20.59±1.11	9.65±0.85	10.4±0.44	1.56±0.07	1.6±0.04
19-Jun-06	11.55±0.78	12.57±0.83	8.1±0.4	7.95±0.39	1.51±0.08	1.24±0.07
1-Aug-06	22.31±1.21	24.96±1.69	12.4±0.69	12.9±0.68	2.09±0.04	1.91±0.06
21-Aug-06	14.56±1.02	18.49±0.97	10±0.71	11.8±0.47	1.53±0.09	1.80±0.11
18-Sep-06	13±2.69	14.15±1.86	5.95±0.54	6.6±0.54	1.53±0.09	1.62±0.08

Table 3.5 Mean percentages of major groups collected in pitfall traps from the Horseshoe Bend Research Station in fire ant inclusion and exclusion plots. Asterisks denote statistically significant differences between inclusion and exclusion plots for the taxa listed at a given sampling week based on mixed model analysis (*P<0.05, **P<0.01, ***P<0.005). Each date represents the day on which traps were set in the field, 2006. Samples in which a specific group was not collected are denoted by (*NA*).

	soil surface fauna									
	1-5Jun		15-19Jun		28-1Jul/Aug		17-21JAug		14-18Sep	
	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion
Coleoptera	*NA*	*NA*	26	74	55	45	21	79***	32	68
Parasitoids	46	54	41	59	39	61	29	71*	20	80*
Mesostigmata	50	50	53	47	55	45	38	62	100	0
Oribatida	42	58*	41	59	47	53	50	50	57	43
Prostigmata	44	56	31	69**	26	74***	26	74***	42	58
Collembola	47	53	49	51	46	54	50	50	51	49
Araneae	43	57	49	51	48	52	36	64*	43	57

Table 3.6 Mean abundance, richness (number of families) and diversity (Shannon H) ±SE collected in pitfall traps from fire ant inclusion and exclusion plots at the Coastal Plain Experiment Station (2007). None of the differences were statistically significant based on mixed model analysis.

	abundance		richness		shannon (H)	
	exclusion	inclusion	exclusion	inclusion	exclusion	inclusion
27-Jul-07	28.83±1.78	32.66±4.04	15.5±1.65	13.5±1.85	2.44±0.10	2.30±0.11
3-Aug-07	33.73±2.41	26.37±1.56	15.25±1.31	13.5±0.95	2.45±0.09	2.37±0.07
10-Aug-07	34.66±2.37	30.63±5.13	14.75±1.32	16.75±1.55	2.40±0.09	2.67±0.19

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Table 3.7 Mean abundance, richness (number of families) and diversity (Shannon H) ±SE per 100g dry soil from soil cores at each sampling date taken from both exclusion and inclusion plots (Horseshoe Bend Research Station). None of the differences were statistically significant based on mixed model analysis.

	abundance		richness		shannon	
	exclusion	inclusion	exclusion	inclusion	exclusion	inclusion
3-Jun-06	14.18±3.83	18.87±4.77	3.81±0.79	4.75±0.62	0.80±0.17	1.02±0.14
30-Jun-06	11.66±5.12	3±1.21	3.22±0.89	1.88±0.48	0.74±0.24	0.46±0.19
1-Aug-06	19.2±2.96	21.75±4.56	6.95±0.73	7±0.94	1.48±0.11	1.49±0.16
28-Aug-06	17.7±2.77	14.95±2.37	7.95±0.78	7.2±0.68	1.76±0.09	1.65±0.08
18-Sep-06	6.55±1.18	6.1±0.79	3.9±0.4	3.6±0.41	1.16±0.1	1.07±0.11

Table 3.8 Mean percentages of major groups collected from soil cores from the Horseshoe Bend Research Station taken from fire ant inclusion and exclusion plots. Asterisks denote statistically significant differences between inclusion and exclusion plots for the taxa listed at a given sampling week (*$P<0.05$, **$P<0.01$, ***$P<0.005$ Each date represents the day on which soil cores were taken from field, 2006. Samples in which a specific group was not collected are denoted by (*NA*).

	soil fauna									
	3-Jun		30-Jun		1-Aug		28-Aug		18-Sep	
	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion
Prostigmata	43	57	*NA*	*NA*	33	67*	53	47	51	49
Mesostigmata	46	54	39	61	47	53	49	51	53	47
Oribatida	40	60	70	30	52	48	58	42	49	51
Collembola	55	45	60	40	51	49	48	52	52	48
Coleoptera	*NA*	*NA*	*NA*	*NA*	77	23	65	35	67	33
Protura	25	75	*NA*	*NA*	41	59	43	57	77	23
Pauropoda	*NA*	*NA*	100	0	21	79	42	58	0	100
Symphyla	*NA*	*NA*	*NA*	*NA*	59	41	78	22	0	100
Japygidae	0	100	*NA*	*NA*	38	62	*NA*	*NA*	100	0
Dipteran larvae	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	29	71
Thysanoptera	*NA*	*NA*	61	39	*NA*	*NA*	45	55	59	41
Parasitoids	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	100	0	*NA*	*NA*

Table 3.9 Mean abundance, richness (number of families) and diversity (Shannon H) ±SE per 100g dry soil from Coastal Plain Experiment Station soil cores at each sampling date taken from both exclusion and inclusion plots. None of the differences were significantly different based on mixed model analysis.

	abundance		richness		shannon	
	exclusion	inclusion	exclusion	inclusion	exclusion	inclusion
17-Aug-07	4.73±2.13	5.85±3.33	3.25±1.03	3±1.35	0.93±0.33	0.74±0.36
3-Sep-07	7.49±1.18	6.85±3.09	4.25±0.63	2.5±0.87	1.38±0.16	0.69±0.3

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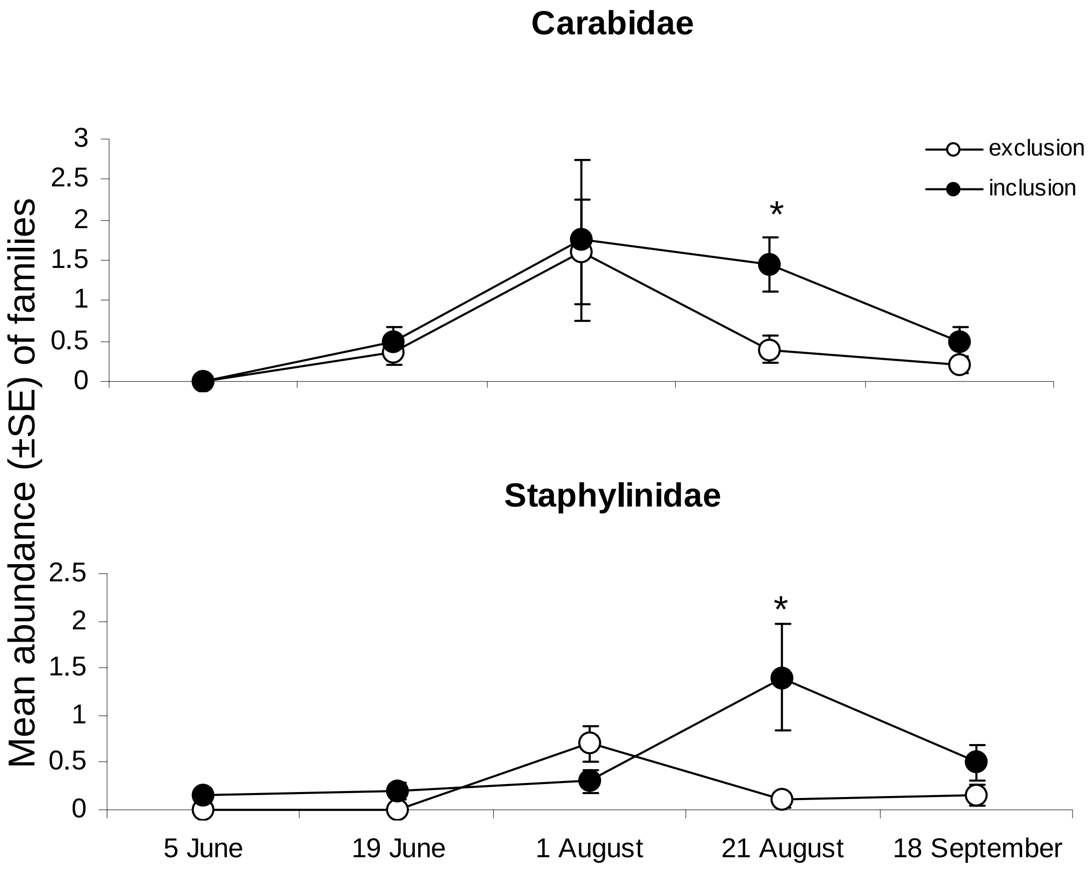

Figure 3.1 Mean abundance (± SE) of coleopteran indicator taxa from pitfall traps during each sampling week (Horseshoe Bend Research Station). Each date represents the day on which traps were set in the field, 2006. Asterisks denote significant differences between abundances from inclusion and exclusion plots based on mixed model analysis (*P<0.05).

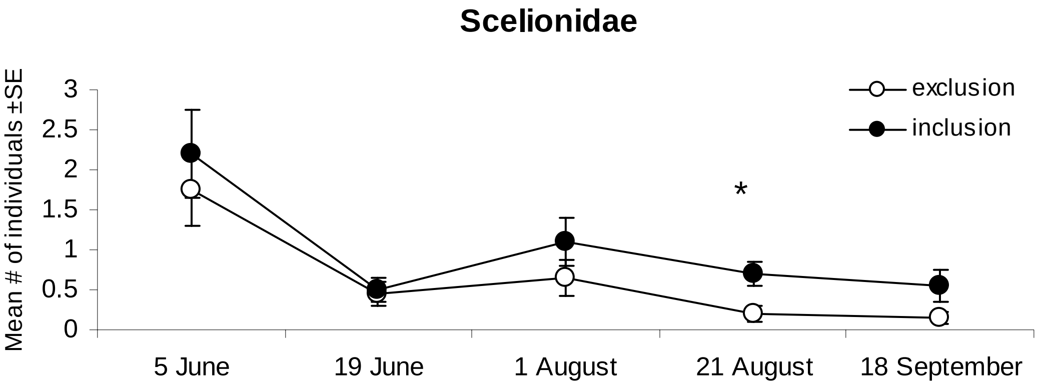

Figure 3.2 Mean abundance (± SE) of the Hymenopteran parasitoid family Scelionidae from pitfall traps during each sampling week (Horseshoe Bend Research Station). Each date represents the day on which traps were set in the field, 2006. Asterisks denote a significant difference between abundance from inclusion and exclusion plots based on mixed model anaysis (*P<0.05).

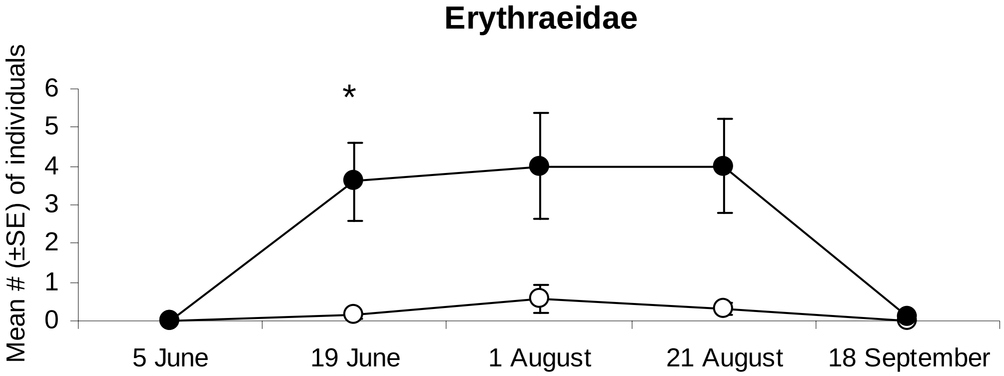

Figure 3.3 Mean abundance (± SE) of erythraeid mites from pitfall traps during each sampling week from fire ant inclusion, filled circles and exclusion plots, open circles (Horseshoe Bend Research Station). Each date represents the day on which traps were set in the field, 2006. Asterisks denote significant differences in abundances between inclusion and exclusion plots based on mixed model analysis (*$P<0.05$).

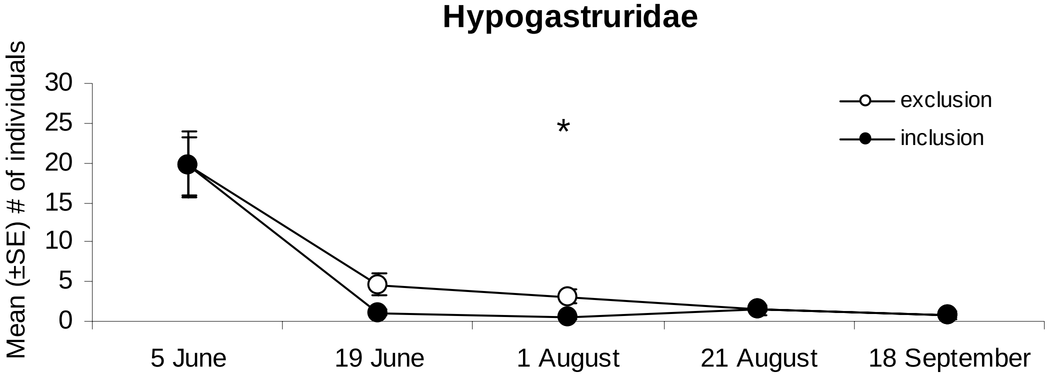

Figure 3.4 Mean abundance (± SE) of hypogastrurids from pitfall traps during each sampling week (Horseshoe Bend Research Station). Each date represents the day on which traps were set in the field, 2006. Asterisks denote significant differences between abundances from inclusion and exclusion plots based on mixed model analysis (*P<0.05).

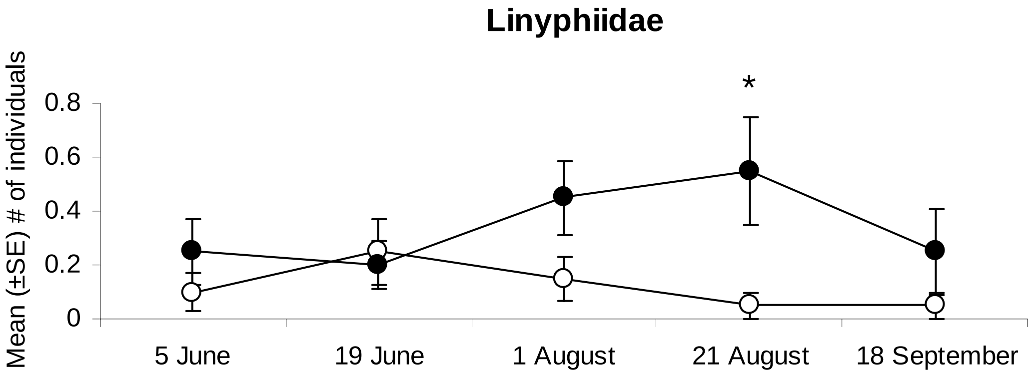

Figure 3.5 Mean abundance (± SE) of the Spider family Linyphiidae from pitfall traps during each sampling week fire ant inclusion, filled circles and exclusion plots, open circles (Horseshoe Bend Research Station). Each date represents the day on which traps were set in the field, 2006. Asterisks denote a significant difference between abundance from inclusion and exclusion plots (*P<0.05).

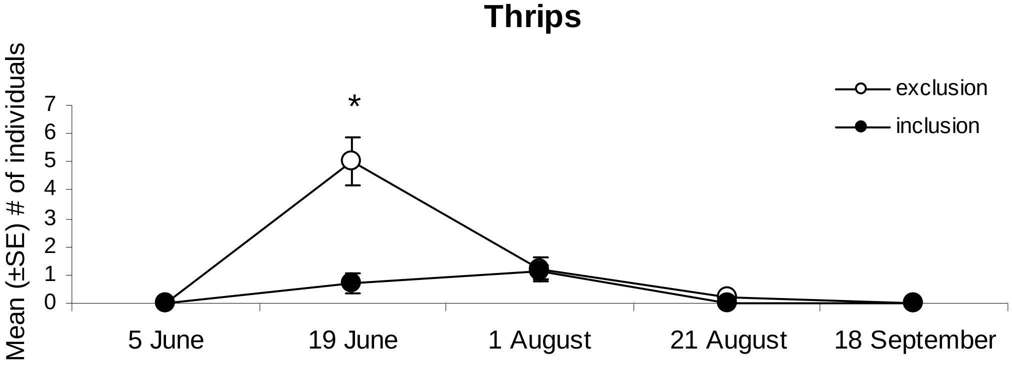

Figure 3.6 Mean abundance (± SE) of the Thrips suborder Terebrantia from pitfall traps during each sampling week (Horseshoe Bend Research Station). Each date represents the day on which traps were set in the field, 2006. Asterisks denote a significant difference between abundance from inclusion and exclusion plots (*P<0.05).

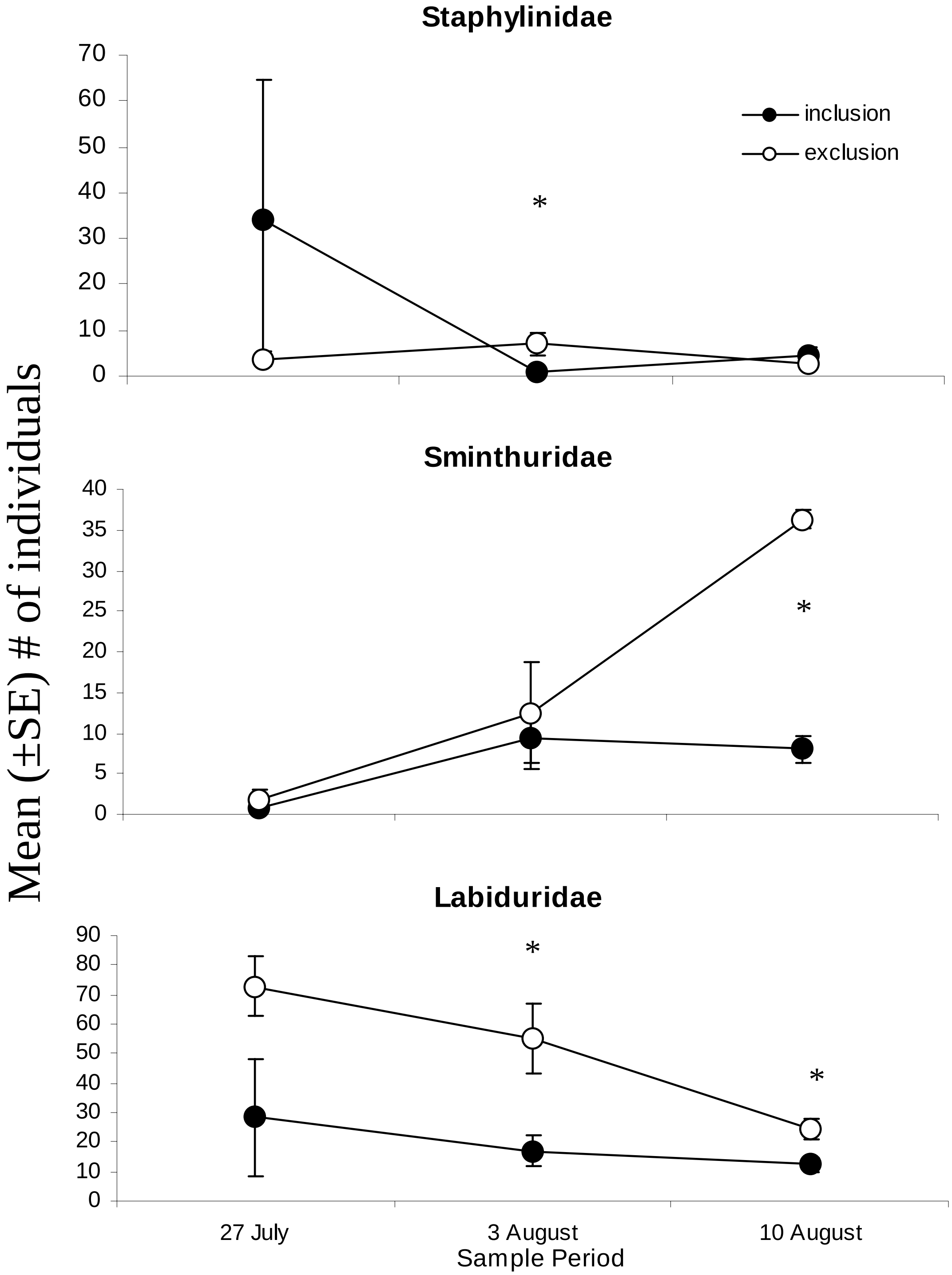
Staphylinidae
70
60
50
40
30
20
10
0
inclusion
exclusion
*
Sminthuridae
40
35
30
25
20
15
10
5
0
*
Labiduridae
90
80
70
60
50
40
30
20
10
0
*
*
27 July
3 August
10 August
Sample Period
Mean (±SE) # of individuals

Figure 3.7 Mean abundance (± SE) of taxa from pitfall traps during each sampling week (Coastal Plain Experiment Station). Each date represents the day on which traps were set in the field, 2007. Asterisks denote significant differences between abundances from inclusion and exclusion plots based on mixed model analysis (*P<0.05).

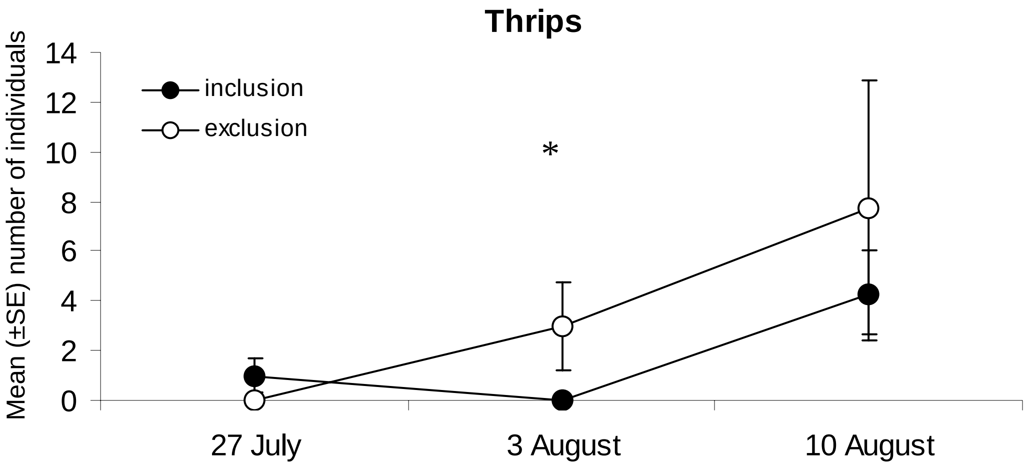

Figure 3.8 Mean abundance (± SE) of the Thrips suborder Terebrantia from pitfall traps during each sampling week (Coastal Plain Experiment Station). Each date represents the day on which traps were set in the field, 2007. Asterisks denote a significant difference between abundance from inclusion and exclusion plots based on mixed model analysis (*P<0.05).

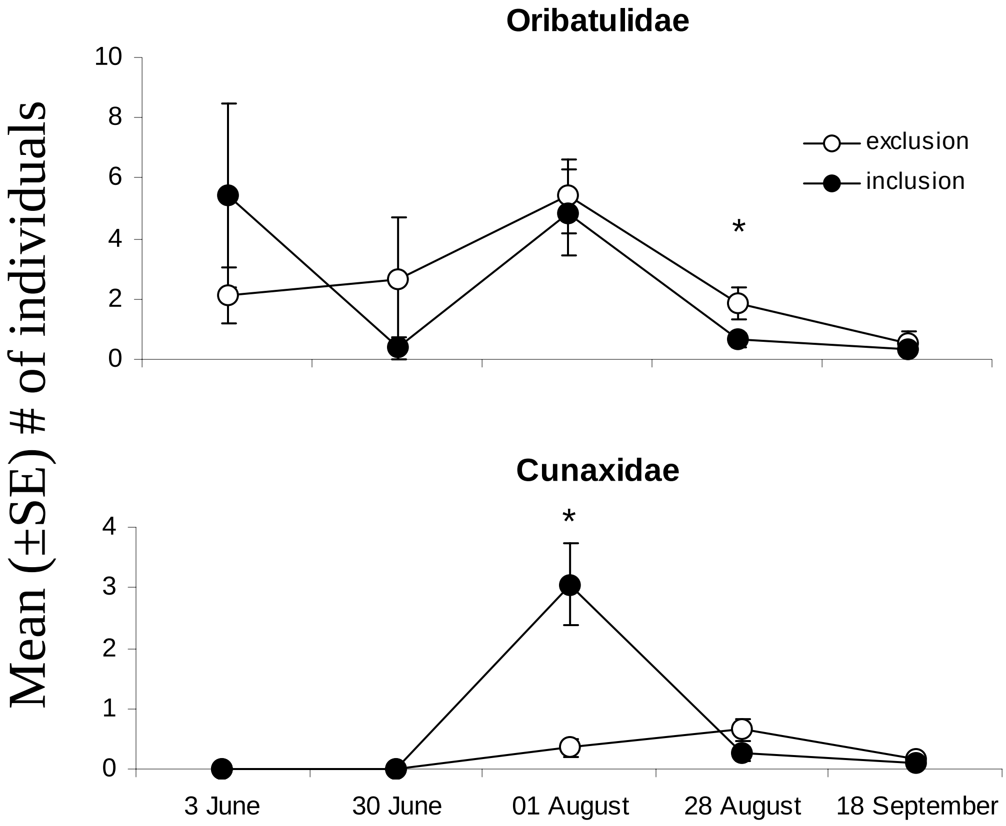

Figure 3.9 Mean abundance (± SE) of Acari per 100 grams of oven dried soil during each sampling week (Horseshoe Bend Research Station). Each date represents the day on which soil cores were taken from field, 2006. Asterisks denote significant differences between abundances from inclusion and exclusion plots based on mixed model analysis (*P<0.05).

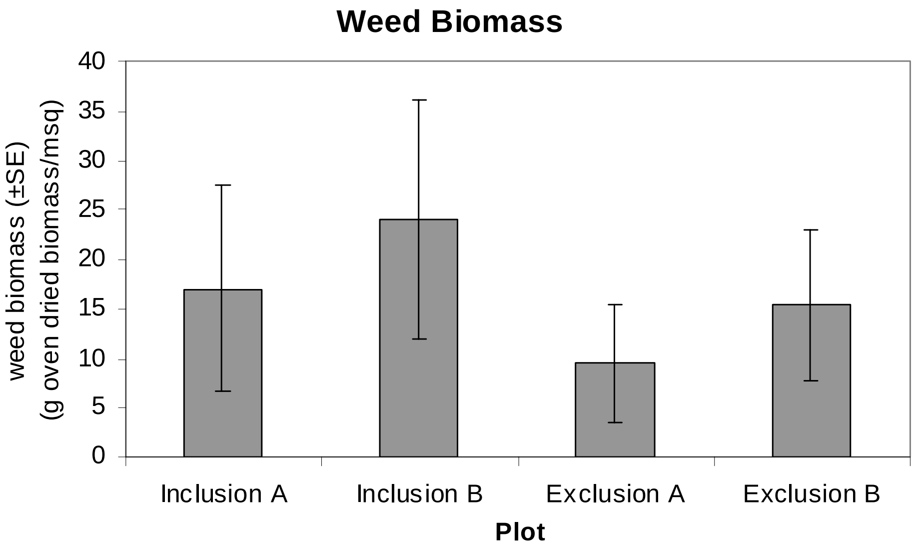

Figure 3.10 Mean (±SD) weed biomass in grams of dry plant matter per square meter. Data represent means of eight one-meter squared samples of aboveground weed biomass per plot. All biomass was oven dried at 50°C.

CHAPTER 4

EGG PREDATION BY THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*, ON FOLIAGE AND AT THE SOIL SURFACE OF A COTTON AGROECOSYSTEM[3]

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[3] Wickings, K.G. and J. Ruberson. To be submitted to *Ecological Entomology*.

ABSTRACT

The red imported fire ant, *Solenopsis invicta* Buren, can have a significant negative effect on agricultural pest; this effect can cross multiple stages of pest ontogeny. There are many areas, however, in which predation by fire ants has not been adequately addressed, including predation at the soil surface and on eggs of non-lepidopteran pests.

Honeydew-producing insects are also known to alter the dietary focus of fire ants. In agricultural systems that host significant populations of honeydew exuding hemipterans, such as aphids and whiteflies, this may lead to a shift in foraging behavior from the soil surface onto crop foliage, subsequently influencing egg predation.

This study examined the contribution of fire ants to predation on egg masses of the beet armyworm, *Spodoptera exigua*, at the soil surface and within cotton foliage as well as on egg masses of two species of stink bug (*Nezara viridula* and *Piezodorus guildinii*) within cotton foliage. Research was conducted during 2006 at the Horseshoe Bend research facility in Athens, GA, and during 2007 at the Coastal Plain Research Site in Tifton, GA.

Fire ants accounted for 20 – 50% of the total beet armyworm egg predation within the first 24 hours of egg release at both the soil surface and in cotton foliage. The impact of fire ants on stink bug egg predation varied by stink bug species. While up to 80% of *N. viridula* eggs were removed in fire ant inclusion plots, no eggs of *Piezodorus guildinii* were removed. Both aphids and whiteflies were significantly more abundant in the presence of fire ants than in their absence. There was no correlation between aphid density and egg predation rates on foliage or at the soil surface.

Increasing abundance of whiteflies, however, coincided with high fire ant activity, an increase in stink bug and beet armyworm egg predation on foliage, and a decrease in beet armyworm egg predation at the soil surface.

Keywords: red imported fire ants, *Solenopsis invicta*, egg predation, *Spodoptera exigua*, *Piezodorus guildinii*, *Nezara viridulus*, aphids, whitefly, cotton

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is known to be a generalist and opportunistic predator in above-ground systems (Tschinkel 2006). While fire ants have been found to directly increase predation in terrestrial systems by 20 to 30%, they are also known to decrease the abundance of many beneficial predators including multiple species from the orders Coleoptera and Neuroptera as well as from the class Arachnida (Eubanks *et al.* 2002; Diaz *et al.* 2004). *Solenopsis invicta* commonly enters into mutualistic exchanges with aphids which have been found to increase the defensive or predatory behavior of fire ants within plant foliage occupied by aphids, subsequently increasing aphid survival (Kaplan & Eubanks 2002). Many studies have investigated the impact of fire ants as egg predators on a variety of lepidopteran pests (e.g., *Helicoverpa zea* (Boddie), *Helicoverpa armigera* (Hübner) and *Heliothis virescens* (Fabr.) (Lepidoptera: Noctuidae)) within cotton foliage and have determined that *S. invicta* can substantially contribute to egg predation, often serving as the top egg predator in the system (Diaz *et al.* 2004; Nuessly & Sterling 1994; McDaniel & Sterling 1979, 1982). Although predation and parasitism efficiencies have been examined for a handful of non-lepidopteran pests, the contribution of red imported fire ants to mortality of these pests has been studied infrequently (Lee *et al*. 1994).

Fire ants also commonly forage at the soil surface, which suggests that they may play an important role as predators within soil foodwebs. Although trophic interactions between *S. invicta* and other soil fauna remain largely overlooked, a handful of studies have investigated such interactions. Vinson (1991) assessed predation events and trophic interactions among detritivores in the presence and absence of *S. invicta* using pieces of rotting fruit as bait stations and determined that in the presence of *S. invicta*, the abundance of fly larvae, sap beetles, rove beetles, and parasitic wasps decreased significantly. Although Vinson's study provides insight into the aggressive nature of *S. invicta* when defending large, high quality food sources, it may be a poor estimate of the impact of these ants as predators at the soil surface, especially in systems where resources are heterogeneously distributed. Members of virtually every order of terrestrial arthropods are known to inhabit soil during certain life-stages (Triplehorn & Johnson 2005). In cotton systems this can include pest arthropods, such as the cotton stainer *Dysdercus* spp. (Hemiptera: Pyrrhocoridae) (Mathews & Tunstall 1994). Similarly, eggs that are laid within crop canopies can become dislodged and fall to the soil surface where they too may become prey for predators of soil foodwebs (Nuessly and Sterling 1994). A few studies suggest that fire ants may make a significant contribution to egg predation at the soil surface (Nuessly and Sterling 1994, Lee *et al*. 1994).

OBJECTIVES

This study examined the contribution of fire ants to egg predation within a cotton crop canopy using egg masses of the beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) and two stink bugs: the redbanded stink bug, *Piezodorus guildinii* Westwood (Hemiptera: Pentatomidae), and the southern green stink bug, *Nezara viridula* Linnaeus (Hemiptera: Pentatomidae). The study also examined the contribution of fire ants to egg predation at the soil surface, again using egg masses of the beet armyworm, *Spodoptera exigua*. This study tested the following hypotheses:

H_1 – eggs of the beet armyworm, *S. exigua*, will be removed at a significantly faster rate from both crop canopies and from the soil surface in the presence of *S. invicta* than in its absence

H_2 – eggs of both the redbanded stink bug, *P. guildinii*, and southern green stink bug, *N. viridula*, will also be removed at a significantly faster rate from cotton foliage in the presence of *S.invicta* than in its absence

H_3 – increased abundance of honeydew-producing hemipterans (cotton aphids, *Aphis gossypii,* Glover (Hemiptera: Aphididae) and cotton whitefly, *Bemisia tabaci*, Gennadius (Hemiptera: Aleyrodidae)) in cotton foliage will lead to:

a) a significant increase in egg removal rates from cotton foliage, and
b) a significant decrease in egg removal rates from the soil surface

RATIONALE

Based on past research we predict that fire ants will make a significant contribution to predation on the eggs of both beet armyworms and stink bugs in cotton foliage. While past research has shown that fire ants do significantly affect predation on eggs of *S. exigua,* this is the first attempt to examine their impact on predation of stink bug eggs (Diaz *et al.* 2004; Ehler 2007). Past research has demonstrated that stink bug eggs are infrequently preyed upon (Ehler 2002), but these studies were not conducted where fire ants were prevalent. We expect stink bug egg predation to be significant in the presence of *S. invicta*. We also expect that due to their foraging activity at the soil surface *S. invicta* will contribute significantly to egg predation in soil food webs. It has also been suggested that seasonal fluctuation in aphid populations in cotton systems may cause a shift in *S. invicta* feeding activity from predation at the soil surface to a diet of hemipteran honeydew in crop canopies (Kaplan & Eubanks 2005). If this is true, we predict that the intensity of predation by *S. invicta* at the soil surface will decrease during periods of high aphid abundance within the cotton canopy.

MATERIALS AND METHODS

Field Sites and Fire Ant Exclusion

This study was conducted at two University of Georgia field stations: the Horseshoe Bend Research Station in Athens, GA, and the Coastal Plain Experiment Station in Tifton, GA, which are approximately 320 km apart. Soil from Horseshoe Bend is a sandy clay loam (64% sand, 12% silt, and 22% clay), while those from the Coastal Plain site are considered a loamy sand (83% sand, 13% silt, and 4% clay) (Parker *et al.* 1988; Bossuyt *et al.* 2002).

Horseshoe Bend is a 0.8 hectare farm composed of eight main plots (four no-till, four conventional till) and thirty two subplots planted with a Bt cotton summer crop and either rye or clover as a winter cover crop. Four conventionally tilled plots measuring 28x28 m each were chosen for this study. All plots were planted with Bt cotton (DPL 555BR, which is genetically modified to express Cry1Ac toxin, from the bacterium *Bacillus thuringiensis* Berliner, and for tolerance to the herbicide glyphosate). Between 15 and 18 May, 2006, a barrier was constructed out of aluminum flashing and buried to a depth of 13 cm to minimize lateral tunneling by ants under the barrier (Stiles & Jones 2001). The placement of this barrier ran evenly between the plots such that two plots were on each side of the fence. All plots on one side of the barrier were treated on 10 June, 2006, with hydramethylnon fire ant bait (Amdro®, Ambrands) at a rate of 1.1 kg of formulated bait per ha to eliminate fire ants (follow-up treatment on 23 July). Vegetation was regularly trimmed in a small band along the barrier wall to minimize ant crossing. Observations were conducted on a daily basis to assess the effectiveness of the exclusion.

The Tifton site is 1.62 hectares also composed of 8 main plots (0.2 ha each) with all plots were managed under conventional tillage for the duration of the current study. All plots were planted with Bt cotton (DPL 555BR) on 4 June 2007. Plots were separated from one another by open gaps of 3 m of bare soil tilled at regular intervals, rather than an aluminum flashing barrier as was used at Horseshoe Bend. The plots were arranged in 4 blocks, each containing one fire ant inclusion plot and one fire ant exclusion plot. Plots were approximately square, and a 10x10m area in the center of each plot was designated for sampling. Fire ant exclusion plots were treated with hydramethylnon ant bait at the rate noted above on 28 June, 16 July, 4 August, and 22 August 2007 to eliminate fire ants. To assess the exclusion treatment, ant detection tests were conducted on 6 August and 2 September. This test consisted of placing three 33-ml test tubes containing a small piece (5 gm) of hotdog in each plot. After 1 hour all tubes were recovered and sealed, and transported back to the lab where the tubes were emptied and the number of ants was recorded.

Experimental design – beet armyworm egg masses

The effectiveness of fire ants as predators using egg masses of the beet armyworm, *S. exigua*, was examined at Horseshoe Bend during the 2006 field season and the Tifton site during 2007. Horseshoe Bend egg mass trials were conducted over four separate, weeklong periods (28 June – 2 July, 25-29 July, 15 – 19 August and 26 – 30 September) in the presence and absence of fire ants. Tifton egg mass trials were conducted over three periods; one 24-hour period on 30 July, and two 3-day trial periods starting on 14 August and 7 September. Egg masses for both years were obtained from a laboratory colony maintained at the University of Georgia Department of Entomology, in Tifton. During the 2006 predation trials, egg masses from laboratory colonies of *S. exigua* were frozen and shipped overnight from Tifton to Athens, where they were immediately processed for the field (freezing prevented eggs from hatching during shipment). Egg mass processing involved identifying egg masses containing between 30 and 50 eggs, and counting and recording the exact number of eggs in the selected masses using a dissecting microscope. The egg masses were then transported to the field where they were either stapled to the underside of cotton leaves or pinned directly to the soil surface. When stapled to cotton leaves, one egg mass was attached to a fully expanded leaf near the plant's mid-height. This resulted in ten egg masses per plot during each sampling week (five in plant foliage (one mass per plant) and five at soil surface). Egg mass locations were marked by placing plastic flagging tape on adjacent branches for ease in relocation. The same procedure (including number of egg masses per plot) was followed for the Tifton, GA, predation trials (2007) except the eggs did not require freezing.

Following the placement of all egg masses, egg removal rates were recorded at set observation periods. At Horseshoe Bend, observation consisted of counting eggs using a 16x magnifying lens. The number of eggs was recorded at 24-hour intervals during the first two trials, however, this interval was reduced to every 12 hours for the final two trials due to a much faster removal rate than anticipated in plots where fire ants were included. At the Tifton site, egg masses were photographed at given observation periods using a digital camera. Photos were taken during the August and September predation trials at 1, 18, 48 and 72 hours after egg release. All photos were given a label and egg counts were subsequently conducted in the laboratory. The July predation trial from the Tifton site involved only one observation after a 24 hour period and therefore, did not require digital photos. Predation efficiencies for both the Horseshoe Bend and Tifton sites were calculated as the proportion of eggs absent from each mass.

Stink Bug Egg Masses

At the Tifton field site, predation trials were also conducted using egg masses of two species of stink bug, the redbanded stink bug, *P. guildinii*, and the southern green stink bug, *N.*

viridula. All egg masses were obtained from the laboratory of John Ruberson. Three separate predation trials were conducted during the 2007 field season (11 – 14 July, 24 – 27 July, and 21 – 25 September); however, the number of eggs obtained for each trial varied due to inconsistent egg production in the laboratory, causing the amount of replication used during the trials to be limited and variable. During the 11 July trial a total of eight egg masses of *N. viridula* were placed in cotton foliage of two treatment plots (four masses per plot) – one excluding ants and one including them (N=1). Thirty-two egg masses of *P. guildinii* were also placed in cotton foliage of the same two treatment plots (16 masses per plot, N=1). One egg mass was stapled to the lower surface of the uppermost, expanded leaf per cotton plant (total of 40 plants utilized). Five egg masses were placed on plants in each of 4 rows of cotton, which were separated from one another by three rows. One egg mass of *N. viridula* and four egg masses of *P. guildinii* were placed in each row. All egg masses were collected on 14 July and egg counts were not conducted between egg deployment and collection (3-day period). During the 24 July trial 20 egg masses of *N. viridula* were divided evenly among four plots (two ant exclusion plots and two inclusion plots, N=2). These eggs were similarly attached to the underside of the uppermost, fully expanded leaf. After deployment, these eggs similarly remained in the field for a 3-day period during which no egg counts were conducted until the final collection day (27 July). During the September predation trial, 34 egg masses were distributed among all eight treatment-plots (four or five per plot, N=4). Egg counts were then made at 1, 72 and 96 hours after all eggs had been deployed.

Predator Observations

The activity of predators was observed during each egg mass count period for both beet armyworm and stink bug predation trials. If, during the course of counting eggs on, or photographing an egg mass, any predators were encountered on those masses, they were identified to species in the field and were recorded either preying upon or simply occupying egg masses.

Aphid and Whitefly Density Estimation

Cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), densities were assessed at the Horseshoe Bend field site on 24 July and 26 September, 2006. Density assessments were conducted by carefully removing two fully expanded leaves (one fully expanded leaf approximately 6-8" below the top of the plant, and one fully expanded leaf from approximately the vertical center of the plant) from each of 10 randomly selected cotton plants at the center of each plot. All leaves from individual plots were carefully bulked within the same labeled, gallon-sized ziplock bag and transported to the laboratory for processing. Once in the laboratory leaves were carefully removed from their bags and all aphids were counted using a mechanical tallying device. After all leaves were processed from each bag, the bag was examined under a dissecting microscope to search for aphids that had fallen from the leaves during transport.

At the Tifton field site the density of cotton aphids was assessed over four weeks in July and August (24, 27 and 30 July, and 3, 6, 13, 17 and 24 August), 2007. On each sampling date the total number of aphids was recorded from each of 10 randomly chosen plants within each plot. The density of sweetpotato whiteflies, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), was also assessed on 29 August, 2007. Whitefly densities were estimated by counting the number of eggs as well as small and large nymphs in a circle, (2.5cm in diameter) between two veins on the main-stem leaf of the fifth node from the top of a cotton plant. This was conducted once on each of 12 randomly selected cotton plants per plot for a total of 96 observations.

Statistical Analyses

Before data were statistically analyzed, the number of eggs present during each observation period was converted to the proportion of eggs absent. Data from beet armyworm egg mass trials from both field seasons were analyzed using the mixed model procedure (Proc Mixed) for repeated measures using SAS software, version 8 of the SAS system for Windows, with ant status (present or absent), observation time, plot and interactions of all three factors serving as fixed effects and with block serving as a random effect (SAS 1999). The number of eggs at the start of each trial was also included in the mixed model analysis and functioned as a covariate.

Due to the varying availability of stink bug egg masses data were analyzed using a one-way analysis of variance for each sampling period. Data from the 11 July sampling period were analyzed using one-way ANOVA (proc glm) with ant status (presence or absence) as the treatment factor using SAS software, version 8 of the SAS system for Windows (SAS 1999). Data from the 24 July and 21 September sampling periods were similarly analyzed using one-way ANOVA (proc glm); however, due to a high degree of variability across treatment plots, comparisons were made between plots from individual treatment blocks in order to search for significant differences that would not have been apparent across the entire field site.

The density of *A. gossypii* was analyzed using mixed model analysis for repeated measures, with ant status (presence or absence) and sample date as fixed effects and plot as a random effect. The density of *B. tabaci* was analyzed using two-way analysis of variance (ANOVA) with ant status (presence or absence) and block (one through four) as fixed effects.

RESULTS

Fire ant exclusion

Visual assessment of fire ant activity at the Horseshoe Bend site revealed that Amdro treatments were successful in reducing the abundance of *S. invicta* as no individuals were observed either on plants or at the soil surface in the exclusion plots. Further, no new nests were detected during the course of the 2006 field season. Similarly, ant activity assessment at the Tifton site demonstrated that Amdro treatments were successful during the 2007 field season (Table 4.1).

Beet Armyworm Egg Predation in Cotton Foliage – Horseshoe Bend

In general, the proportion of eggs absent on plants at each date was significantly lower in ant exclusion plots than in plots where ants were included. During the 28 June trial, egg removal rates were significantly affected by the exclusion of ants, with removal rates being lower in exclusion than in inclusion plots during the first observation period ($t_{3.11}$=5.86, P=0.009, Figure 4.1). A similar pattern developed during the 25 July trial, but there were no significant differences in removal rates between exclusion and inclusion plots during any of the observation periods ($F_{2, 4.6}$=0.9, P=0.5, Figure 4.1). These two dates involved 24 hour intervals between observation-periods, which resulted in more than 50% of all eggs being consumed before observation period one in both inclusion and exclusion plots. During the 15 August trial, the proportion of eggs absent was also significantly affected by fire ant removal resulting in lower removal rates from exclusion plots than from inclusion plots at 12, 24, 36 and 48 hours after egg release ($t_{4.68}$=17.91, P<0.0001; $t_{4.68}$=11.16, P=0.0002; $t_{4.68}$=6.12, P=0.002; $t_{4.68}$=3.23, P=0.03, Figure 4.1). Shortening the amount of time in between observation periods revealed that although fire ants were able to exploit most, if not all, eggs within the first 12 hours of each trial predators in the absence of fire ants were only able to exploit approximately 25% of all eggs during the same amount of time. During the 26 September trial, however, this pattern disappeared and ant removal had no influence on egg mass removal rates during any of the observation periods ($F_{4, 7.62}=0.49$, $P=0.74$, Figure 4.1).

Tifton – Beet Armyworm

Similar to the 2006 Horseshoe Bend predation trials, *S. exigua* egg removal rates were generally faster in the presence of fire ants during the 2007 Tifton sampling season. During the 30 July predation trial all egg masses were removed from cotton foliage within the 24 hour assessment period in all plots, and we were not able to detect any differences in the rate of egg removal within this period. In the 14 August egg mass trial the proportion of eggs absent at each observation period was generally lower in fire ant exclusion plots; however, this difference was not apparent upon mixed model analysis as there were no significant effects of ants or the ants x observation interaction ($F_{1, 6}=0.72$, $P=0.42$; $F_{4, 84}=1.50$, $P=0.21$, Figure 4.2). Analysis of the 7 September predation data revealed a significant interaction effect ($F_{4, 82}=2.38$, $P=0.05$) and predation rates were significantly higher in fire ant inclusion plots than in exclusion plots during observation periods two and three ($t_{14.3}=2.38$, $P=0.03$; $t_{14.2}=2.16$, $P=0.04$, Figure 4.2). During the 7 September predation trial there was also a significant effect of plot among fire ant inclusion plots during observation period one ($F_{6, 80}=6.71$, $P<0.0001$, Figure 4.5A).

Tifton - Stink Bugs

Surprisingly, egg masses of the redbanded stink bug, *P. guildinii* in cotton foliage suffered no attrition by predators, including fire ants. Unlike the eggs of *P. guildinii*, eggs of *N. viridula* were readily fed upon by *S. invicta* and the big-eyed bug *Geocoris punctipes* Say (Hemiptera: Geocoridae). Actual egg removal rates, however, varied greatly among dates as well as treatment blocks. During the 11 July stink bug egg trial egg removal rates did not differ between exclusion plot one and inclusion plot one after three days ($F_{1,6}$=2.95, P=0.13). During the 24 July trial there were similarly no differences between exclusion and inclusion plots for either block one or block two ($F_{1,8}$=1.95, P=0.2; $F_{1,8}$=3.6, P=0.09). Predation rates did, however, differ significantly during the September stink bug egg mass trial (Table 4.2). Although there were no significant differences in predation rates between inclusion and exclusion plots from blocks one, two or three, there were significantly more eggs absent from fire ant inclusion plots 72 hours post-deployment ($F_{1,8}$=6, P=0.04). At 96 hours post-egg deployment, significantly more eggs were found with their contents removed (shell left intact) in fire ant exclusion plots than in inclusion plots ($F_{1,8}$=5.69, P=0.04); however, total predation (proportion of eggs removed, emptied and chewed) was significantly higher in inclusion plots than exclusion plots ($F_{1,8}$=11.52, P=0.009, Table 4.2).

Predation at Soil Surface – Horseshoe Bend

The proportion of eggs absent during each observation period on the soil surface was generally higher in ant inclusion plots than in exclusion plots and this trend tended to be consistent across all sampling dates. During the June sampling period there were no significant differences between ant exclusion and inclusion plots during any of the observation periods ($F_{2,2.92}$=0.77, P=0.54, Figure 4.3). The proportion of eggs absent during the July sampling period was lower in exclusion than in inclusion plots but this pattern was only significant for observation period one ($t_{4.25}$=2.81, P=0.05, Figure 4.3). During the August sampling period the proportion of eggs absent was significantly lower in exclusion than in inclusion plots at 12, 24 and 36 hours after egg release ($t_{11.5}$=13.66, P<0.0001; $t_{11.5}$=6.04, P<0.0001; $t_{11.5}$=5.35, P=0.0002, Figure 4.3). Unlike the results for predation on plant foliage, the proportion of eggs absent during the September sampling period was significantly lower in ant exclusion than inclusion plots and remained so over all five observation-periods ($t_{2.97}$=6.20, P=0.008; $7_{2.97}$=4.03, P=0.02; $t_{2.97}$=4.45, P=0.02; $t_{2.97}$=4.26, P=0.02; $t_{2.97}$=3.67, P=0.03, Figure 4.3). The September trial was terminated after the fifth survey date due to the fact that any remaining egg masses were dried out and considered to be unsuitable as prey during period six.

Tifton

Beet armyworm egg predation at the soil surface was high in both fire ant inclusion and exclusion plots for July, August and September sampling periods and all eggs were removed within 24 hours of egg deployment at the soil surface in both inclusion and exclusion plots. During August and September (virtually all eggs were removed within the first 18 hours), the increased frequency of egg mass observations allowed us to assess subtle differences in egg removal rates among ant inclusion and exclusion plots. During the August egg predation trial, we detected significant ant and ant x observation interaction effects ($F_{1, 6}$=11.84. P=0.01; $F_{4, 50}$=31.84, P<0.0001) and the proportion of *S. exigua* eggs absent one hour after egg deployment was significantly higher in fire ant inclusion plots than in exclusion plots ($t_{29.8}$=10.87, P<0.0001, Figure 4.4). Similarly, during the September egg trial, there were significant effects of ants and the ant by observation interaction ($F_{1, 6}$=13.53, P=0.01; $F_{4, 82}$=18.06, P<0.0001) and the proportion of eggs absent one hour after egg deployment was higher in fire ant inclusion plots than in exclusion plots ($t_{46.8}$=9.07, P<0.0001, Figure 4.4). Furthermore, during the September egg predation trials there was a significant plot effect detected among fire ant inclusion plots during observation period one ($F_{6, 80}$=2.24, P<0.05, 4.5B)

Aphid and Whitefly Density

The type 3 test for fixed effects based on mixed model analysis revealed a significant effect of fire ant exclusion on cotton aphid density at the Horseshoe Bend site ($F_{1,47.1}$=13.9, P=0.0005, Figure 4.6). This effect was significant during both July and September ($t_{49.2}$=2.44, P=0.018; $t_{49.2}$=2.83, P=0.006). Although aphid abundance appeared to increase between July and September in all plots, this trend was not significant ($F_{1, 51.4}$=0.08, P=0.7). Similarly, at the Tifton site there were significantly more individuals of *A. gossypii* on whole plants in fire ant inclusion plots than in exclusion plots during each sample date ($F_{1, 78}$=25.85, P<0.0001; $F_{1, 78}$=24.8, P<0.0001; $F_{1, 78}$=11.5, P=0.001; $F_{1, 78}$=21.86, P<0.0001; $F_{1, 78}$=15.35, P=0.0002; $F_{1, 78}$=29.67, P<0.0001; $F_{1, 78}$=18.27, P<0.0001; $F_{1, 38}$=4.2, P=0.04, Figure 4.7). In general, the density of *B. tabaci* was higher in fire ant inclusion plots (Figure 4.8); however, abundances of eggs and small nymphs were significantly higher in inclusion plots three and four than in one and two ($F_{7, 88}$=21.07, P<0.0001; $F_{7, 88}$=17.95, P<0.0001, Figure 4.9). In addition, the abundance of large nymphs was significantly higher in inclusion plot four than in any other plot ($F_{7, 88}$=16.05, P<0.0001, Figure 4.9).

DISCUSSION

Solenopsis invicta is considered an omnivore/generalist predator and can be extremely aggressive in both procuring and defending food (Tschinkel 2006). This study demonstrated that fire ants make a dramatic contribution to egg predation in cotton systems. These results are similar to those found by Diaz *et al.* (2004) who determined that fire ants are the most significant predators of both beet armyworm, *S. exigua*, and bollworm, *Helicoverpa zea* (Boddie), in cotton systems. This study is the first to demonstrate the strength of fire ant predation on eggs of *S. exigua* at the soil surface. Although beet armyworm eggs are not laid at the soil surface, they did provide us with a useful method for assessing food removal rates by predators. It is also important to note that eggs laid in foliage often become dislodged, falling to the soil surface. This experiment confirms the findings of a small number of studies that have similarly examined egg predation by *S. invicta* at the soil surface. Nuessly and Sterling (1994) determined that fire ant predation resulted in the recovery of only 20 and 10% of all eggs of *H. zea* 24 and 48 hours, respectively, after initial egg placement at the soil surface in a Texas cotton field. However, the study was only run during the month of July making it difficult to generalize over an entire season. Similarly, Lee *et al.* (1994) determined that after 72 hours *S. invicta* had removed 60-70% of all eggs of the mosquito *Psorophora columbiae* Dyar and Knab (Diptera: Culicidae), from the soil surface of an experimental rice paddy in Texas.

This study is also the first to assess the impact of fire ant predation on eggs of stink bugs. While fire ants significantly affected predation rates of *S. exigua* eggs across both field sites, predation on stink bug eggs by fire ants at the Tifton site was less striking and varied considerably among treatment blocks. Ehler (2002, 2007) observed that although predators readily fed upon nymphs of *Nezara viridula*, they rarely fed upon *N. viridula* eggs. In the current study we observed predation on eggs of *N. viridula* by both *S. invicta* and *G. punctipes*. However, eggs of *P. guildinii* were not predated for the duration of egg predation trial (3 days). The avoidance of *P. guildinii* eggs by predators has also been observed in laboratory feeding trials (Ruberson, unpubl. data) and suggests that there may be defensive chemicals secreted onto the surface of *P. guildinii* eggs, some of which deter predation. Bundy & McPherson (2000) observed a great deal of variation in the surface architecture of stink bug eggs which may also influence the ability of predators to feed on the eggs of particular species. These factors may have strong implications for pest management given that *P. guildinii*, originally from South and

Central America, appears to be expanding its range in the southern US, and is becoming a significant pest of US soybeans (Panizzi & Slansky 1985; J. Temple, Louisiana State Univ., personal comm.).

It has also been suggested that the close ecological relationship between *S. invicta* and cotton aphids may cause a seasonal trophic shift in fire ant feeding from the soil surface to cotton canopies (Kaplan and Eubanks 2005). Although we observed significantly more cotton aphids in fire ant inclusion plots during both sampling months at the Horseshoe Bend field site, aphid abundance did not differ between sampling months. Predation efficiencies on cotton foliage at Horseshoe Bend significantly decreased in September in both the presence and absence of *S. invicta*, coinciding with a significant drop in average nighttime temperature (Figure 4.10). This suggests that the primary factors limiting predation of beet armyworm eggs by fire ants during the course of our experiment at the Horseshoe Bend field site may be abiotic (e.g., temperature).

At the Tifton field site, aphid abundance was also significantly higher in fire ant inclusion plots than in exclusion plots. Furthermore, aphid abundance fell dramatically over the aphid sampling period within fire ant inclusion plots, dropping steadily from an average of 96 ± 14.4 aphids per plant on 24 July to 6 ± 2.8 on 24 August. This change in aphid abundance did not coincide with a change in egg predation rates from either cotton foliage or the soil surface, and therefore, did not support our third hypothesis. Populations of the whitefly *B. tabaci* appeared in August just as cotton aphids were disappearing during the 2007 growing season. While the abundance of whitefly eggs and small nymphs was significantly higher in fire ant inclusion plots three and four than in plots one and two, the abundance of large whitefly nymphs was significantly higher in inclusion plot four than in all other plots. Ant activity assays also revealed that the fire ant inclusion plot from block four was by far, the most active of all the inclusion plots (Table 4.1). In addition, inclusion plot number four was the only block in which predation rates on southern green stink bug eggs were significantly higher in the fire ant inclusion plot than in the exclusion plot (Table 4.2). Mixed model analyses also revealed that during the September beet armyworm egg mass trials, fire ant inclusion plot number four had significantly lower egg predation rates at the soil surface but higher predation rates on cotton foliage. These results suggest that an increase in whitefly activity, possibly initiated by high activity of fire ants, may have produced a feedback, causing fire ants to shift foraging activity away from the soil surface and onto cotton foliage, and offering partial support for hypothesis three.

In conclusion, the impact of fire ants on egg predation in cotton systems depends on a number of factors. This study demonstrates that predators can easily exploit eggs of the beet armyworm in cotton systems and that the presence of fire ants alone is enough to significantly affect predation rates. Our results further suggest that honeydew producing insects may be capable of altering fire ant foraging behavior, causing a shift in predation from the soil surface to foliage; however, this effect may vary in intensity based on the identity as well as the density of the dominant honeydew producer in the system.

Predation on stink bug eggs was generally lower than that on beet armyworm eggs and in the case of *Piezodorus guildinii*, was non-existent. However, predation on eggs of *Nezara viridula* was significantly affected by fire ants in plots with high ant activity. Increasing ant activity was also associated with an increase in the abundance of the whitefly *B. tabaci*. While many researchers have investigated the influence of cotton aphids on the predatory behavior of fire ants, the influence of whiteflies has been poorly examined.

Future research in this area should investigate the mechanisms underlying the species level discrimination in predation of stink bug eggs by *S. invicta.* Given the contrasting effects on egg predation and whitefly abundance during the current study, future research should also continue to explore the net impact of fire ants on control of multi-species pest complexes. Members from most insect orders have life stages that occur within soil; therefore, predation by fire ants within this habitat may affect multiple trophic groups, including agricultural pests. The potential link between plant and soil trophic interactions observed in this study should also be examined more rigorously.

REFERENCES

Bossuyt H., Six J. & Hendrix P.F. (2002). Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. *Soil Science Society of America Journal*, 66, 1965-1973.

Bundy C.S. & McPherson R.M. (2000). Morphological examination of stink bug (Heteroptera: Pentatomidae) eggs on cotton and soybeans, with a key to genera. *Annals of the Entomological Society of America*, 93, 616-624.

Diaz D., Knutson A. & Bernal J.S. (2004). Effect of the red imported fire ant on cotton aphid population density and predation of bollworm and beet armyworm eggs. *Journal of Economic Entomology*, 97, 222-229.

Ehler L.E. (2002). An evaluation of some natural enemies of Nezara viridula in northern California. *Biocontrol*, 47, 309-325.

Ehler L.E. (2007). Impact of native predators and parasites on Spodoptera exigua, an introduced pest of alfalfa hay in northern California. *Biocontrol*, 52, 323-338.

Eubanks M.D., Blackwell S.A., Parrish C.J., Delamar Z.D. & Hull-Sanders H. (2002). Intraguild predation of beneficial arthropods by red imported fire ants in cotton. *Environmental Entomology*, 31, 1168-1174.

Kaplan I. & Eubanks M.D. (2002). Disruption of cotton aphid (Homoptera: Aphididae) - Natural enemy dynamics by red imported fire ants (Hymenoptera: Formicidae). *Environmental Entomology*, 31, 1175-1183.

Kaplan I. & Eubanks M.D. (2005). Aphids alter the community-wide impact of fire ants. *Ecology*, 86, 1640-1649.

Lee D.K., Bhatkar A.P., Vinson S.B. & Olson J.K. (1994). Impact of foraging red imported fire ants (Solenopsis invicta) (Hymenoptera, Formicidae) on Psorophora columbiae eggs. *Journal of the American Mosquito Control Association*, 10, 163-173.

Mathews G.A. & Tunstall J.P. (eds.) (1994). *Insect Pests of Cotton*. University Press, Cambridge.

McDaniel S.G. & Sterling W.L. (1979). Predator determination and efficiency on Heliothis virescens (Heliothis virescens (F) Lepidoptera, Noctuidae) eggs in cotton using P-32(2). *Environmental Entomology*, 8, 1083-1087.

McDaniel S.G. & Sterling W.L. (1982). Predation of Heliothis virescens (F) eggs on cotton in east Texas. *Environmental Entomology,* 11, 60-66.

Nuessly G.S. & Sterling W.L. (1994). Mortality of Helicoverpa zea (Lepidoptera, Noctuidae) eggs in cotton as a function of oviposition sites, predator species, and desiccation. *Environmental Entomology*, 23, 1189-1202.

Panizzi A.R. & Slansky F. (1985). Review of phytophagous pentatomids (Hemiptera, Pentatomidae) associated with soybean in the Americas. *Florida Entomologist*, 68, 184-214.

Parker M.B., Gaines T.P., Plank C.O., Jones J.B., Walker M.E., Boswell F.C. & Dowler C.C. (1988). The effects of limestone on corn, soybeans and peanuts grown on a Tifton soil at various for seventeen years. . *Georgia Agricultural Experiment Station Research Bulletin*.

SAS (1999-2001). SAS System for Windows. In. SAS Institute inc. Cary, NC.

Stiles J.H. & Jones R.H. (2001). Top-down control by the red imported fire ant (Solenopsis invicta). *American Midland Naturalist*, 146, 171-185.

Triplehorn C.A. & Johnson N.F. (2005). *Borror and Delong's Introduction to the Study of Insects*. 7 edn. Thomson Brooks/Cole, Belmont, CA.

Tschinkel W.R. (2006). *The fire ants*. Belknap Press, Cambridge.

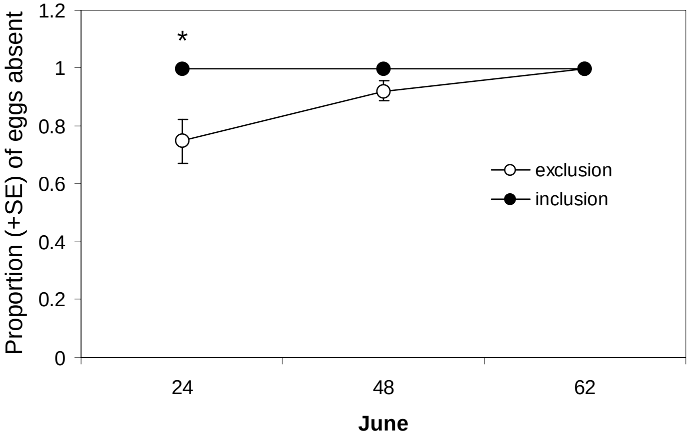
Vinson S.B. (1991). Effect of the Red Imported Fire Ant (Hymenoptera, Formicidae) on a Small Plant-Decomposing Arthropod Community. *Environmental Entomology*, 20, 98-103.

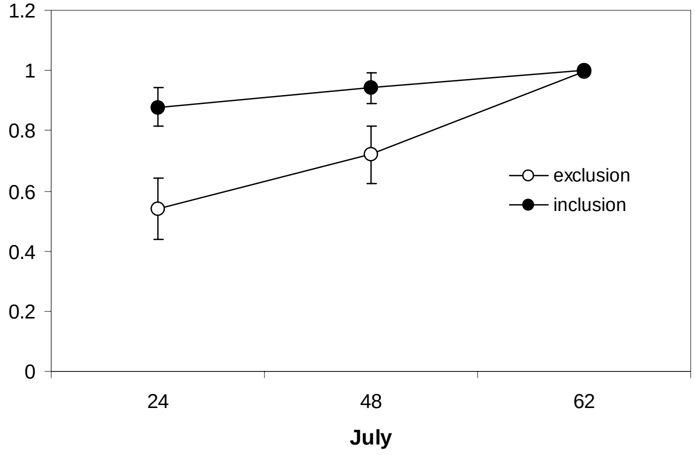
Table 4.1 Fire ant exclusion efficacy test. Data are means (±SE) of the number of fire ants collected in test tubes each containing 3g. of hotdog after 1 hour at the soil surface and on cotton foliage of fire ant inclusion and exclusion plots at the Coastal Plain Experiment Station. Each plot received 3 test tubes and numbers in brackets denote the number of test tubes in which fire ants were detected in a particular plot. Dates for which this test was not conducted are denoted by *(na)*.

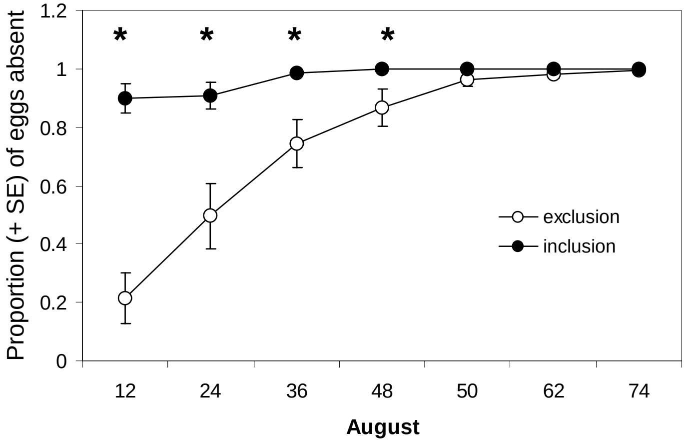
		total # ants collected in test tubes			
		soil surface		cotton foliage	
	block	inclusion	exclusion	inclusion	exclusion
6-Aug-07	one	18 (1)	0	0	0
	two	13 (1)	0	0	0
	three	2 (1)	0	0	0
	four	66 (3)	0	34 (1)	0
2-Sep-07	one	87 (1)	0	*na*	*na*
	two	103 (1)	0	*na*	*na*
	three	164 (1)	0	*na*	*na*
	four	52 (2)	0	*na*	*na*

Table 4.2 Proportion (±SE) of *Nezara viridula* eggs preyed upon in fire ant inclusion and exclusion plots of each block at 72 and 96 hours after eggs were initially deployed on 21 September, 2007 at the Coastal Plain Experiment Station. Predation type refers to the method by which eggs were fed upon. In cases where egg contents were removed the eggshell remained in place. Asterisks denote significant differences between inclusion and exclusion plots based on one-way analysis of variance (ANOVA, *P<0.05).

	proportion of eggs preyed upon at given observation time				
	time since deployment (hrs)	predation type	inclusion		exclusion
block 1	72	eggs removed	0.005±0.005		0.019±0.01
	96	eggs removed	0.015±0.01		0.013±0.008
block 2	72	eggs removed	0.27±0.24		0
	96	eggs removed	0.039±0.02		0
block 3	72	eggs removed	0.065±0.06		0.035±0.03
	96	eggs removed	0.065±0.06		0.047±0.04
block 4	72	eggs removed	0.6±0.24	*	0.072±0.07
	96	eggs removed	0.8±0.2	*	0.081±0.07
	96	contents only removed	0	*	0.013±0.005


Proportion (+SE) of eggs absent
1.2
1
0.8
0.6
0.4
0.2
0
*
exclusion
inclusion
24
48
62
June


1.2
1
0.8
0.6
0.4
0.2
0
exclusion
inclusion
24
48
62
July


Proportion (+ SE) of eggs absent
1.2
1
0.8
0.6
0.4
0.2
0
* * * *
exclusion
inclusion
12
24
36
48
50
62
74
August

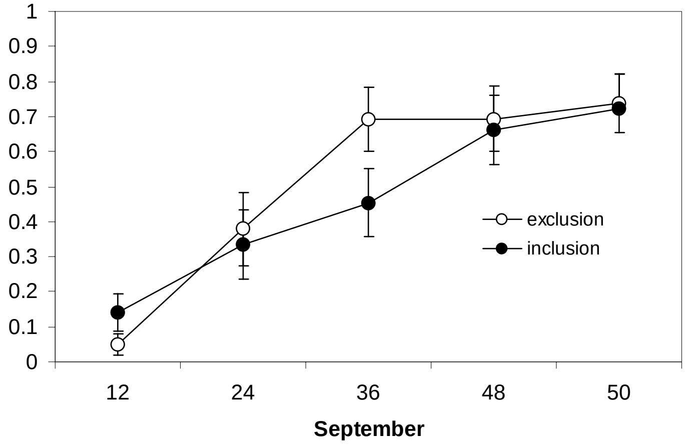

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
exclusion
inclusion
12
24
36
48
50
September

Figure 4.1 Beet armyworm egg predation from cotton foliage at Horseshoe Bend (2006). The proportion of eggs absent (±SE) at either 24 (June and July) or 12-hour observation intervals (August and September). Asterisks denote significant differences between the proportion of eggs absent from inclusion and exclusion plots for a given observation period based on Mixed Model analysis (*$P<0.05$).

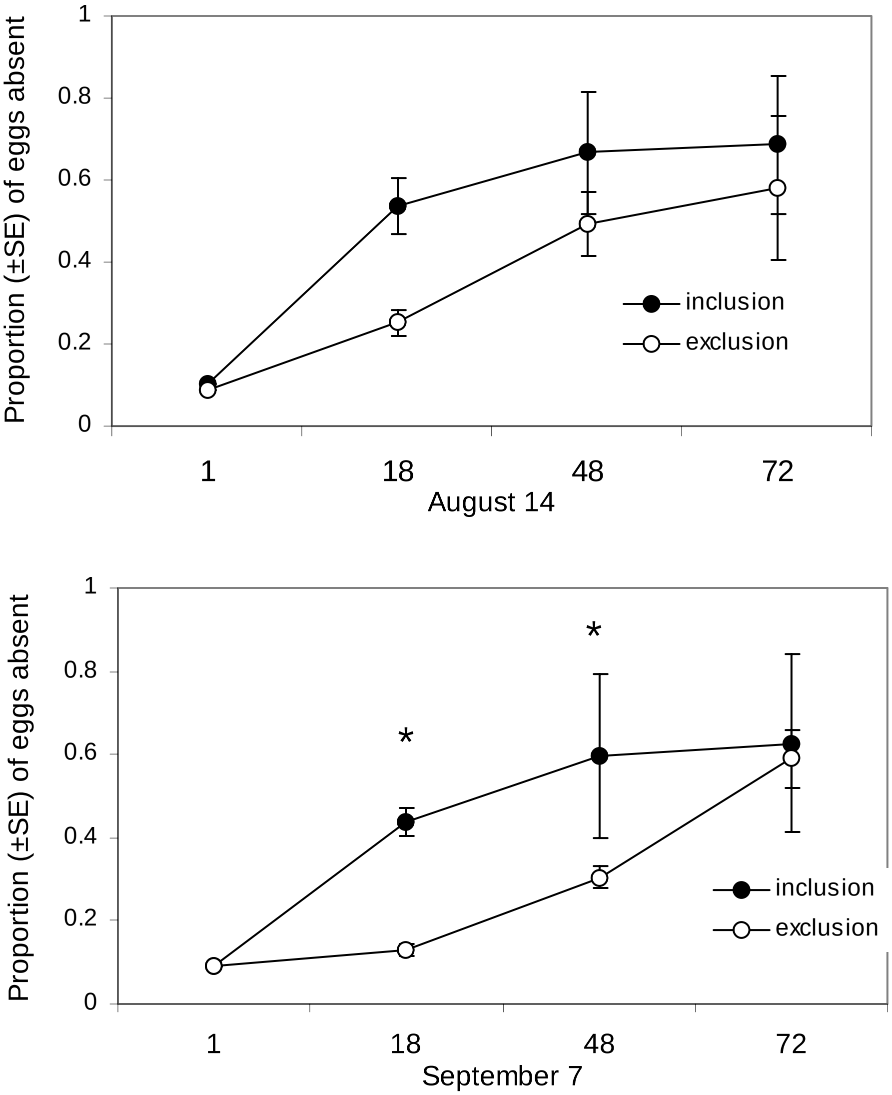
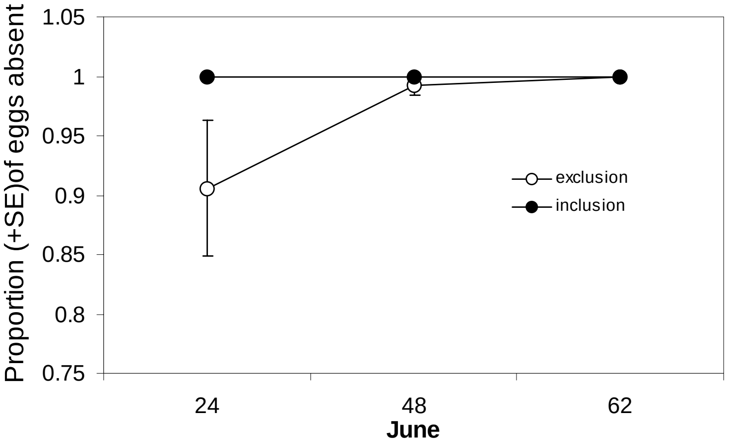
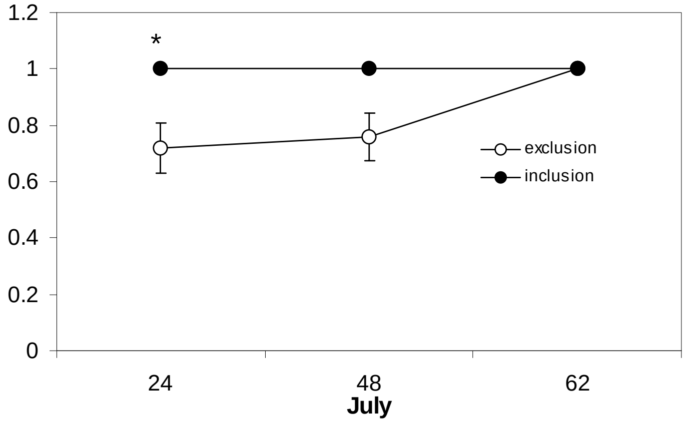
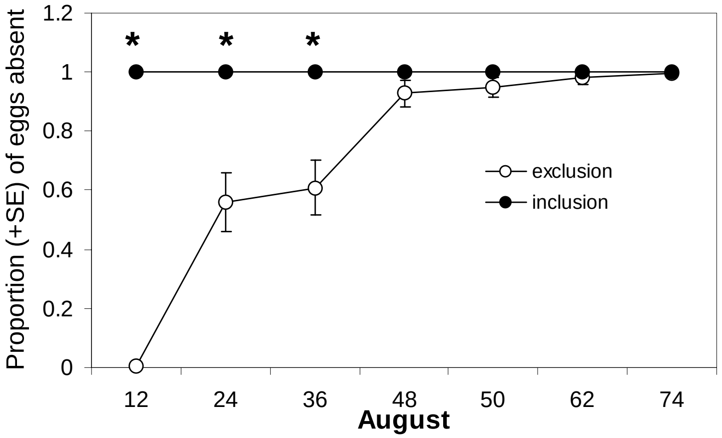


Figure 4.2 Beet armyworm egg predation from cotton foliage in Tifton, GA (2007). The proportion of eggs absent (±SE) at 1, 28, 48 and 72 hours after initial egg deployment (August and September). Asterisks denote significant differences between the proportion of eggs absent from inclusion and exclusion plots for a given observation period based on Mixed Model analysis (*P<0.05).


Proportion (+SE)of eggs absent
1.05
1
0.95
0.9
0.85
0.8
0.75
24
48
62
June
exclusion
inclusion


1.2
1
0.8
0.6
0.4
0.2
0
*
24
48
62
July
exclusion
inclusion


Proportion (+SE) of eggs absent
1.2
1
0.8
0.6
0.4
0.2
0
* * *
12
24
36
48
50
62
74
August
exclusion
inclusion

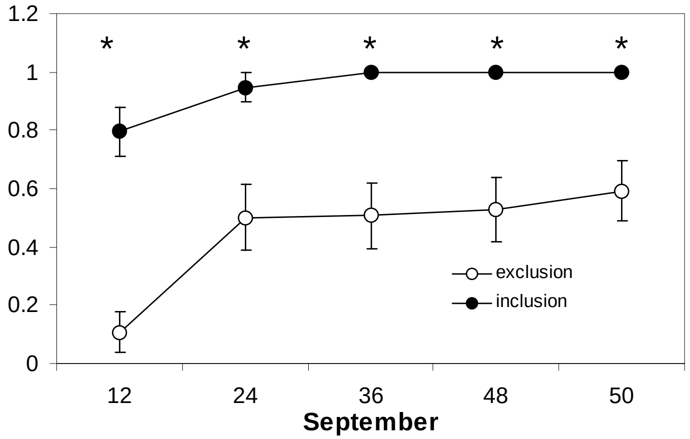

1.2
1
0.8
0.6
0.4
0.2
0
* * * * *
12
24
36
48
50
September
exclusion
inclusion

Figure 4.3 Beet armyworm egg predation at the soil surface at Horseshoe Bend (2006). The proportion of eggs absent (±SE) at either 24 (June and July) or 12-hour observation intervals (August and September). Asterisks denote significant differences between the proportion of eggs absent from inclusion and exclusion plots for a given observation period based on Mixed Model analysis (*$P<0.05$).

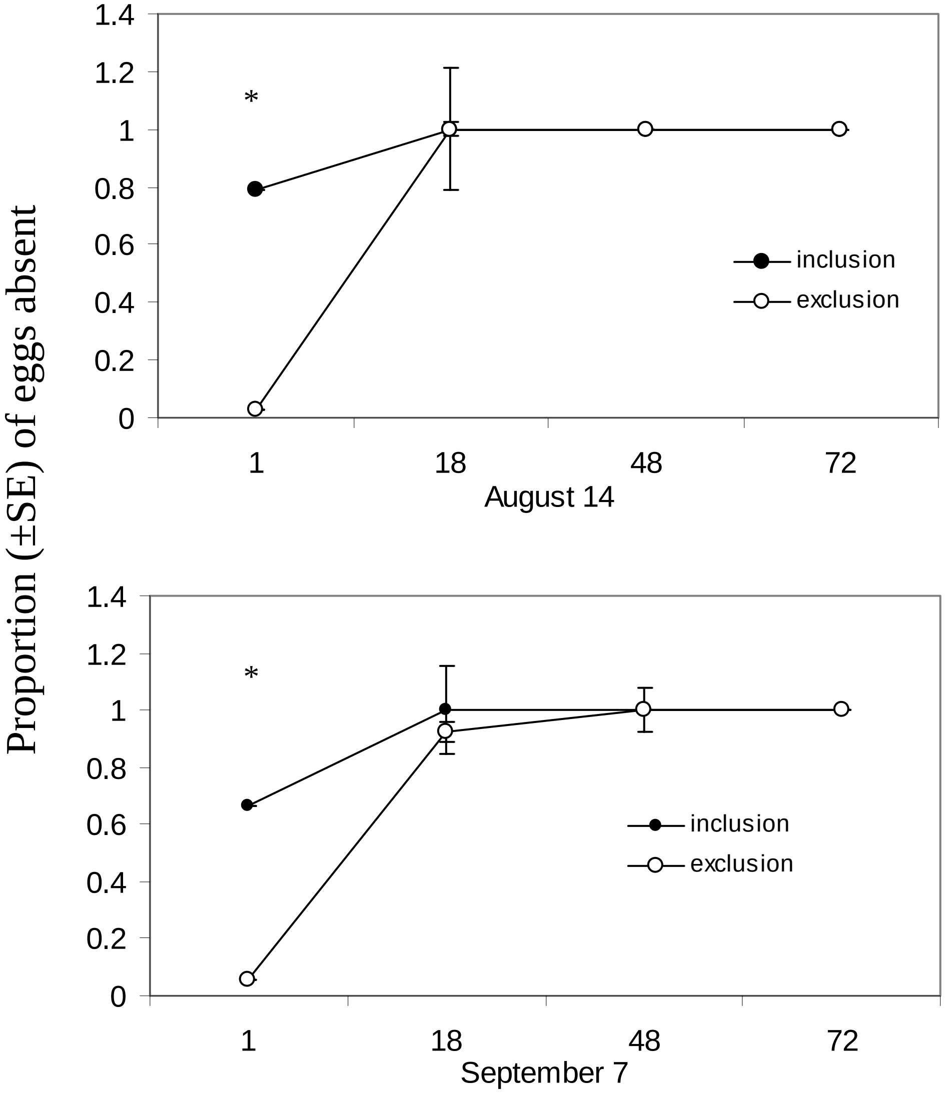


Figure 4.4 Beet armyworm egg predation from soil surface in Tifton, GA (2007). The proportion of eggs absent (±SE) at 1, 28, 48 and 72 hours after initial egg deployment (August and September). Asterisks denote significant differences between the proportion of eggs absent from inclusion and exclusion plots for a given observation period based on Mixed Model analysis (*P<0.05).

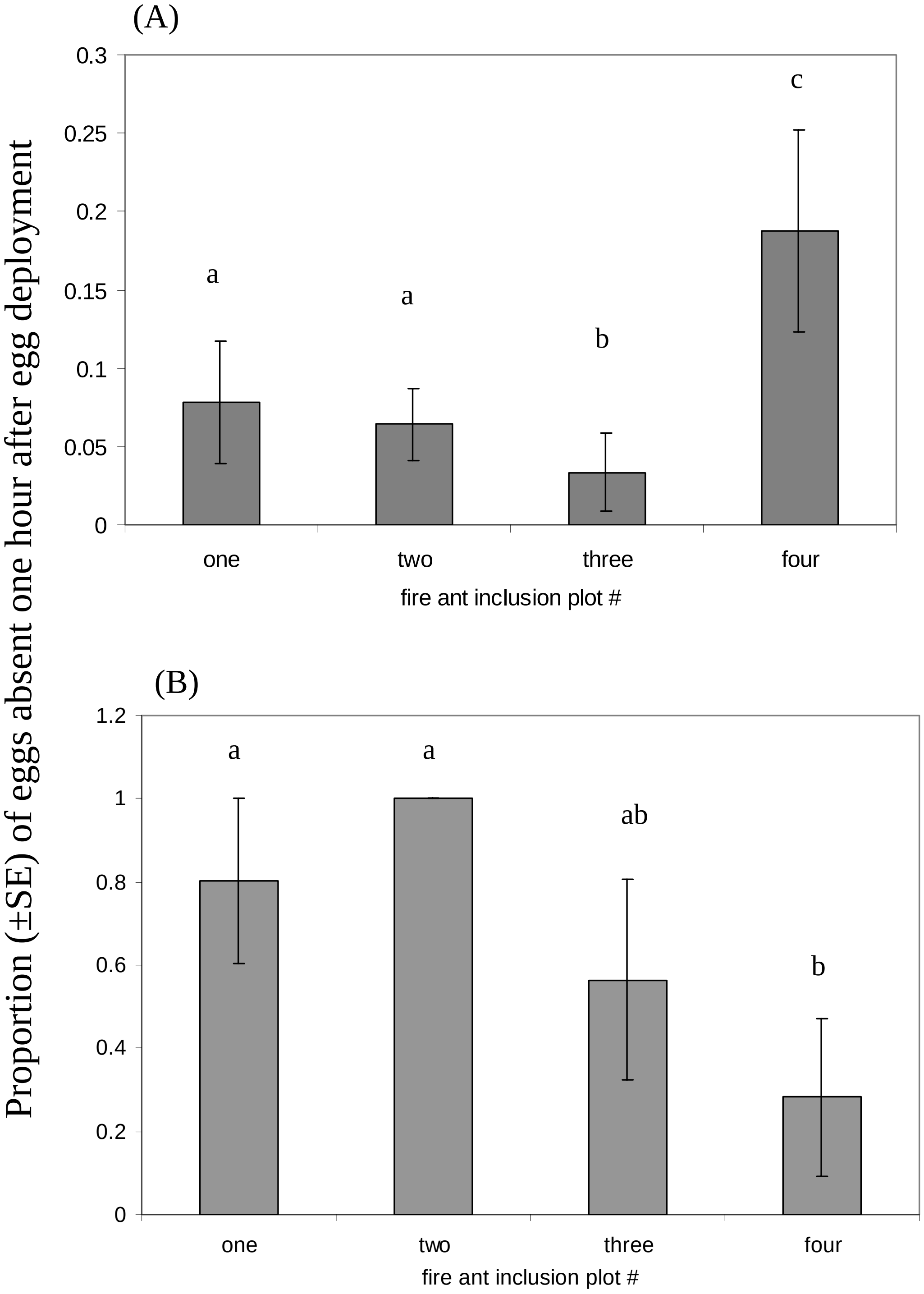

Proportion (±SE) of eggs absent one hour after egg deployment
(A)
0.3
0.25
0.2
0.15
0.1
0.05
0
a
a
b
c
one
two
three
four
fire ant inclusion plot #
(B)
1.2
1
0.8
0.6
0.4
0.2
0
a
a
ab
b
one
two
three
four
fire ant inclusion plot #

Figure 4.5 Egg predation from cotton foliage (A) and at the soil surface (B) in Tifton, GA (2007). The proportion of eggs absent (±SE) at 1 hours after initial egg deployment (September). Letters denote significant differences between given plots at the 1hr observation period based on Mixed Model analysis (*P<0.05).

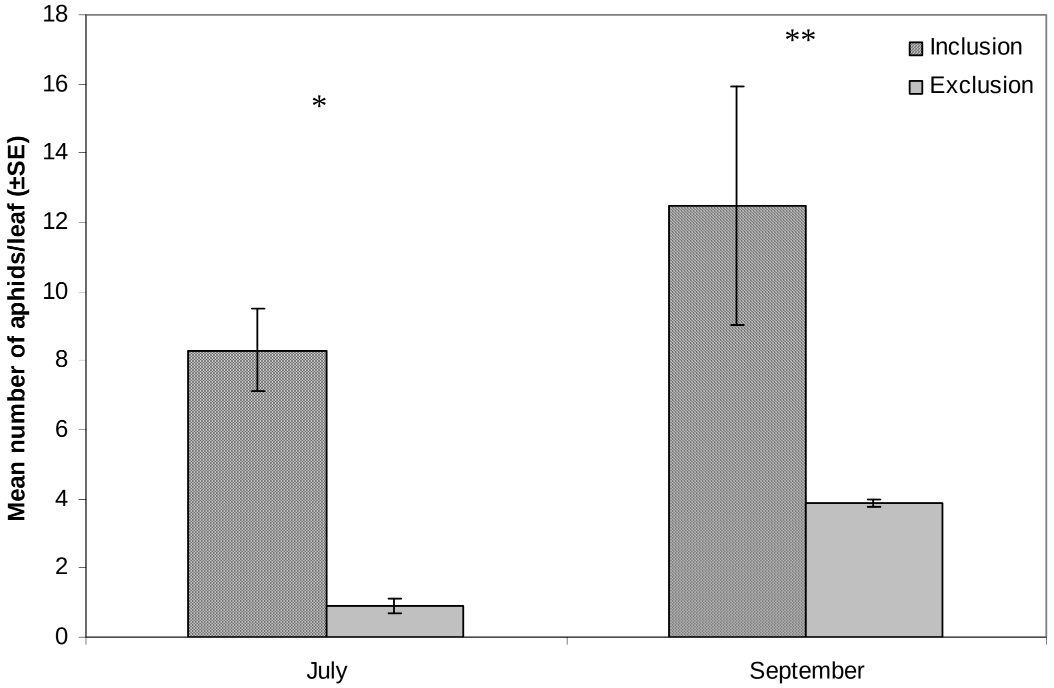


Figure 4.6 Average density of cotton aphids (*Aphis gossypii*) per cotton leaf from fire ant inclusion and exclusion plots during July and September 2006. Significant differences are based on mixed model analysis (*P<0.05, **P<0.01).

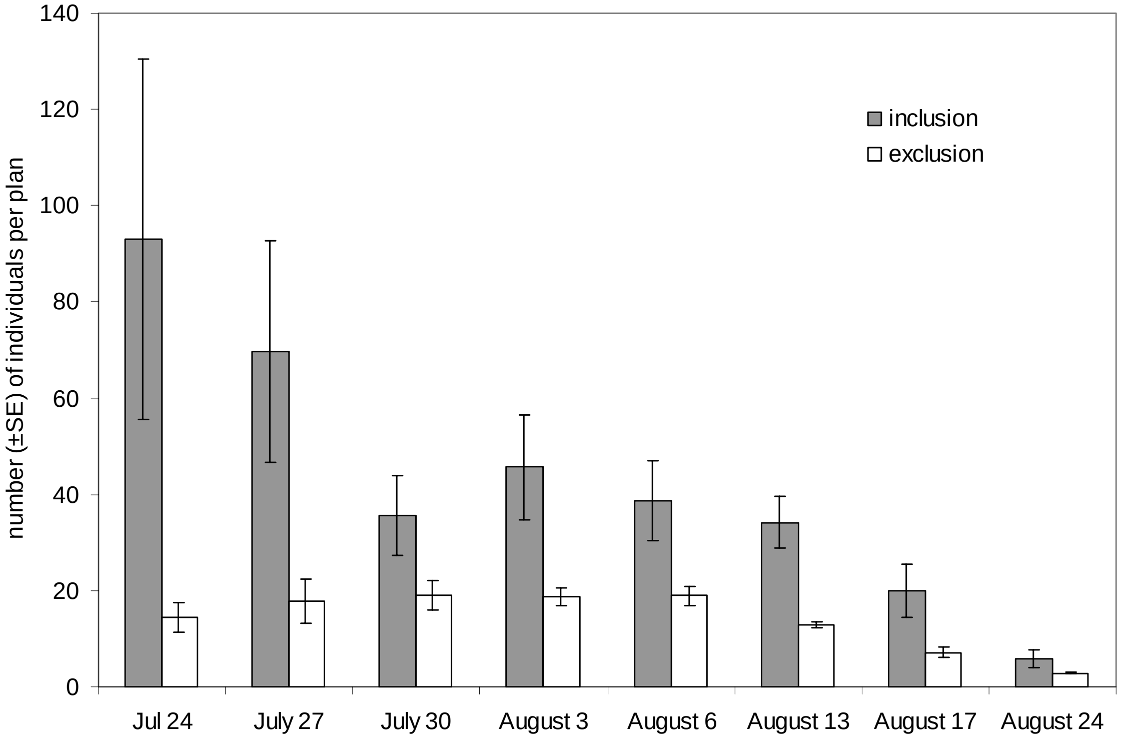


Figure 4.7 Mean (±SE) number of cotton aphids per cotton plant per sampling date from the Tifton, GA, 2007. Bars represent fire ant inclusion and exclusion plots. At each date, the difference between abundance from inclusion and exclusion plots is significantly different based on mixed model analsysis (P<0.0.5).

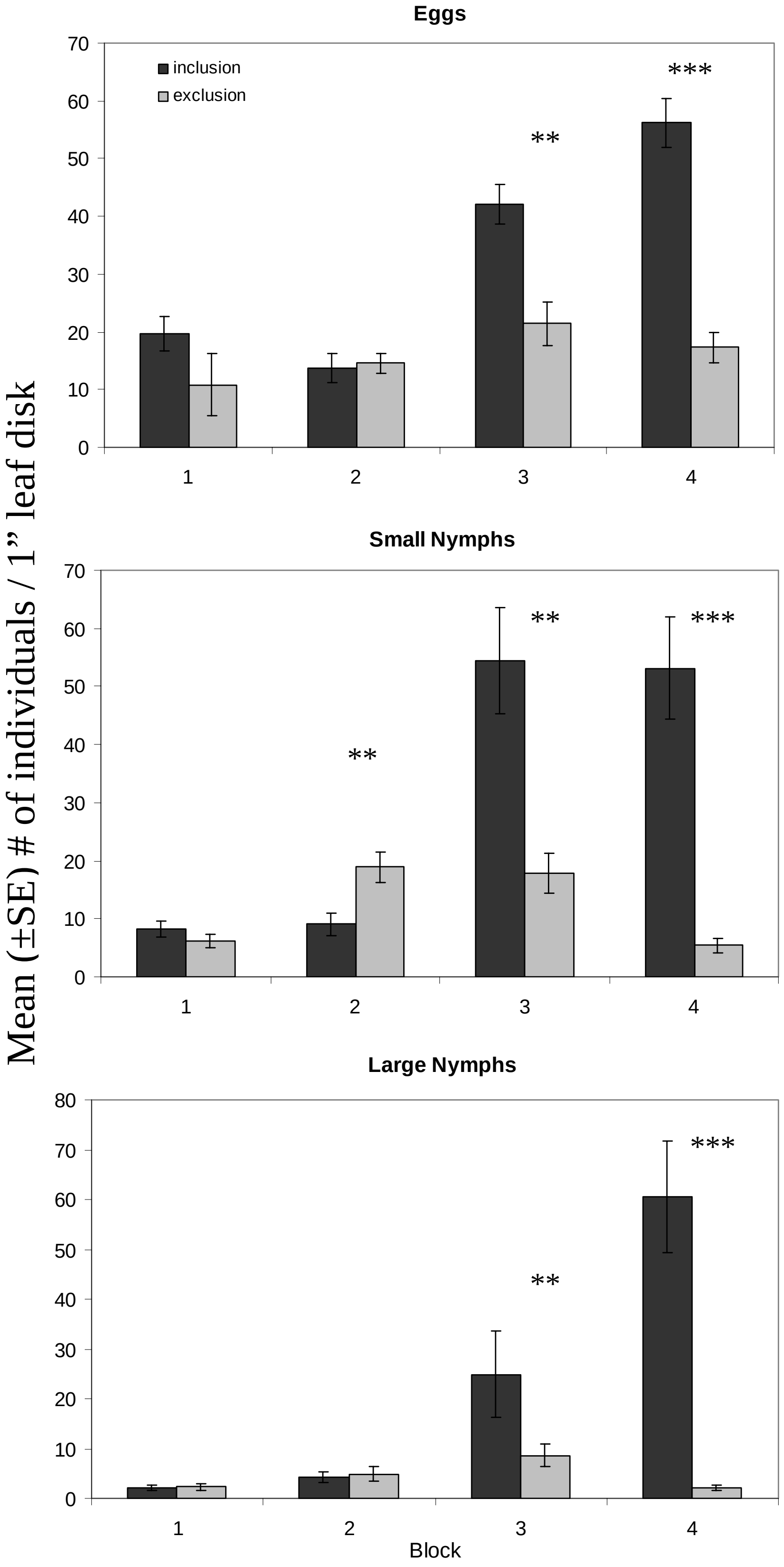

Eggs
inclusion
exclusion
70
60
50
40
30
20
10
0
**
***
1
2
3
4
Small Nymphs
70
60
50
40
30
20
10
0
**
**
***
1
2
3
4
Large Nymphs
80
70
60
50
40
30
20
10
0
**
***
1
2
3
4
Block
Mean (±SE) # of individuals / 1” leaf disk

Figure 4.8 Mean (±SE) number of whitefly (*Bemesia tabaci*) eggs as well as small and large nymphs counted on 12 cotton leaf disks (1 inch in diameter) per fire ant inclusion plot on August 29th, 2007 from Tifton, GA. Asterisks denote significant differences based on one-way analysis of variance between fire ant inclusion and exclusion plots of individual blocks (*P<0.05, **P<0.01, ***P<0.001).

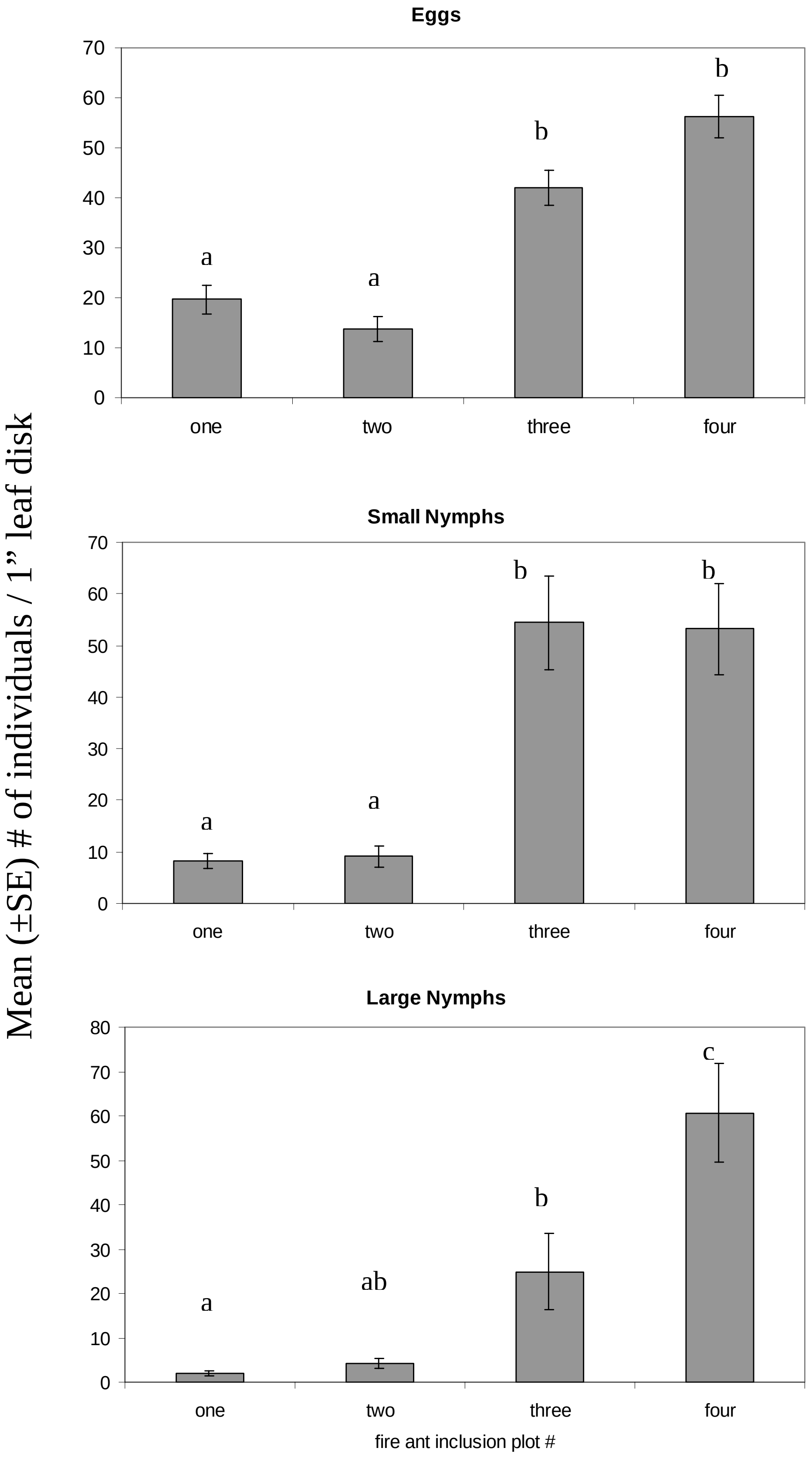

Eggs
70
60
50
40
30
20
10
0
a
a
b
b
one
two
three
four
Small Nymphs
70
60
50
40
30
20
10
0
a
a
b
b
one
two
three
four
Large Nymphs
80
70
60
50
40
30
20
10
0
a
ab
b
c
one
two
three
four
fire ant inclusion plot #
Mean (±SE) # of individuals / 1” leaf disk

Figure 4.9 Mean (±SE) number of whitefly (*Bemesia tabaci*) eggs as well as small and large nymphs counted on 12 cotton leaf disks (1 inch in diameter) per fire ant inclusion plot on August 29th, 2007 from Tifton, GA. Letters denote significant differences between fire ant inclusion plots at the 1hr observation period based on Mixed Model analysis *($P<0.05$).

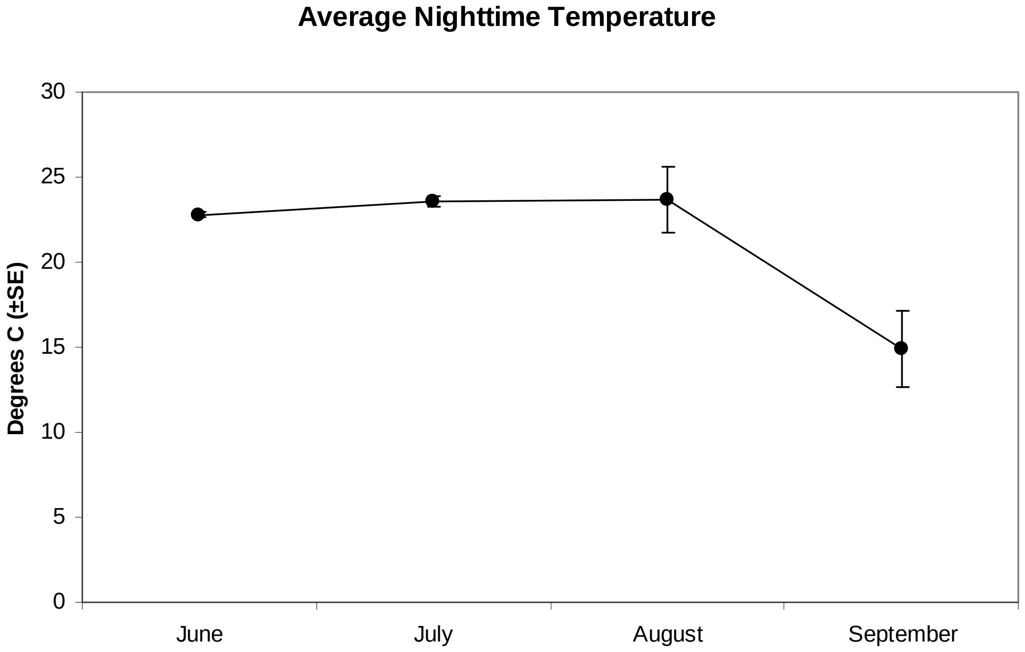


Figure 4.10 Average temperature (°C±SD) between 9:00 pm and 6:00 am during egg mass removal periods during each of the four sampling months for 2006.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

Agroecosystem structure is shaped by a complex of natural and anthropogenic factors, prominent among which are invasive species and the recent release of pesticide-incorporated transgenic plants. Genetically modified (GM) crops and invasive species also have both generated a great deal of interest within the field of ecology and much of this interest has been directed towards their impact on biodiversity. The impact of GM crops on non-target arthropods in agricultural systems has been studied extensively and, in general, they have been found to have very little influence over non-target communities (O'Callaghan *et al.* 2005; Romeis *et al.* 2006). Most of these studies are limited to simple comparisons between GM and non-GM crops. However, these crops are grown in a complex matrix of agronomic management strategies that include variation in management techniques such as tillage regimes and cover crops. To begin addressing potential interactions among crop choices and management strategies it is necessary to understand the relative contributions of all management factors being used within a single agroecosystem and the role of those factors in shaping the arthropod community structure.

Invasion by exotic species is common in disturbed habitats with low levels of species richness such as agroecosystems (Hooper et al. 2005). Past research shows that invasive arthropods and other invertebrates can alter many components of agricultural systems. For example, the red imported fire ant *Solenopsis invicta* Buren, has been found to contribute to the control of many cotton pests (Lofgren 1986). Another invasive predator, the multicolored Asian ladybeetle *Harmonia axyridis* Pallas, has been found to out compete a native coccinellid species *Cycloneda sanguinea* (L.) on Florida citrus farms (Michaud 2002). The impact of invasive invertebrates in agricultural systems can also go beyond pest control. For example, European lumbricid earthworms *Amynthus* spp. have been found to significantly affect soil processes

(Hendrix *et al.* 2006); however, many aspects of the impact of invasives in agroecosystems have not been explored.

The experiments conducted during the course of this dissertation examined the impact of genetically modified crops and invasive species on arthropod communities above- and below-ground in a cotton agroecosystem. First, the impact of genetically-modified Bt cotton was examined relative to the effects of tillage and cover crop identity; management strategies within which the use of Bt cotton was nested (Chapter 2). Second, the effect of the red imported fire ant, *S. Invicta*, was examined on arthropod communities above- and below-ground, as well as on egg predation in cotton foliage and at the soil surface using a series of fire ant exclusion experiments (Chapters 3 and 4).

Chapter 2 demonstrated that tillage and cover crop identity can have much greater effects on arthropod communities than can Bt cotton. Although their relative importance varied based on year and sample type, tillage and cover crop appeared to have the largest impact on arthropods, both at the whole-community and individual family levels. Tillage strategy had significant effects on 16 different arthropod families and similar to the results of past experiments, most arthropods were more abundant in no-till than in conventionally tilled plots (Stinner & House 1990; Wardle D.A. 1995). Although cover crop effects were limited to one year of the study (April 2005), 12 different arthropod families were significantly affected, 11 of which were more abundant in clover than in rye. Previous research has demonstrated a high degree of variability among different cover crop species regarding their effects on arthropod communities, and in many cases, crop morphology had a stronger impact on arthropods than did crop species (Bugg & Dutcher 1989; Tremelling *et al.* 2003). Although the effects of cover crops on arthropod communities are highly variable among cover crop species, arthropod abundance is often higher in leguminous covers than in graminaceous cover crops (Tillman *et al.* 2004). This functional partitioning of cover crops supports the observations of the current study, as the abundance of many families increased in clover compared to that in rye plots.

There were also significant effects of both Bt cotton and its residue on specific arthropod families. For instance the mite suborder Prostigmata was significantly more abundant in non-Bt cotton whereas crickets (Orthoptera: Gryllidae) were more abundant in Bt cotton. Further, the family Miridae (chiefly *Lygus lineolaris,* and *Halticus bractatus*) was more abundant in plots with Bt cotton residue during the cover crop season. All of the effects of Bt cotton, however, were restricted to one growing season and strong evidence is lacking to suggest a mechanism linking the dynamics of these particular taxa to the known characteristics of Bt cotton. Many interactions were also detected in the present study indicating that the impact of tillage and cover crop identity significantly altered the impact of Bt cotton on arthropod communities, but none of these effects were consistent and many were significant in opposing directions from one season to the next.

Transgenic crops remain controversial and like all types of agricultural production practices, pose potential environmental risks. The results presented in this dissertation demonstrate that Bt cotton may have some context specific impacts on non-target arthropod communities when examined under varying cover crop and tillage practices. However, the effect of Bt was negligible relative to the role of tillage and cover crop choice in shaping the non-target arthropod community of this particular system.

In Chapter 3 the impact of *Solenopsis invicta* was examined on both soil- and surface-active arthropods. This was the first assessment of the role of fire ants as predators in soil food webs, and as predicted, *S. invicta* did not appear to have a significant effect on total arthropod abundance, richness or diversity, either above or belowground. There were, however, a number of taxa that responded independently to the removal of fire ants. For instance, the abundance of thrips as well as three surface-active detritivores (two collembolan families and one oribatid mite) increased in the absence of fire ants. While research has shown that fire ants often prey upon Collembola as well as some detritivorous beetles, this is the first observation of a significant decrease in the abundance of Collembola and oribatid mites in the presence of fire ants (Wilson & Oliver 1969; Vinson 1991). The abundance of multiple entomophagous groups was also significantly affected by the removal of fire ants; however, conflicting patterns were detected between two field sites suggesting that the effect of fire ants on soil arthropod communities may be context specific. While the abundance of multiple predators (tiger beetles, erythraeid and cunaxid mites and linyphiid spiders) and parasitic scelionid wasps increased in the presence of fire ants at the Horseshoe Bend field site, the most abundant predator at the Tifton field site, *Labidura riparia,* decreased in abundance in the presence of fire ants. While past research has demonstrated that the abundance of many entomophagous insects can increase in the presence of fire ants, other examples exist to support our observed decrease in earwig abundance (Morrison & Porter 2003; Calixto *et al.* 2006). Staphylinid beetles also showed conflicting responses to fire ant removal between the two field sites. At Horseshoe Bend, seven morphospecies of staphylinids were collected and, at the family level, their abundance increased in the presence of *S. invicta.* Only two staphylinid morphospecies were commonly collected at the Coastal Plain Experiment Station and, contrary to our findings at Horseshoe Bend, staphylinid abundance decreased in the presence of fire ants. These conflicting results may be attributable to species level differences among taxa from our field sites, however, as discussed in

Chapter 3, other differences between the sites, such as weed biomass and soil structure may be the primary reason for the differences observed (Stinner & House 1990; Andersen 1999).

Chapter 4 demonstrated that fire ants also make a significant contribution to egg predation at the soil surface in cotton systems. This study was also the first to assess the impact of fire ant predation on eggs of stink bugs. Predation on stink bug eggs was generally lower than that on beet armyworm eggs and in the case of the stink bug *Piezodorus guildinii*, was non-existent. In contrast, predation on eggs of *Nezara viridula* was significantly affected by fire ants in plots with high ant activity. Increasing ant activity, however, was also associated with an increase in the abundance of the whitefly *Bemisia tabaci*. While many researchers have investigated the influence of cotton aphids on the predatory behavior of fire ants, the influence of whiteflies has not been considered (Kaplan & Eubanks 2002, 2005). The results of this study suggest that a great deal of variation exists in the intensity of egg predation by fire ants. Our results further suggest that honeydew-producing insects may be capable of altering fire ant foraging behavior, causing a shift in predation from the soil surface to foliage, but this effect may vary in intensity based on the identity and density of the dominant honeydew producer in the system.

In summary, tillage and cover crop identity are more important in shaping arthropod communities than are the effects of Bt cotton. Furthermore, the effect of Bt cotton on arthropod communities is not consistently altered within the context of different management strategies (tillage strategy and cover crop type). Conservation tillage contributes to enhanced ant populations in cotton, and the red imported fire ant can have a significant effect on many trophic groups within soil arthropod communities of cotton agroecosystems; however, factors such as weed density and arthropod species identity may alter the overall direction of this effect. The red imported fire ant can also serve as a significant egg predator in cotton systems, both in cotton foliage and at the soil surface; however, there is considerable variation in the intensity of egg predation by fire ants among eggs from different taxa. Finally, honeydew-producing hemipterans may significantly influence the predation of eggs by fire ants, both at the soil surface and within cotton foliage.

FUTURE DIRECTIONS

It is clear from past research and from the results of the research conducted during the course of this dissertation that the impact of Bt cotton on arthropod communities is subtle to non-existent (O'Callaghan *et al.* 2005). The results presented here also, however, demonstrate that there may be significant context-specific differences in the effects of Bt cotton on arthropod communities. Given the variation among cotton production systems even within the state of Georgia, further investigations of the impact of Bt cotton on arthropod communities within each of these contexts may be necessary. In the current study, the strength of context specific effects was likely distorted by the small size of experimental field units. Subsequently, the examination of context specific effects should also be explored at the full farm-scale level, involving multi-hectare fields.

During the course of this dissertation I was unable to adequately assess the impact of Bt cotton on soil fauna communities. Past research has found little effect of Bt cotton on soil arthropod communities (O'Callaghan *et al.* 2005), but more recent studies have demonstrated that Bt cotton can have significant effects on soil microbes and processes, including microbial enzyme activity and decomposition rate (Flores *et al.* 2005; Knox *et al.* 2007; Sun *et al.* 2007; Yan *et al.* 2007). These findings may warrant further study on the impact of Bt crops on soil food webs.

Future research on the impact of fire ants on soil arthropod communities of cotton agroecosystems is also necessary. Past research, including results from the current experiments (Chapter 2), has shown that many of the agricultural management factors examined during the course of this dissertation are known to influence fire ant densities. For example, fire ant abundance has been found to increase under conservation tillage strategies compared to conventional tillage (Ruberson *et al.* 1997; Tillman *et al.* 2004). Chapter 2 of the current study also demonstrated that fire ants can be more abundant in clover than in rye, a pattern also observed by Tillman *et al.* (2004). Our assessment of the impact of fire ants on soil foodwebs was conducted in conventionally tilled fields only. Given the potential of weeds to influence the direction of fire ant induced effects on predators observed in the current study, as well as the differences observed in past research on the impact of tillage on weed density, a likely next step would include an examination of the effects of *S. invicta* on soil food webs across agroecosystems with varying intensities of soil management, and subsequently, varying weed community compositions.

Future research in this area should also investigate the mechanisms underlying the species level discrimination in predation of stink bug eggs by *S. invicta*. Given the contrasting effects on egg predation and whitefly abundance during the current study, future research should also continue to explore the net impact of fire ants on control of multi-species pest complexes. Members from most insect orders are known to lay their eggs on or near the soil surface, and ground-level predation by fire ants may therefore affect multiple trophic groups, including agricultural pests. The potential link between plant and soil trophic interactions observed in this study involving honeydew-producing hemipterans should also be examined more rigorously.

REFERENCES

Andersen A. (1999). Plant protection in spring cereal production with reduced tillage. II Pests and beneficial insects. . *Crop Protection*, 18, 651-657.

Bugg R.L. & Dutcher J.D. (1989). Warm season cover crops for pecan orchards - Horticultural and entomological implications. *Biological Agriculture & Horticulture*, 6, 123-148.

Calixto A., Dean A., Knutson A. & Harris M. (2006). Density changes of two earwigs, Labidura ripade (Pallas) and Euborellia annulipes (Lucas) following fire ant reduction in Mumford, Texas. *Southwestern Entomologist*, 31, 97-101.

Flores S., Saxena D. & Stotzky G. (2005). Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry*, 37, 1073-1082.

Hendrix P.F., Baker G.H., Callaham M.A., Damoff G.A., Fragoso C., Gonzalez G., James S.W., Lachnicht S.L., Winsome T. & Zou X. (2006). Invasion of exotic earthworms into ecosystems inhabited by native earthworms. *Biological Invasions*, 8, 1287-1300.

Kaplan I. & Eubanks M.D. (2002). Disruption of cotton aphid (Homoptera: Aphididae) - Natural enemy dynamics by red imported fire ants (Hymenoptera: Formicidae). *Environmental Entomology*, 31, 1175-1183.

Kaplan I. & Eubanks M.D. (2005). Aphids alter the community-wide impact of fire ants. *Ecology*, 86, 1640-1649.

Knox O.G.G., Gupta V., Nehl D.B. & Stiller W.N. (2007). Constitutive expression of Cry proteins in roots and border cells of transgenic cotton. *Euphytica*, 154, 83-90.

Lofgren C.S. (1986). The Economic Importance and Control of Imported Fire Ants in the United States. In: *Economic Impact and Control of Social Insects* (ed. Vinson BS). Praeger Publishers New York, pp. 227 - 256.

Michaud J.P. (2002). Invasion of the Florida citrus ecosystem by Harmonia axyridis (Coleoptera: Coccinellidae) and asymmetric competition with a native species, Cycloneda sanguinea. *Environmental Entomology*, 31, 827-835.

Morrison L.W. & Porter S.D. (2003). Positive association between densities of the red imported fire ant, Solenopsis invicta (Hymenoptera: Formicidae), and generalized ant and arthropod diversity. *Environmental Entomology*, 32, 548-554.

O'Callaghan M., Glare T.R., Burgess E.P.J. & Malone L.A. (2005). Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology*, 50, 271-292.

Romeis J., Meissle M. & Bigler F. (2006). Transgenic crops expressing Bacillus thuringiensis toxins and biological control. *Nature Biotechnology*, 24, 63-71.

Ruberson J.R., Phatak S.C. & Lewis W.J. (1997). Insect populations in a cover crop/strip till system. In: *Proceedings, Beltwide Cotton Conference*. National Cotton Council Memphis, TN, pp. 1121-1124.

Stinner B.R. & House G.J. (1990). Arthropods and other invertebrates in conservation-tillage agriculture. *Annual Review of Entomology*, 35, 299-318.

Sun C.X., Chen L.J., Wu Z.J., Zhou L.K. & Shimizu H. (2007). Soil persistence of Bacillus thuringiensis (Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzyme activities. *Biology and Fertility of Soils*, 43, 617-620.

Tillman G., Schomberg H., Phatak S., Mullinix B., Lachnicht S., Timper P. & Olson D. (2004). Influence of cover crops on insect pests and predators in conservation tillage cotton. *Journal of Economic Entomology*, 97, 1217-1232.

Tremelling M.J., McSorley R. & Gallaher R.N. (2003). Effects of winter cover crops on the soil surface invertebrate community. *Soil and Crop Science Society of Florida Proceedings*, 62, 77-82.

Vinson S.B. (1991). Effect of the Red Imported Fire Ant (Hymenoptera, Formicidae) on a Small Plant-Decomposing Arthropod Community. *Environmental Entomology*, 20, 98-103.

Wardle D.A. (1995). Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. *Advances in Ecological Research*, 26, 105-183.

Wilson N.L. & Oliver A.D. (1969). Food Habits of Imported Fire Ant in Pasture and Pine Forest Areas in Southeastern Louisiana. *Journal of Economic Entomology*, 62, 1268-&.

Yan W.D., Shi W.M., Li B.H. & Zhang M. (2007). Overexpression of a foreign Bt gene in cotton affects the low-molecular-weight components in root exudates. *Pedosphere*, 17, 324-330.