Red Imported Fire Ant Influences on White Grub Populations and Soil Foraging Characteristics in Managed Turfgrass

by

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Abstract

White grubs (Coleoptera: Scarabaeidae) are a significant pest of managed turfgrass throughout the United States and are difficult to monitor because of their subterranean habitat. Recent reports have stated that white grubs are becoming an increasing problem in southern turfgrass. Selective removal of common turfgrass ants using labeled insecticides can cause localized outbreaks of turfgrass pest. However, white grubs and other hypogeal insects are reported to escape predation of *Solenopsis invicta* (Buren) and seemingly co-exist in close proximity. After two years of field experiments examining interactions between *S. invicta* and white grubs, as well as examining this ant's soil foraging characteristics, it was found that the control of *S. invicta* has no influence on white grub populations in turfgrass. Further investigation showed *S. invicta* does not forage within the soil for prey as previous research on other common turfgrass ants suggests. Experimental design flaws identified and addressed during this research highlights factors affecting previous studies on ant predation of subterranean pests that likely resulted in biased data.

Dedication

I would like to dedicate this thesis and its work to my parents, Doug and Jill, brother Chandler, and sister Wesley Anne. Also, I would like to dedicate this work to Coach Laverty who sparked my interest in turfgrass.

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Chapter I

GENERAL INTRODUCTION

Introduction to Turfgrass

The turfgrass industry has grown into a major industry in the United States with 27.6 million acres of total turfgrass, 21 million of which are home lawns (Borman et al. 2001). In addition, the United States golf industry creates approximately 2 million jobs and generates $195 billion dollars toward the economy (SRI International 2008). In 2003, Alabama produced 23,000 acres of sod and generated $200 million in revenue competing with cotton, the state's top cash crop (Adrian et al. 2004).

Warm-Season Turfgrass

Warm-season turfgrasses are monocot plants that grow best during the warm months of late spring through early fall. There are many cultivars and varieties of warm season turfgrass, but generally they are grouped according to genera. The heat tolerant grasses that dominate the Southeastern US are; *Cynodon* spp. (bermudagrass), *Stenotaphrum secundatum* (Walter) Kuntze (St. Augustinegrass), *Zoysia* spp. (zoysiagrass), *Eremochoa ophiuroides* (Munro) Hack (centipedegrass), and *Paspalum vaginatum* (seashore paspalum). The favorable foliage canopy, dense root system, and aggressive growth of these poaceae plants create an aesthetically and functionally suitable surface for homes, golf courses, and sports fields. All of these grasses are perennial plants and are dormant during cooler winter months. In general, these grasses have fair

cold tolerance and will not be killed if planted below the transition zone, but some such as *S. secundatum*, *P. vaginatum*, and *E. ophiuroides* have a lower cold temperature tolerance and must be planted in more tropical areas. The favored grasses for highly managed turf, particularly golf courses and sports fields, are the *Cynodon* hybrids. These hybrids are very aggressive in their growth with many rhizomes and stolons allowing for rapid expansion and rapid recovery of damaged areas.

Insect Management in Warm-Season Turfgrass

Because of warm-season turfgrass's rapid growth during spring, summer, and early fall, these grasses sometimes have the ability to tolerate higher pest densities than cool season grasses in other areas of the country where peak plant vigor is different. These differences in growth patterns result in different level and timing of pest problems in cool and warm-season grasses. As a result pest management decisions in these two systems vary making traditional thresholds, such as economic thresholds and action thresholds, difficult to apply to both warm and cool-season grasses. A common rule of thumb for turfgrass is that greater pest densities can be better tolerated when the grass plant is at its healthiest, indicating that nominal thresholds are a very important aspect of insect pest management decision making. In addition, cultural and physical management strategies that turf specialists implement play a major role in whether the turf will show signs of damage from insects.

Despite the ability of these grasses to tolerate various pest densities, turfgrass is unlike many other agriculture systems where crop values are measured by yields, land acreage, or price per bushel. Instead in most scenarios such as golf courses and most home lawns, "crop value" of the turfgrass plant is based more on aesthetics and functionality than yield. Because of this, there

is often a low to zero tolerance for insect pest damage, whereas published economic thresholds for white grubs can range from five to 10 larvae per square foot causing visible damage to the turf plant (Merchant et al. 2004, Miller 2011).

Scouting for white grub populations to make accurate management decisions requires digging through the soil, damaging the turf plant, and consuming valuable man hours. It is important to properly identify white grub species because insecticides vary in efficacy. Cowles et al. (1999) found that the labeled rate of halofenozide (Mach 2 1.5G), a common grubicide, was ineffective against the Asiatic garden beetle.

The subterranean habitat of white grubs creates an even greater control problem for turf specialists. Insecticides may become bound in the thatch layer of the turf, liquid formulations can volatilize before reaching the pest, granular formulations needing to be watered in may receive too much or too little irrigation, the white grub may be deeper in the soil profile than the insecticide is able to penetrate, and/or the insecticide may be degraded by microbes (Copper 1990).

Traditional chemical control options for insect pests include liquid spray, granular, and fumigant insecticides that can be applied as a preventative or curative application, and sometimes even both. Preventative applications of insecticides are applied during egg laying periods and are effective by remaining active either within the turf plant or soil for several weeks killing the emerging larva. Curative applications are administered if late season outbreaks occur and typically have no long-term residual activity. These curative applications are effective, but higher amounts of active ingredients are usually required due to the larger size of late instar white grubs. Damage from mammalian or avian predators can signify that a curative control

application is warranted but should not be relied upon as a signal for insecticide application (Duble 2004).

White Grubs

White grubs (Coleoptera: Scarabaeidae) are among the most problematic pests in turfgrasses (McCarty 2005). They cause severe economic, agronomic and aesthetic losses to turf growers throughout the United States, and have proven difficult to control for a number of reasons. There are approximately ten different species of white grubs that are major pests of turf (McCarthy 2005). In the southeast, these include the *Cyclocephala lurida* Bland (Southern masked chafer), *Phyllophaga* spp. (May/June beetle), *Cotinis nitida* (Linnaeus) (green June beetle), *Popillia japonica* Newman (Japanese beetle), *Ataenius spretulus* (Haldeman) (black turfgrass Ataenius), *Euetheola rugiceps* (LeConte) (sugarcane beetle), *Hybosorus illigeri* Reiche, and now the *Maladera castanea* Arrow (Asiatic garden beetle) (Buss 2006a, 2008, Cobb 1998, Held and Ray 2009). Raster patterns can be used to identify most white grubs to genera and some to species (Richter 1966).

Biology of Research Taxa

Cyclocephala lurida, *Cotinis nitida*, *Popillia japonica*, *Euetheola humilis rugiceps* (LeConte), and *Maladera castanea* are all univoltine scarab beetles with similar life cycles. Adult flight typically begins in June to early July and eggs are laid in the soil by mid July. As eggs hatch neonate, first instar larvae feed on organic matter then small root fibers. In late fall, or at the first frost, the larvae will burrow 5 to 20 cm into the soil to overwinter. When soil

temperatures begin to increase in the spring to about 10° C, larvae will move back up into the turfgrass root zone to continue feeding. In April to May the 3rd instar larvae will pupate and emerge as an adult around May or June (Potter 1998).

Ataenius spretulus are multivoltine insects producing at least two generations per year. The first generation begins its life cycle when adult females lay eggs in March to early April. After hatching, the grubs go through three larval instars, pupate and emerge as adults by July. These second generation adults will mate and create a second generation emerging August to October. Instead of laying eggs in the soil, they will overwinter along the edges of wood lots or golf courses and mate the following spring (Potter 1998).

Phyllophaga species have a prolonged two to three year life cycle which begins in May when adult females burrow in the soil to lay eggs. Depending on environmental conditions eggs will hatch in approximately one month and neonate larvae will begin to feed on organic matter and small root fibers as they grow larger. As soil temperatures begin to decrease in October *Phyllophaga* 2nd instar larvae will move deeper into the soil to overwinter. In the spring when soil temperatures begin to increase, these larvae will move back into the root zone of the turfgrass to continue feeding, molting into a 3rd instar larva. By the end of fall most larvae will pupate and emerge as adults the following spring (Potter 1998).

Little is known about the biology of *Hybosorus illigeri* but a Florida study showed that there are two generations per year (Woodruff 1973, Buss 2006b). Adult flight begins in April and continues through October but peak flights are May to June and August to September. It is suggested that the smaller second flight is a result of not all of the larvae pupating at the same time and emerging as adults with the first flight (Buss 2006b).

White Grub Damage to Turfgrass

Of these scarab larvae species, non-native Japanese beetle and the Asiatic garden beetle tend to be the most destructive on a per larvae basis. However, populations of native species could be greater causing the same, if not more, damage (Tashiro 1987). White grubs feed on turfgrass root systems causing retarded growth, aesthetic damage, and eventual chlorosis of the plant. These species feed on the root system of the turfgrass plant as larvae, sometimes causing the turfgrass to be rolled back like carpet. The amount of above-ground damage shown by the turf plant can vary not only due to the number of white grubs present but also to agronomic practices such as fertility, soil type, organic matter, and amount of soil moisture (Buss 2006a).

Indirect damage caused by mammalian or avian animals digging through turfgrass while searching for white grubs may be the most common damage seen in the Southeastern US (McCarty 2005). This type of damage can be most problematic because there are no known thresholds for the number of white grubs needed to attract these predators and severe damage can occur in just one night.

Some adult scarabs such as *P. japonica* and *M. castanea*, also feed on ornamental vegetation. However, *E. humilis rugiceps* beetles actually cause severe damage to turf as an adult (Lockwood and Brandenburg 2010), and *C. nitida* beetle larvae are mainly a pest by mechanical movement through the soil (Potter 1998).

Ants in Turfgrass

Biology of S.invicta

Solenopsis invicta Buren was first introduced into the port of Mobile, Alabama over 70 years ago (Vinson 1997). *Solenopsis invicta* is native to South America and was introduced into

the United States with no natural predators. Because of this introduction into a new environment, lack of natural enemies, and the aggressive swarming tendencies of *S. invicta*, populations were able to grow and spread with little resistance from native ant species. Currently there are over 320 million acres covering 13 states in the southeastern United States where *S. invicta* is present (Oi et al. 1994). These ants are omnivorous pests in turfgrass. They cause medical problems to livestock, domestic animals, and humans due to their painful sting. Economic loss occurs when cultivation, harvesting, etc. equipment is damaged from large above ground mounds. Irrigation and electrical circuit boxes have been shown to attract *S. invicta*, which can short electrical equipment, destroying it. Also, aesthetic damage from unsightly mounds in turf and mower scalping causes problems for turf specialists (Vinson 1997).

A single mound of *S. invicta* can measure up to 1 m in diameter and 0.5 m in height. Polygyne colonies have multiple queens and are usually smaller than colonies with a single queen. These monogyne mounds are the most common colonies in Alabama. Both colonies can contain 100,000 to 500,000 workers. Polygyne colonies are much more dense than monogyne mounds reaching densities of up to 1900 mounds per ha (Oi et al. 1994). *Solenopsis invicta* are social insects with the entire colony working as a group performing various roles such as foraging for food, caring for developing young, providing colony security, and even tending to dead ants.

Such large populations allow the *S. invicta* to be an aggressive predator of many different insects, reptiles, and mammals (Vinson 1997). Foraging ants searching for food are told whether to search for protein or carbohydrate based food sources by the queen. Ants usually search for food when there is no rain and air temperatures are 22°C to 36°C. *Solenopsis invicta* can forage for food more than 30 m from their colony's central location. When food is found, ants begin to

lay a recruitment pheromone trial to guide other workers to bring the food resources back to the colony (Oi et al. 1994, Suckling et al. 2010).

Damage and Control

Ants are considered pests of turfgrass because their mounds cause aesthetic eyesores and are physically damaging to mowing equipment. A Texas study found that over a million dollars was spent on damaged golf course equipment due to *S. invicta* (Lard et al. 2001), and turfgrass specialists spent more than $12 million annually on *S. invicta* control in Alabama (Graham and Gaylor 1999). Also, in the case of *S. invicta*, medical problems are of major concern (Vinson 1997). In 0.6 to 16% of all human stings *S. invicta* causes an anaphylactic reaction (Kemp et al. 2000).

Turfgrass specialists cannot tolerate unsightly or dangerous *S. invicta* mounds and use multiple insecticidal applications per year to provide tolerable suppression. These methods include applications of broadcast bait formulations which are retrieved by foraging ants and brought back to the colony. Baits work well because they contain a sublethal dose of active ingredient which allows the foraging ant to return safely to the colony. As more bait is brought back to feed the developing immature ants, queen, and workers through trophallaxis, the amount of poison builds to a lethal dose killing the entire colony (Oi and Oi 2006). Baits vary in their initial speed of kill as well as the length of *S. invicta* control. For example, hydramethylnon bait formulations have been shown to take two to four weeks for initial suppression, whereas bifenthrin, a contact insecticide, can only require a day or two for results (Flanders 2010). Another method of control is a non-selective contact insecticide, in which a granular or liquid application is delivered to an entire or partially infested area. These products either repel the

foragers or kill them with a toxicant. Rarely do these applications kill the queen(s), therefore killing the entire colony requires repeated applications (Oi et al. 1994).

Because of the potential damage *S. invicta* can cause in turfgrass environments many turf managers have overlooked their potential benefits. For example, *Prosapia bicincta* (Say) (twolined spittlebug) is a common surface pest of turfgrass. Out of nine common turfgrass insect predators, *S. invicta* consumed the greatest number of *P. bicincta* eggs and was the only predator of nymphs concealed in their spittle mass (Nachappa et al. 2006). Turfgrass hosts many ant species. Low maintenance lawns, for example hosts 18 species compared to 13 species on golf course fairways (López and Potter 2003). Other *Solenopsis* species such as *S. molesta* are another common ant in turfgrass.

Chapter II

RED IMPORTED FIRE ANT INFLUENCES ON WHITE GRUB POPULATIONS IN MANAGED TURFGRASS

Abstract

White grub (Coleoptera: Scarabaeidae) and red imported fire ant, *Solenopsis invicta* (Buren), interactions are examined to determine if the control of *S. invicta* would lead to localized secondary pest outbreak of *Popillia japonica* Newman and other white grub species populations in managed turfgrass. Using three different *S. invicta* insecticides; bifenthrin, fipronil, and hydramethylnon, *S. invicta* populations were suppressed and plots then inoculated with female *P. japonica* during the summer of 2009 and 2010. Control plots were left untreated to compare populations of *S. invicta* and *P. japonica* to treated plots. Adjustments to treated plot size were made from 2009 to 2010 to account for edge effect. A separate study using roundabout islands of turfgrass plots within surrounding asphalt was conducted. Islands were treated with fipronil and hydramethylnon to exclude *S. invicta*. Treated islands where then compared to adjacent controls where *S. invicta* were present. Through these studies in 2009 and 2010 it was found that the reduction of *S. invicta* with the previously mentioned insecticides does not increase *P. japonica* or other scarab species populations in managed turfgrass.

Introduction

White grubs (Coleoptera: Scarabaeidae) are one of the most destructive insect pests of turfgrass and their subsurface habitat creates monitoring and control problems for many turf specialists (McCarty 2005). Recently, there have been claims (Brandenburg 2006, Buss 2006b) that problems with white grubs are increasing in southern states. One hypothesis to explain this reported increase is that aggressive management of red imported fire ants, *Solenopsis invicta* (Buren) in southern turfgrass removes a significant predator of turf pests. Many genera of ants have been found to be predators of insect pests in several agroecosystems throughout the world (Way and Whoo 1992). A survey of ant diversity in home lawns and golf courses has shown that as many as 18 turf-inhabiting ant species can be present and two of these collected species are significant predators of turfgrass insects such as white grubs (López and Potter 2000, López and Potter 2003, Zenger and Gibb 2001).

In the southeastern United States, *S. invicta* is a natural enemy of insect pests in corn, cotton, soybean, and turfgrass (Eubanks 2001, Way and Whoo 1992, Nachappa et al. 2006). Within turfgrass, *S. invicta* was the only observed predator able to remove *Prosapia bicincta* (Say) (two-lined spittlebug) nymphs from their spittle masses (Nachappa et al. 2006). Also, after removal of *S. saevissima richteri* Forel (or *S. invicta*) in field plots using heptachlor, populations of *Labidura riparia* (Pallas) (striped earwig) were higher than untreated controls (Gross and Spink 1969).

However, in the turfgrass industry *S. invicta* is considered a pest for its destructive mounds, damage to electrical equipment, and their dangerous stings (Vinson 1997). For these reasons, *S. invicta* is often controlled in managed turfgrass which can lead to secondary pest out breaks (Gross and Spike 1969). When 82.7% of the common ant species, *Lasius neoniger*, was

selectively removed from two golf courses in Kentucky, a significantly higher abundance of *P. japonica* larvae were found (López and Potter 2000). Based on this aforementioned research showing that ants can be significant natural enemies of insect pests, it is hypothesized that *S. invicta* are also significant predators of white grubs in turfgrass and that the reduction in *S. invicta* following insecticide applications will cause a localized increase in white grub abundance. The objective of this study is to test this hypothesis using three different insecticides labeled for control of *S. invicta*.

Materials and Methods

Sources of Test Insects

Female *P. japonica* adults were field collected using a Trece trap, baited with a baitpack containing one food lure and one sex lure (Great Lake IPM, Vestaburg, MI) but only the food lure was used to decrease number of collected males. Traps were collected throughout the summer either daily or every other day depending on temperature. Adults were immediately placed in placed in a 44 cm L x 31 cm W x 17 cm H Sterilite 15 Quart Latch Box (Sterilite Corporation, Townsend, MA) with approximately 7.5 cm of field soil filled from the bottom. Prior to adults being placed into these containers, the field soil was autoclaved at 120° C for three, 60 minute cycles, dried, sifted using a 710 µm sieve, and moistened. Adult *P. japonica* were fed field collected rose petals and watered daily in the containers. *Popillia japonica* eggs were collected from these containers every other day.

2009 Field Trial

A field experiment was conducted on two golf courses (Links and Short) at Grand National on the Robert Trent Jones Golf Trail in Opelika, AL. *Cynodon dactylon* L. Pers x *Cynodon transvaalensis* Burtt-Davy ('Tifway') was the primary turfgrass at each site. This hybrid bermudagrass was cut weekly to every other week as needed at a height of 5 cm in early summer and increased to 6 cm as summer temperatures increased. Soil texture analysis, texture class, percent organic matter, and pH were recorded at each site. Soil texture analysis showed that site 1 (Links Course) contained a mean of 45.17% sand, 25.54% silt, 29.30% clay (sandy clay loam), 1.13% OM, and a pH of 6.72 (n=4) and site 2 (Short Course) contained 55.52% sand, 19.99% silt, 27.0% clay (sandy clay loam), 1.08% OM, and a pH of 6.31 (n = 4). Precipitation levels were measured using 50 mL vials (Fisherbrand, polypropylene, 29 O.D. x 115 mm L) as described in Chapter IV. Air and soil temperatures were recorded at four locations per site on 30 min intervals using a datalogger (HOBO Pro v2 2x External Temperature Data Logger, Onset Computer Corporation, Bourne, MA).

Each course was a separate test site with four replicates of four treatments, 10 × 10 m, with a minimum buffer of 10 m between plots located on each hole. Treatments included bifenthrin applied at 224.17 kg/ha (Talstar EZ, 0.2% ai G, FMC Corp., Philadelphia, PA), fipronil at 97.51 kg/ha (TopChoice, 0.0143% ai G, Bayer Environmental Science, Research Triangle Park, NC), and hydramethylnon at 2.24 kg/ha (Amdro Pro Fire Ant Bait, 0.73% ai G, BASF, Research Triangle Park, NC) applied at labeled rates for control of *S. invicta*, and an untreated control.

Fifty mL centrifuge vials were inserted into the soil by using a 2.22 cm soil probe, removing a 13.0 cm core from the soil. The vial was then inserted into the hole so that the open

vial was flush with the soil surface during hot dog baiting (Figure 2.1). One week before treatment, all plots were sampled for *S. invicta* populations using the hot dog method (Jones et al. 1998). For these population samplings vials were arranged in an "X" pattern in the 10 x 10m sampling plot with 1 m between each vial and nine vials inserted into each plot (Figure 2.2). Approximately 9 g of hot dog (Bar-S Food Co., Phoenix, AZ) per vial was used as a bait attractant for <1 h before vial collection. *Solenopsis invicta* populations were sampled during appropriate *S. invicta* foraging conditions with temperatures between 22°C and 36°C and no rain.

Figure 2.1. Fifty mL polypropylene vials used for baiting (top) and vial fully inserted with hot dog present (bottom).

Figure 2.2. Within each plot, vials were arranged on 1 m spacing in a “X” pattern.

All treatments were applied on May 19 with ≥4 h passing before an irrigation cycle of >1.25 cm to allow ants time to forage the bait. Because of the small amount of insecticide needed for the treated plot sizes, a traditional broadcast spreader could not be used to ensure even coverage. Therefore, each application of fipronil and bifenthrin was added to the appropriate amount of dried sand to create an equal volume of sand and insecticide mixture so an even rate was applied to the entire plot. Because hydramethylnon was formulated as a bait, no sand was mixed with these applications. All three products were applied by gloved hand in a crisscross pattern for an even application.

At 1, 2, 4, and 8 wk after treatment (WAT) the hot dog baiting method, as previously described, was used to sample for reductions in *S. invicta* populations following treatment. Treatment effects on *S. invicta* populations among sites, replicates, and time were analyzed using a repeated measures analysis of variance using a log(+1) transformation (MANOVA) followed

by an one-sided Dunnett's test (Statistix 8, Tallahassee, FL) compared to the untreated control (Zar 1999). All data are presented as untransformed means ±SEM.

One month after application, 10 of the aforementioned field collected, female *P. japonica* were placed into a 15 cm diameter PVC pipe, 20 cm tall, 1 m from plot center, and covered with a screen (Figure 2.3). Females were identified by examining the front tibia for a rounded spine which is unique to females (Fleming 1972). Each plot received two pipes inserted ≥15 cm into the soil using an 11.3 kg weight plate and/or sledge hammer, and the location of each pipe was measured to the middle (approximately 1.2 – 1.7 m) of existing centrifuge vials so that white grub samplings later in the season were in the inoculated area (Figure 2.4). Into each pipe, 10 *P. japonica* females were placed into each pipe and left for >16 h to allow time for oviposition. After this time only the pipes were collected but *P. japonica* females were left in the soil.

Figure 2.3. Installation of PVC pipe (top) for inoculating field plots with *P. japonica* females for oviposition (bottom).

Figure 2.4. Inoculated area was determined by locating the middle of two existing vials in each sampling plot so white grub sampling in August was exact to the inoculation area.

In August, 4 wks after inoculation, a 60 cm L x 60 cm W x 15 cm deep sample of turf from each inoculated area in each plot was sampled with a spade for white grubs. The turf and soil was destructivly sampled and all scarab larvae present were collected in 90% ethanol solution. In the lab, white grubs were identified to species and counted. Data from all species of white grubs as well as all *P. japonica* collected were transformed using log(+1) to account for variance. The effect of treatment on white grub abundance among sites was determined using split-plot design analysis of variance (ANOVA) followed by a one-sided Dunnett's test ($P < 0.05$) (Statistix 8, Tallahassee, FL) (Zar 1999).

2010 Field Trial

The 2009 field experiment was repeated using the same experimental design but the plot size was increased to reduce a potential edge effect. In 2010 two golf courses, Saugahatchee Country Club, Auburn, AL and Grand National were used with four replicates on each. Soil texture analysis showed that site 1 (Saugahatchee CC) contained 49.08% sand, 19.08% silt, 31.88% clay (sandy clay loam), 3.16% OM, and a pH of 6.24 (n = 4) and site 2 (Grand National GC) contained a mean of 52.5% sand, 10.95% silt, 36.88% clay (sandy clay loam), 1.55% OM, and a pH of 6.73 (n=4). The dominant turfgrass on each site was *C. dactylon* L. Pers x *C. transvaalensis* Burtt-Davy, with mowing heights, soil analysis, temperature, and precipitation recording methods the same as in 2009.

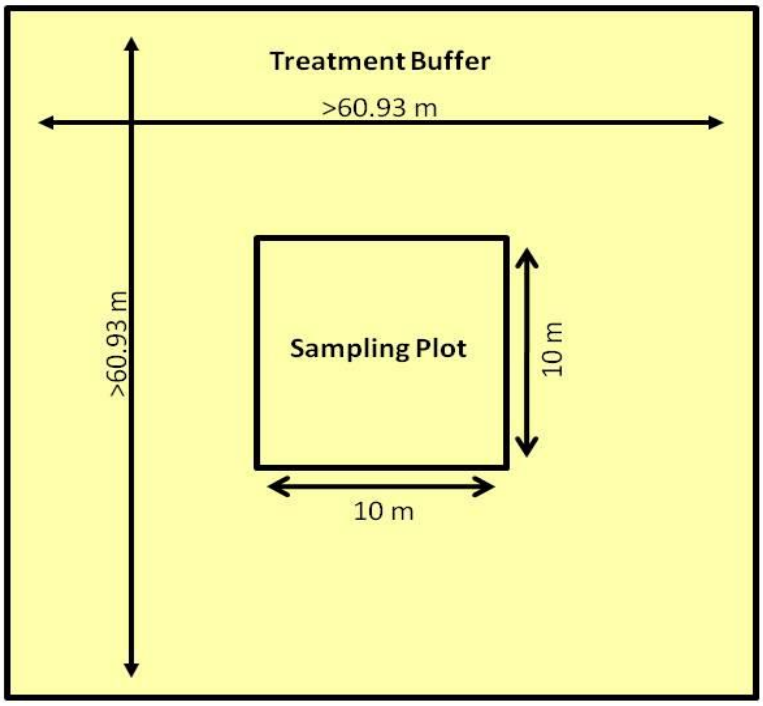

Figure 2.5. In 2010, the treated area was increased to 61 x 61 m with a 10 x 10 m area centered for sampling for consistency between years.

Plot size did increase to 61 x 61 m treated area (Figure 2.5). Because of this large size, many holes had only one plot centered in the rough differing from 2009 when one entire replicate was placed on one hole. On both sites, six holes contained two or more plots but each had an untreated buffer zone of >30 m between them. Fipronil, bifenthrin and hydramethylnon were applied (same formulations and rated as in 2009) using a GT-77 Herd Seeder (Herd Seeder Co., Inc., Logansport, IN, 46947) attached to the back of a gas powered workcart (aka Mule), which was calibrated independently for each product before application. A GPS lightbar (*Ag*GPS EZ-Guide 500, Trimble Navigation Limited, Sunnyvale, CA) was used to monitor speed and swath width of the spreader (Figure 2.6).

A 10 x 10 m plot (Figure 2.5) in the center of each treated area was sampled to keep the sample area as in 2009 for consistency between years. One replicate contained four plots treated with one of the three aforementioned insecticides in addition to an untreated control. Plots were assigned to treatments based on number of *S. invicta* collected in pre-treatment samples (Drees et al. 2005). For example, after ranking all plots from highest number of pretreatment collected *S. invicta*, the first four plots were randomly assigned treatments and grouped into replicate one. Replicate two contained the plots with collected *S. invicta* populations ranked five through eight and randomly assigned treatments.

Figure 2.6. In 2010, all applications were guided using a GPS lightbar on a commercial spreader.

Solenopsis invicta populations were sampled pre-treatment using the hot dog bait method as in 2009, and post-treatment samples were collected at 1, 2, 5, and 9 WAT. Changes in sampling dates from 2009 were due to unfavorable sampling weather and golf tournaments. Treatment effects on *S. invicta* populations among sites, replicates, and time were analyzed using repeated measures analysis of variance using a log(+1) transformation (MANOVA) and a one-sided Dunnett's test as before (Statistix 8, Tallahassee, FL) (Zar 1999).

Approximately 5 WAT, 15 field-collected female *P. japonica* were caged over plots to oviposite as done in 2009. The number of females per inoculation was increased to increase the number of white grubs present during sampling. Eight weeks after inoculation, two 60 cm L x 60 cm W x 15 cm deep sections of turf were excavated underneath the inoculated points. These

areas were then destructively sampled for white grubs (Figure 2.7), and all scarab larvae were collected to ethanol, identified, and counted as in 2009. The effect of treatments on *P. japonica* and white grub abundance was determined using split-plot design analysis of variance (ANOVA) followed by a one-sided Dunnett's test ($P < 0.05$) (Statistix 8, Tallahassee, FL) (Zar 1999).

Figure 2.7. Turf and the underlying soil was excavated and examined for scarab larvae, and any present were collected into alcohol for identification in the lab.

2010 Exclusion Trial

Roadway island plantings of *Zoysia* sp. (zoysiagrass) (Figure 2.8) were located along Robert Trent Jones Trail, Opelika, AL and maintained at approximately 6.25 cm throughout the summer. Six island plots, with a minimum of 2.7 m of surrounding asphalt, were chosen as

exclusion plots and five control plots were located in adjacent perimeter areas. Replicates (n = 5) were assigned by grouping an island plot and an adjacent plot in the perimeter (Figure 2.8). Island plots were treated with fipronil (TopChoice, 0.0143% ai G, Bayer Environmental Science, Research Triangle Park, NC) at a rate of 97.51 kg/ha and received >1.25 cm of irrigation within 5 h.

Pre and post hot dog samplings, to determine ant populations as previously described, used four 50 mL vials per plot placed in a rectangle within the center of the island. Five WAT and prior to *P. japonica* inoculation, an application of hydramethylnon at a rate of 2.24 kg/ha was made to all island plots further suppressing ants. On 5 August, plots were inoculated with 15 female *P. japonica* in PVC pipes and white grubs were sampled approximately 6 wk after inoculation, as previously described in the 2009/2010 Field Trials. All scarab larvae present were collected, counted, and identified in the lab. The number of white grubs in island plots and adjacent plots were compared using a one sided paired t-test ($P < 0.05$) (Statistix 8, Tallahassee, FL) (Zar 1999).

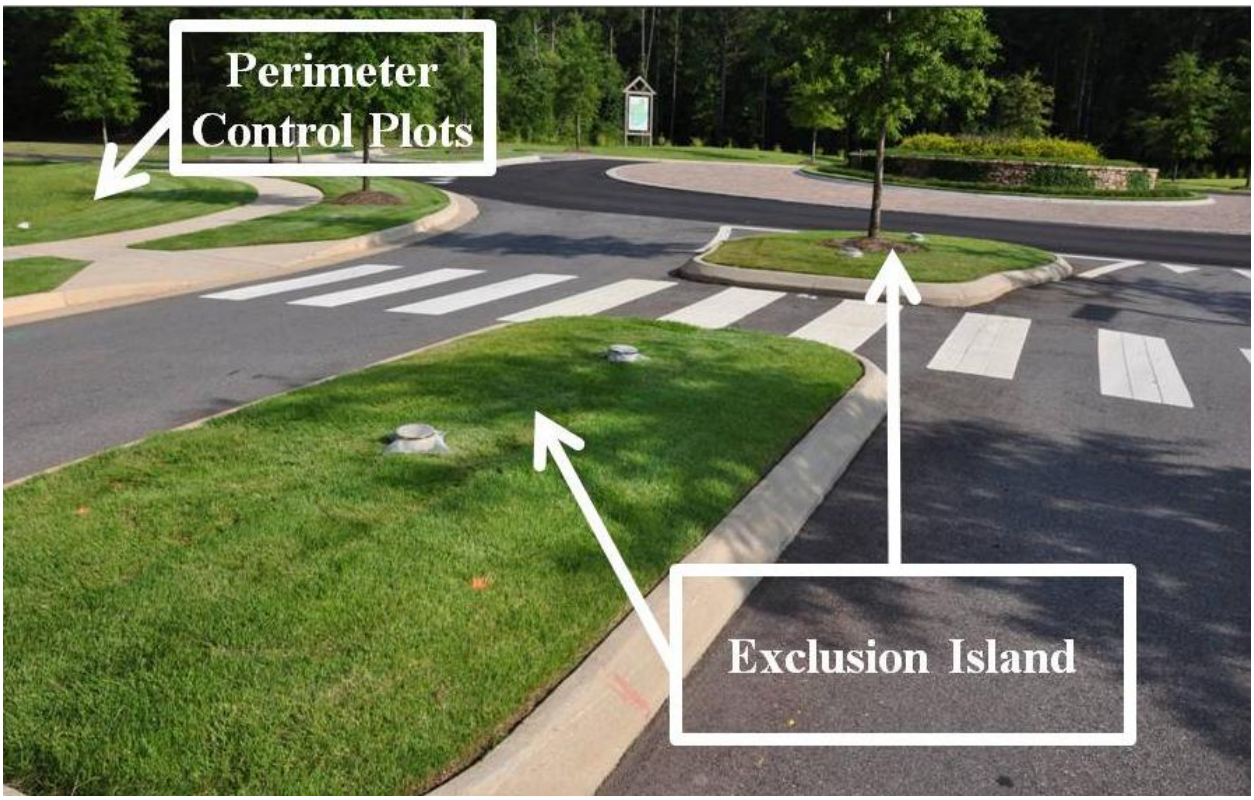

Figure 2.8. Treated island, plots were surrounded by an adjacent asphalt border ≥2.7 m and adjacent perimeter plots were exposed to surrounding and populations.

Results

2009 Field Trial

Of the collected ants (n = ~30,000), >95% were *S. invicta*. There was no differences between sites (MANOVA, $F = 0.37$, $P < 0.7731$, df = 3, 1) so all site data was pooled. There was significant difference over time (MANOVA, $F = 12.73$, $P < 0.000$, df = 4) (MANOVA, $F = 2.73$, $P < 0.004$, df = 12, 3). All insecticides significantly reduced populations of *S. invicta* within 1 wk of application (Table 2.1). Populations of *S. invicta* in treated plots except hydramethylnon were significantly less than untreated plots at the time of inoculation. Bifenthrin suppressed *S. invicta* populations below that of untreated during the entire study whereas populations fluctuated in fipronil treated plots (Figure 2.9).

Table 2.1. 2009 *S. invicta* population means, ±SEM, and significantly different treatments compared to the control.

Treatment\ formulation	Rate kg product/ha	Mean (±SEM) no. of *S.invicta*				
		Time of sample[a] (WAT)				
		0	1	2	4	8
Control	Untreated	1340.8 ± 297.8	737.6 ± 225.7	1070.4 ± 468.1	689 ± 348.3	1169 ± 445.2
TopChoice 0.0143 G	97.51	402.6 ± 188.7*	152.3 ± 98.5*	132.9 ± 81.4	0.13 ± 0.13*	432.9 ± 182.1
Talstar EZ 0.2 G	224.17	1455.3 ± 477.5	0.13 ± 0.13*	1.6 ± 1.63*	0.3 ± 0.2*	177.5 ± 105.2*
Amdro 0.73 bait	2.24	563.5 ± 271.6*	199.6 ± 193.2*	507.8 ± 235.3	750.9 ± 299.7	588.5 ± 264.7

Means presented are actual means. Within a column, means followed by an asterisk were significantly different from the untreated control (Dunnett's test, $P < 0.05$).

[a] Pitfall traps baited with hot dog then exposed for 1 h on each plot at each sample timing.

At 1 WAT, $F = 9.25$; $P < 0.0004$; df = 7, 21. At 2 WAT $F = 5.21$; $P < 0.0051$; df = 7, 21. At 4 WAT, $F = 11.83$; $P < 0.0001$; df = 7, 21. At 8 WAT, $F = 2.71$; $P < 0.0708$; df = 7, 21.

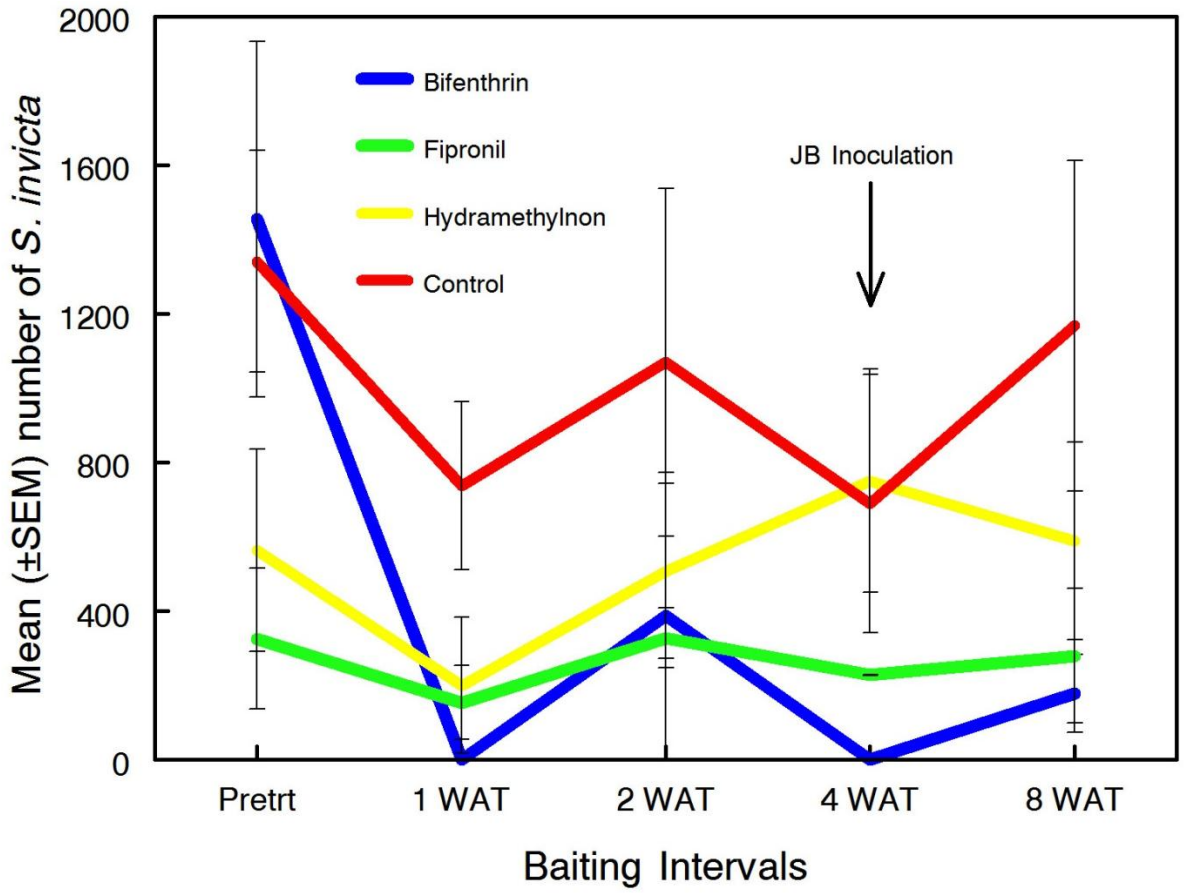

Figure 2.9. Populations of *S. invicta* monitored with hot dog baits before and after treatment in 2009.

No differences in number of white grubs (ANOVA, split-plot design, $F = 0.34$, $P = 0.799$, df = 1, 3) or *P. japonica* alone (ANOVA, split-plot design, $F = 0.39$, $P = 0.8279$, df = 1, 3) were detected between sites. Both sites were pooled for further data analysis. There were significant differences in white grub abundance between treatments (ANOVA, $F = 3.40$, $P = 0.0366$, df = 7, 21). Plots treated with fipronil had significantly more total white grubs than other treatments but not the untreated control (ANOVA, LSD, $P < 0.05$). All treatments had no significant mean differences in collected grubs when compared to the control using a one-sided Dunnett's test ($P < 0.05$) (Figure 2.10). When just *P. japonica* collected larvae were analyzed

there was no significant difference between treatments (ANOVA, $F = 2.26$, $P = 0.1108$, df = 3, 21) (Figure 2.19). Six different species; *Popillia japonica*, *Phyllophaga* sp., *Cyclocephala lurida* Bland, *Ataenius spretulus* (Haldeman), *Eutheola humilis rugiceps* (LeConte), and *Hybosorus* sp. were found in plots across the two different test sites. On site 1 (Links Course), the dominant scarab species collected was *Hybosorus* sp. and on site 2 (Short Course) *P. japonica* was the most abundant species (Figure 2.11).

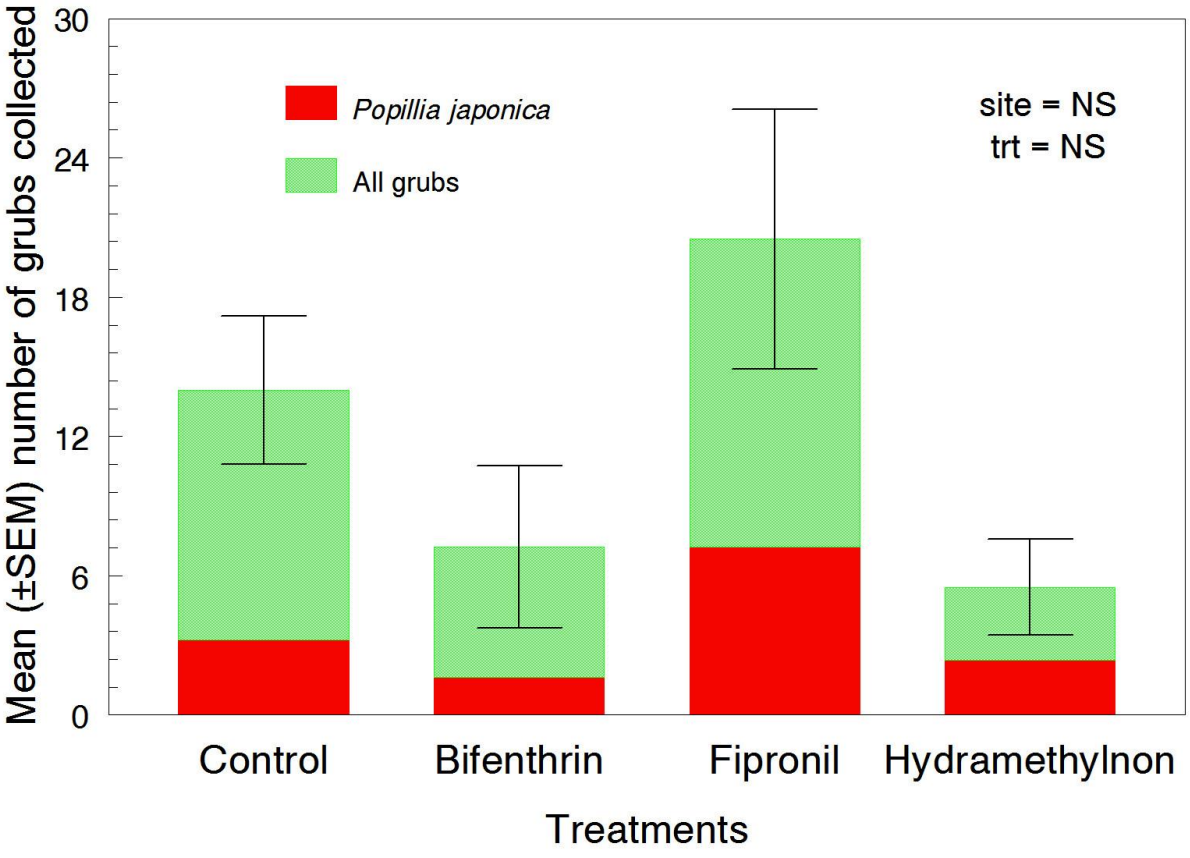

Figure 2.10. Abundance of white grubs collected following removal of ants with each insecticide in 2009.

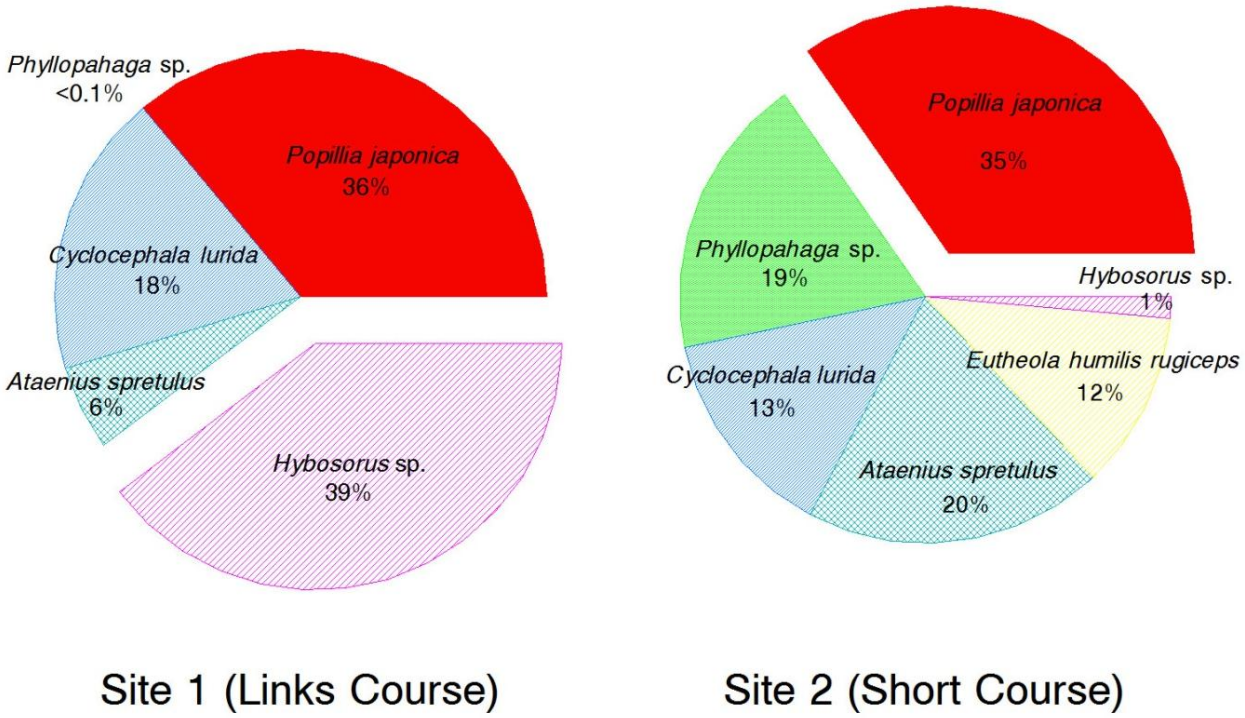

Figure 2.11. Diversity of scarab larvae collected in field plots during 2009.

Table 2.2. Mean air and soil temperatures (°C) on field sites in 2009.

	Site		Mean	±SEM	SD	Min	Median	Max
2009	Links Course	Air	34.22	0.0546	6.26	5.23	22.85	42.68
		Soil	26.74	0.0332	3.91	15.80	26.48	40.80
	Short Course	Air	23.96	0.0645	5.14	7.85	23.16	40.43
		Soil	26.21	0.0415	3.30	15.92	26.18	35.72

Mean weekly precipitation throughout the summer on site 1 (Links Course) was 38.89 mm (range = 86.0 mm to 0.0 mm, median = 26, n = 12) and site 2 (Short Course) was 38.87 mm (range = 87.0 mm to 2.0 mm, median = 36, n = 12). Soil and air temperature data from the entire summer on both sites is shown in table 2.2.

2010 Field Trial

Similar to 2009, >95% of ants (~40,000) collected were *S. invicta*. There was no significant difference in *S. invicta* populations between sites so both sites were pooled for further analysis (MANOVA, $P < 0.05$, $F = 0.12$, $P = 0.9449$, df = 3, 1) (Table 2.3). There was significant difference over time (MANOVA, $F = 12.15$, $P < 0.000$, df = 4) (MANOVA, $F = 3.25$, $P < 0.0005$, df = 12, 3). Populations of *S. invicta* were significantly lower in the treated plots compared to untreated plots during the time of inoculation and remained low in all treated plots until nine WAT when populations of *S. invicta* treated with hydramethylnon began to increase (Figure 2.12).

Table 2.3. 2010 *S. invicta* population means, ±SEM, and significantly different treatments compared to the control.

Treatment\ formulation	Rate kg product/ha	Mean (±SEM) no. of *S. invicta*				
		Time of sample[a] (WAT)				
		0	1	2	5	9
Control	Untreated	804.9 ± 417.7	434.1 ± 222.4	329.5 ± 217.5	560.1 ± 223.2	1166.9 ± 520.5
TopChoice 0.0143 G	97.51	491.9 ± 368.6	0*	0*	1.1 ± 0.9*	1.4 ± 1.1*
Talstar EZ 0.2 G	224.17	270.8 ± 79.9	80 ± 52.6*	1.6 ± 1.63*	0.5 ± 0.5*	93.4 ± 90.9*
Amdro 0.73 bait	2.24	209.5 ± 114.2*	0*	0*	164.9 ± 160.9*	298.1 ± 115.9

Means presented are actual means. Within a column, means followed by an asterisk were significantly different from the untreated control (Dunnett's test, $P < 0.05$).

[a] Pifall traps baited with hot dog then exposed for 1 h on each plot at each sample timing.

At 1 WAT, $F = 6.44$; $P < 0.0029$; df = 7, 21. At 2 WAT $F = 5.12$; $P < 0.0082$; df = 7, 21. At 5 WAT, $F = 12.01$; $P < 0.0001$; df = 7, 21. At 9 WAT, $F = 29.58$; $P < 0.0001$; df = 7, 21.

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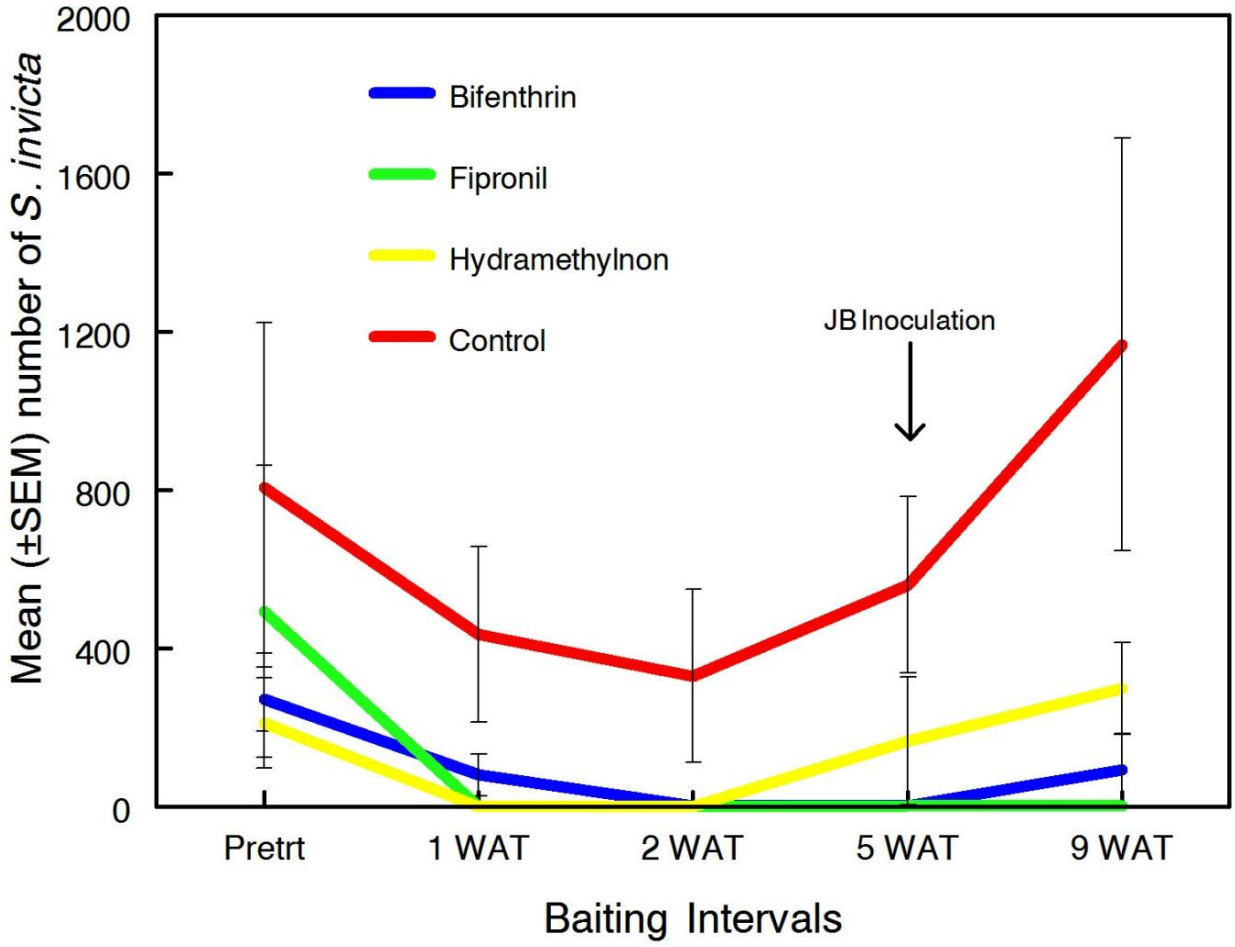

Figure 2.12. Populations of *S. invicta* monitored with hot dog baits before and after treatment in 2010.

There were no differences between treatments for total white grubs collected (split-plot ANOVA, $F = 0.60$, $P = 0.6257$, df = 1, 3 for total white grubs) or *P. japonica* (split-plot ANOVA, $F = 0.74$, $P = 0.5408$, df = 1, 3) on either site. All sites were pooled for subsequent data analysis. There were no significant differences between treatments of total white grubs (ANOVA $F = 1.70$, $P = 0.1980$, df = 7, 21) or *P. japonica* ($F = 1.51$, $P = 0.2411$, df = 7, 21). The untreated control had the greatest number of white grubs with 8.25 per plot (Figure 2.13)

and fipronil contained the lowest with 2.25. *Popillia japonica* was the dominant species of scarab larvae collected on either site (Figure 2.14).

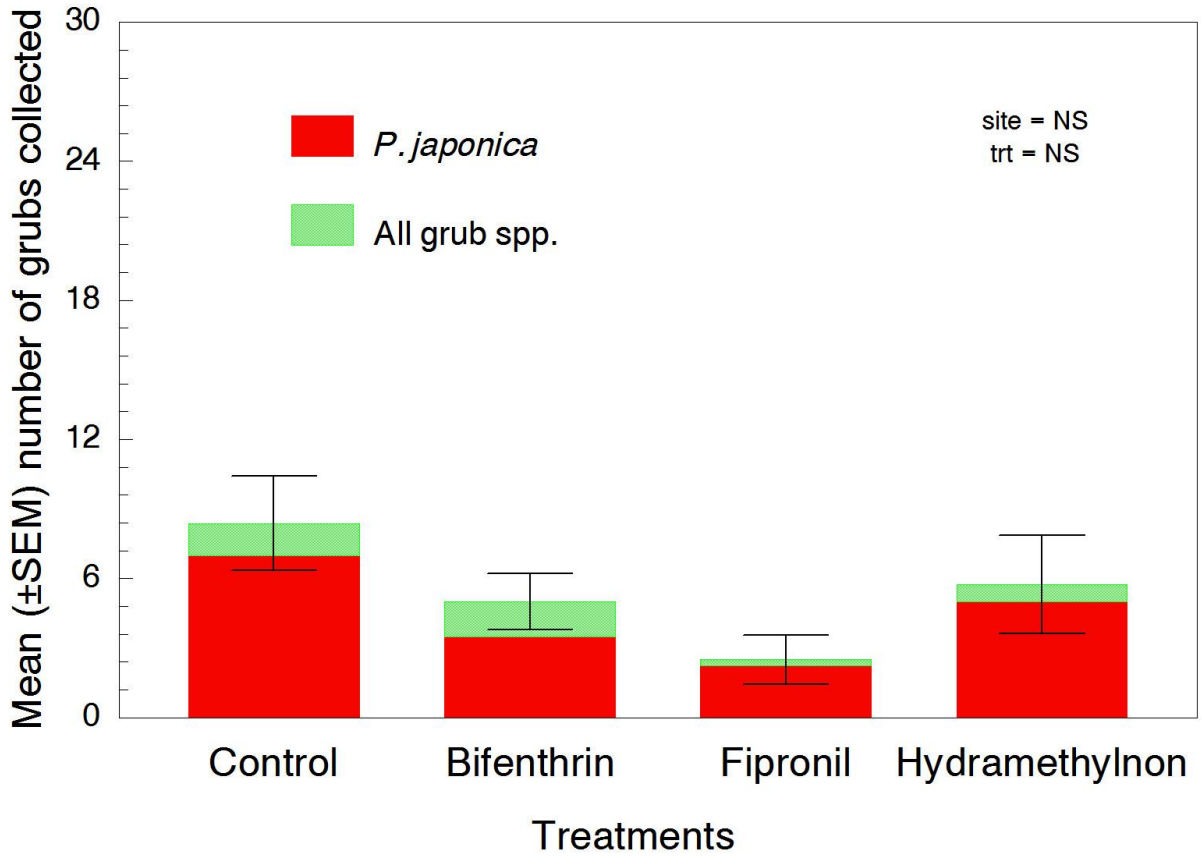

Figure 2.13. Abundance of white grubs collected following removal of ants with each insecticide in 2010.

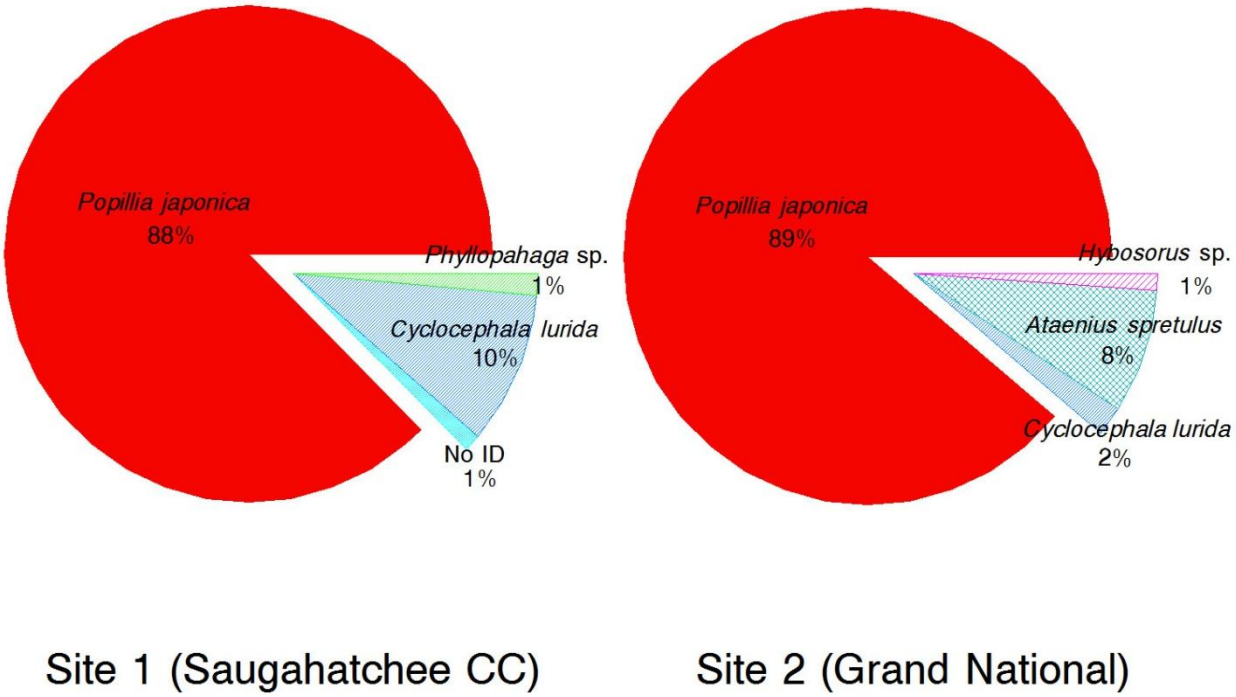

Figure 2.14. Diversity of scarab larvae collected in field plots during 2010.

Table 2.4. Mean air and soil temperatures (°C) on field sites in 2010.

	Site		Mean	±SEM	SD	Min	Median	Max
2010	Saugahatchee CC	Air	25.69	0.0531	4.55	14.55	24.61	37.34
		Soil	27.42	0.0490	4.20	18.18	27.55	38.25
	Grand National	Air	25.07	0.0503	5.71	3.38	25.72	39.49
		Soil	25.23	0.0412	4.67	13.02	25.77	40.63

Mean weekly precipitation throughout the summer on site 1 (Saugahatchee CC) was 45.08 mm (range = 0.0 mm to 90.0 mm, median = 45, n = 5) and site 2 (Grand National) was 61.58 mm (range = 0.0 mm to 90.0 mm, median = 75, n = 5. Soil and air temperature data from the entire summer on both sites is shown in table 2.4

2010 Exclusion Trial

Plots isolated and treated still had low numbers of *S. invicta* (range 0 to 240) present after treatments. One replicate was excluded due to the extremely dry soil in the treated plot and extremely wet soil of the corresponding control plot. On average 7.25 and 2.25 larvae were found in treated and control plots respectively (t = 1.55, *P* = 0.9220 df = 9). *S. invicta* were the most abundant ant species present in baited samples, as were *P. japonica* in white grub samples.

Discussion

The results of all field tests failed to support the hypothesis that selective removal of *S. invicta* would result in a localized increase in scarab larvae. With this hypothesis untreated control plots were expected to have the lowest number of collected white grubs. Instead, control plots contained the greatest number of larvae, excluding the 2009 fipronil treated plots which contained the highest number of white grubs that year and will be discussed in more detail.

S. invicta population fluctuations in the 2009 sampled plots were of importance in preparation for the 2010 field season. With such high variation in the *S. invicta* populations during 2009, there was concern *S. invicta* had created inconsistent results by foraging into sampling plots from outside the treated areas. Prior to 2009, it was suggested that these buffer zones be increased to reduce possible edge effect, but concerns with assigning a full replicate to a single golf hole for consistency made this not possible. A 5 m treated buffer zone was not large enough to prevent *S. invicta* from foraging into the sampling plots during hot dog baiting. To solve this edge effect in 2010, the treated plot area was increased to 61 x 61 m area and the plot treatment assignment method was also changed to yield more consistent *S. invicta*

populations throughout the summer. It was determined that reducing the possibility of any edge effect was of more importance than assigning each replicate to a single hole. Increases in 2010 treated plot sizes reduced edge effect yielding better results through the summer. The only plots to have a gradual increase in *S. invicta* 5 and 9 WAT were the hydramethylnon treated plots and this increase can be expected with bait formulations which have no residual value. Also, the adjustment to treatment assignments reduced variability seen in 2009.

Fipronil was the only treated plot with significantly different number of white grubs collected when compared to the three other treatments (ANOVA LSD, $P < 0.04$) in 2009. However, when using a one-sided Dunnett's test to compare collected white grubs in treated plots to the untreated control, no significant difference was detected. One of these fipronil plots contained 33.5% (55 of 164 white grubs n = 8) of all the collected white grubs in sampled plots and after removal of this replicate, no statistical difference was detected between treatments ($F = 2.20$, $P = 0.1238$, df = 3,6). White grub means changed as follows after omitting this replicate; bifenthrin 7.86, fipronil 15.57, hydramethylnon 6.14, and the untreated control contained a mean of 14.43 white grubs (n = 7). All sites were pooled and transformation of collected white grub numbers was not needed for this analysis. Fipronil treated plots went from containing the greatest number of white grubs in 2009 to containing the fewest in 2010. Overall sampled white grub populations were lower in 2010 than the first trial year. There are a number of factors that may have played a role in this, but an unusually cold winter in 2010 may have favored the more cold hardy species such as *P. japonica* which had increased flight activity in 2010 (Potter and Held 2002, *person. observ.*) while reducing the number of other species.

Other notable data from 2009 was the scarab larvae diversity from the sampled plots with six different genera collected. *A. spretulus* was the most damaging and difficult to control insect

pest in a 1994 collection study but little is known about the biology and damage potential within warm season turfgrass (Gelernter and Stowell 1994). In Michigan, *A. spretulus* was found to be a univoltine pest but the number of generations per year increased to three in Southern California during samplings (Johanningsmeier 1999, Gelernter and Stowell 1994) showing how biology can change in different regions. *Hybosorus* sp. larvae were collected in abundance in 2009 and again very little is known about this species in turfgrass (Woodruff 1973). Adults were found to cause physical damage while emerging out of turfgrass on golf courses in Florida but little was observed on larval feeding habits (Ocampo 2002).

Elimination of *S. invicta* and any other ant species from isolated island plots in the 2010 Exclusion Trial was documented during the first hot dog baiting after treatment. However, during the second post-treatment baiting, small populations of *S. invicta* were collected. An application of hydramethylnon was made to each island plot to ensure *S. invicta* would be eliminated and one week after this treatment no *S. invicta* were detected during baiting. Since no differences were found between grub numbers in treated and control plots, this trial further showed that *S. invicta* play no role in influencing white grub populations.

My results are inconsistent with previously published data (López and Potter 2001, Gross and Spink 1969, Cook 2003) that each show the control of other ant species (*S. molesta* and *L. neoniger*), including *S. invicta* results in insect outbreaks. However, Gross and Spink (1969), and Cook (2003), were examining above ground insect interactions. Subterranean interactions among predators and prey can greatly vary because of this hypogeal habitat in addition to the experimental design chosen to quantify these interactions.

López and Potter (2000) reduced ant populations within turfgrass using an application of granular fipronil, with curative applications of abamectin ant bait as needed, to conclude that an

82.7% mean reduction in ants resulted in significantly fewer *P. japonica* larvae in treated plots when compared to untreated controls. However, during *P. japonica* inoculation 15 male and 15 female *P. japonica* adults were placed into a 25 cm diameter PVC pipe inserted 25 cm deep into the soil for 5 d to allow for oviposition. Thirty adults in such high density over a period of 5 d may lead to an aggregation effect by foraging ants recruiting other ants. Further examination into these experimental design factors are discussed in Chapter III.

However, there are data that agree with our findings. White (1940) sampled for *P. japonica* larvae at pasture, roadside, cemetery, and golf course sites in New Jersey. These samplings were conducted in areas both infested and adjacent areas where no ants were present. After 35 samples in 1934, 213 more grubs per ft^2 were found in sampled areas with ants present than samplings without ants with 210 grubs collected. Similar results were found in 1935 when grub samplings at eight sites contained 180 total grubs per ft^2 in both areas with ants present and absent (White 1940). These data uses the most natural experimental conditions possible.

With three experiments conducted over two years, none of the plots treated with insecticides for *S. invicta* control had significantly different white grub populations when compared to the untreated control. However, one treatment (fipronil) in 2009 had significantly more white grubs collected than the two other treatments. One replicate contained 33% of all the total grubs collected from both sites in 2009. When this replicate was excluded no significant difference between treatments was detected. A possible explanation for this may be that *A. spretulus*, which accounted for 60% of the collected species in this plot, can be positively correlated with the amount of organic matter by weight in the soil (Williamson et al. 2005). This plot contained over 71% (n = 16) of the total *A. spretulus* collected on both sites in 2009 and may have contained more organic matter than other samplings.

In conclusion, with the presented research, the control of *S. invicta* does not produce localized increases in scarab larvae populations within managed turfgrass. Therefore, alternate hypotheses to explain the reported increase in white grubs in the southeast (Brandenburg 2006, Buss 2006b) will need to be examined. It is hypothesized that above ground food resources do not justify soil foraging for prey by *S. invicta*. Scarab larvae were still abundant in the untreated control plots showing that white grubs can seemingly co-exist in soils despite the abundance of *S. invicta* as noted by White (1940) and J. Oliver (*person. comm.*).

Chapter III

FORAGING OF *SOLENOPSIS INVICTA* BUREN AND IMPLICATIONS ON PREDATION OF SOIL-DWELLING PESTS

Abstract

White grub (Coleoptera: Scarabaeidae) and red imported fire ant, *Solenopsis invicta* Buren, interactions were examined to determine if common turfgrass scarab pest species and their different life stages have varying susceptibility to *S. invicta* predation. Life stage susceptibility tests were performed by subjecting *Popillia japonica* Newman eggs and adults to active *S. invicta* foraging territory for 24 h in 2009 and 2010. Other life stages used were 2nd and 3rd instar *Cyclocephala* spp. and *Phyllophaga* spp. larvae. Additional field collected *Cyclocephala lurida* Bland, *Eutheolus humili rugiceps* (LeConte), and *Cotinus nitida* (Linnaeus) were subjected to active *S. invicta* foraging territory for 24 h in 2009 and 2010. *P. japonica* eggs were the most susceptible scarab life stage to *S. invicta* predation with 70% loss, and large 2nd to 3rd instar larvae had 15% reduction in 24 h. Adults of all species had little to no loss due to predation, most likely due to their hardened exoskeleton and ability to escape predation via flight. After these experiments, noting differences in predation rates, it was found that certain experimental design factors considerably influence *S. invicta* foraging tendencies often creating skewed data. The following discusses these predation rates and how these discovered design factors influence experimental results.

Introduction

Generalist predators including ants, spiders, and predatory beetles are the most important natural enemies of arthropod pests in turfgrass (Potter 2001). Various ant species, including *Solenopsis invicta* Buren, are predators of surface and soil dwelling turfgrass pests (López and Potter 2000, Zenger and Gibb 2001, Nachappa et al. 2006) and direct pests of potato crops in Florida (Adams et al. 1988). *Solenopsis invicta* is so voracious, it is the only predator observed to physically remove *Prosapia bicincta* (Say) (twolined spittlebug) nymphs from their nymph spittle mass showing their unique foraging ability. Other ant species such as *S. molesta* can remove as much as 84% of *Cyclocephala lurida* Bland (southern masked chafer) eggs and 45% of larvae. Ants, particularly *S. invicta*, are important in regulating populations of turfgrass pests thus reducing the frequency of pest outbreaks (Potter 2001).

Root-feeding white grubs (Coleoptera: Scarabaeidae) are one of the most destructive pests of turfgrass. Their subsurface habitat creates monitoring and control problems for turfgrass managers (McCarty 2005). Most turf-infesting scarabs are univoltine with all life stages often present temporally in the soil. For example, during adult *P. japonica* flight, typically late spring into summer, females enter and exit the soil repeatedly to lay eggs alternating between egg laying and feeding (Potter and Held 2002). Eggs are generally laid in the upper 7.5 cm of soil and hatch into neonate larvae in 10 to 14 days. After approximately 4 weeks larvae, will develop to the 3rd instar and in late fall, as soil temperatures drop, will overwinter 5 to 15 cm deep in the soil until soil temperatures increase in spring. These fully developed larvae will feed on the turfgrass rootzone for another 4 to 8 wks then begin pupation which can last 7 to 17 d before final adult emergence (Potter and Held 2002).

White grubs also exist in relatively small, isolated patches and most have limited horizontal movement once in the soil (Potter and Held 2002). Given this long exposure in a relatively confined space, the biology of white grubs provides ample opportunity for predation by generalist predators. As many as 73% of *P. japonica* eggs can be removed by ants in 72 h (Zenger and Gibb 2001) potentially significantly reducing populations in one season (López and Potter 2000).

Despite the abundance and importance of ants as predators, populations of *P. japonica* were similar in areas where ants, but not *S. invicta*, were present or absent (White 1940). Ants, particularly *S. invicta*, may reduce the abundance of natural enemies (Eubanks 2001, Coppler et al. 2007) or cause interference with other biological controls (Chantos et al. 2009). For example, suppression of populations of the *Antonina graminis* Maskell (rhodesgrass mealybug) by an introduced parasitoid is compromised by the presence of *S. invicta* defending these mealybugs against parasitism in exchange for honeydew (Chantos et al. 2009). As previously stated, *S. invicta* are significant predators of surface pests but there has been less emphasis on soil-dwelling pests such as white grubs.

The objectives of this study were to determine *S. invicta* predation rates on various species and life stages of common scarab pests of turfgrass and to investigate general soil foraging characteristics using food resources under field conditions.

Materials and Methods

Sources of Test Insects

Female *P. japonica* were field collected using a Trece trap, baited with a baitpack containing one food lure and one sex lure (Great Lake IPM, Vestaburg, MI) but only the food

lure was used to decrease number of collected males. Traps were collected throughout the summer either daily or every other day depending on temperature. Adults were immediately placed in placed in a 44 cm L x 31 cm W x 17 cm H Sterilite 15 Quart Latch Box (Sterilite Corporation, Townsend, MA) with approximately 5 cm of field soil filled from the bottom. This field soil was autoclaved at 120° C for three, 60 minute cycles, dried, sifted using a 710 µm sieve, and moistened. Adult *P. japonica* were fed field collected rose petals and watered daily in the containers. *Popillia japonica* eggs were collected from these containers every other day.

In October 2009, a 77% and 23% respective mix of 3rd instar *Phyllophaga* spp. and *Cyclocephala* spp. larvae were collected from damaged patches of *Zoysia* spp. (zoysiagrass) on a sod farm in Hurtsboro, AL. Larvae were placed into a container containing a 1:1 mix of autoclaved, sifted soil and ground peat moss and held overnight in a growth chamber at 28 °C. In September 2009, adult *Cotinis nitida* (L.) were collected by aerial nets on the campus of Auburn University in Auburn, AL and held with fresh peaches in a screened cage outdoors until needed for experiments. Adult *Eutheola humilis rugiceps* (LeConte) and *C. lurida* were collected using a bucket-type black light trap (BioQuip, Rancho Dominguez, CA) at the Auburn University Turfgrass Research Unit, Auburn, AL. Adults of these species were held in the 1:1 mix of autoclaved and ground peat moss soil until needed for experiments.

Egg Susceptibility Trial

In 2009, two field trials were conducted at Grand National on the Robert Trent Jones Golf Trail in Opelika, AL to assess predation rates of *S. invicta* on *P. japonica* eggs buried in the soil under managed turfgrass. The dominant turfgrass species was *Cynodon dactylon* L. Pers × *Cynodon transvaalensis* Burtt-Davy ('Tifway') maintained at approx. 5 cm. Mounds of *S.*

invicta were located and determined to be active using the potato chip test (Figure 3.1) (Jones et al. 1998). All experiments were conducted within the *S. invicta* foraging temperature range (22 to 36 °C) (Vinson 1997) determined by a datalogger measuring soil temperature (at 5 cm) on 30 min intervals (HOBO Pro v2 2x External Temperature Data Logger, Onset Computer Corp., Bourne, MA). Trials were performed in July 2009.

Figure 3.1. Prior to each *S. invicta* predation and foraging experiment, *S. invicta* mounds were determined active using the potato chip test from Jones et al. (1998).

Sterilized and sifted field soil, as previously described, was covered to the depth of 1 cm in the bottom of three 6 cm diam. × 1.5 cm deep plastic petri dish (VWR International, Radnor, PA). Into each, 15 *P. japonica* eggs, collected <24 h prior, were placed onto the top of the soil

(Figure 3.2). Each replicate consisted of two such petri dishes and one closed petri dish with eggs sealed using Parafilm as a control for egg hatch (Figure 3.2). A 10 cm deep hole (10 cm diam.) was made using a standard golf course cup cutter 2 m from the *S. invicta* mound center. Dishes with eggs were placed into the bottom of the hole then cores were gently replaced. Eight replicates, two open and one closed dish, were used.

Figure 3.2. Top photo depicts *P. japonica* eggs on top of autoclaved soil in petri dish prior to being enclosed with the excavated soil core. Bottom picture shows control petri dish enclosed with Parafilm seal at the bottom of the hole.

After 24 h, cores were removed and all dishes were collected. The soil at the bottom of each core was also examined to ensure no *P. japonica* eggs remained stuck to the bottom of the core. Petri dishes were then capped with lids, sealed with Parafilm, and transported to the lab. In the lab, the soil of each petri dish was sifted and egg recovery or hatch was recorded. The number of neonate larvae was counted to determine the percent hatch of eggs, and all remaining eggs were counted to find the percent loss due to *S. invicta* predation.

Larval Susceptibility Trial

Active *S. invicta* mounds were located in a lawn on the Auburn University campus comprised mainly of *Eremochloa ophiuroides* (Munro) Hack (centipedegrass). Mounds were gauged active again using the potato chip test. Two meters from the center of a mound, two PVC pipes (15 cm diam., >20 cm tall) were inserted to a depth of 10 cm into the turf using a 11.3 kg weight. To allow foraging in the soil, 5 mm diam. holes, drilled 3 to 5 cm apart, were made in the sides of each pipe around the entire circumference and from the bottom to the top of the PVC pipe. Each mound was treated as a replicate with two PVC pipes per mound arranged in opposite directions relative to the mound (Figure 3.3). This experiment was conducting within the *S. invicta* foraging temperature range with dataloggers as previously described.

Figure 3.3. Perforated 15 cm PVC pipe inserted >10 cm deep and 2 m from the middle of an active *S. invicta* mound to determine loss of scarab larvae and adults.

Ten scarab larvae were placed into each pipe, and allowed to burrow into the soil. If any larva did not burrow into the soil within 1 min it was replaced with another larva. Once all 10 larvae were in the soil, the pipe was covered with a metal mesh screen to exclude avian or mammalian predation. Pipes were exposed for 24 h, then the pipe with the core of turf and soil inside were removed and destructively sampled to recover the larvae. To safeguard against movement of grubs outside of the pipe, a 60 × 60 × 15 cm deep section of turf was excavated and destructively sampled whenever 10 larvae were not found in a pipe. All larvae collected were preserved in ethyl alcohol and transported to the lab to record percent loss.

Adult Susceptibility Trial

Four species of Scarabaeidae adults were tested to examine their susceptibility to *S. invicta* predation within the soil. Experiments were conducted as previously described for the larval trial. Two, PVC pipes were placed 2 m from an active mound located in the roughs at the Links and Short courses on Grand National (Figure 3.3). Hybrid bermudagrass was the dominant turf species maintained at 6 cm. Temperature was monitored using dataloggers and only conducted when air temperatures were within the previously stated foraging range.

Ten adults were placed into each PVC pipe and allowed to burrow in the soil. Any adults that did not burrow into the soil within 1 min were replaced with a new adult. Scarabaeidae adults were left in the soil for 24 h and then the cores were removed, and destructively sampled. Remaining adults were collected and preserved in ethyl alcohol, and percent loss was recorded. As with the larval trial, if all of the adults that were originally placed into the pipe were not recovered a 60 x 60 x 15 cm deep section of turf was destructively sampled for adults that may have burrowed under the 10 cm deep pipe. Experiments with *P. japonica* and *C. nitida*, were conducted on separate days in 2009, and each was replicated 8 times (using 8 and 4 mounds respectively). Trials with *E. humilis rugiceps* and *C. lurida*, also conducted on separate days but in 2010, were replicated 8 and 5 times respectively (each around 4 mounds).

Temporal Foraging Trial

In 2010, a trial to examine subterranean foraging rates of *P. japonica* eggs over time, at a constant depth within the soil, was conducted. In the lab, 50 mL centrifuge vials (Fisherbrand, polypropylene, 29 mm diam. × 115 mm) were prepared with 16, 5 mm diam. holes drilled into the walls to allow *S. invicta* to enter and exit freely (Figure 3.4). Each vial was filled with

autoclaved, sifted, and then moistened soil at a depth of 6 cm from the bottom. Fifteen *P. japonica* eggs, collected from the previously described lab colony, were then transferred into each vial. The eggs were then covered with the same soil filling the remaining volume in the vial. Another set of 50 mL vials with no holes were similarly prepared to measure the amount of egg hatch (Figure 3.4). Each treatment contained a perforated and non-perforated vial and there were eight replicates.

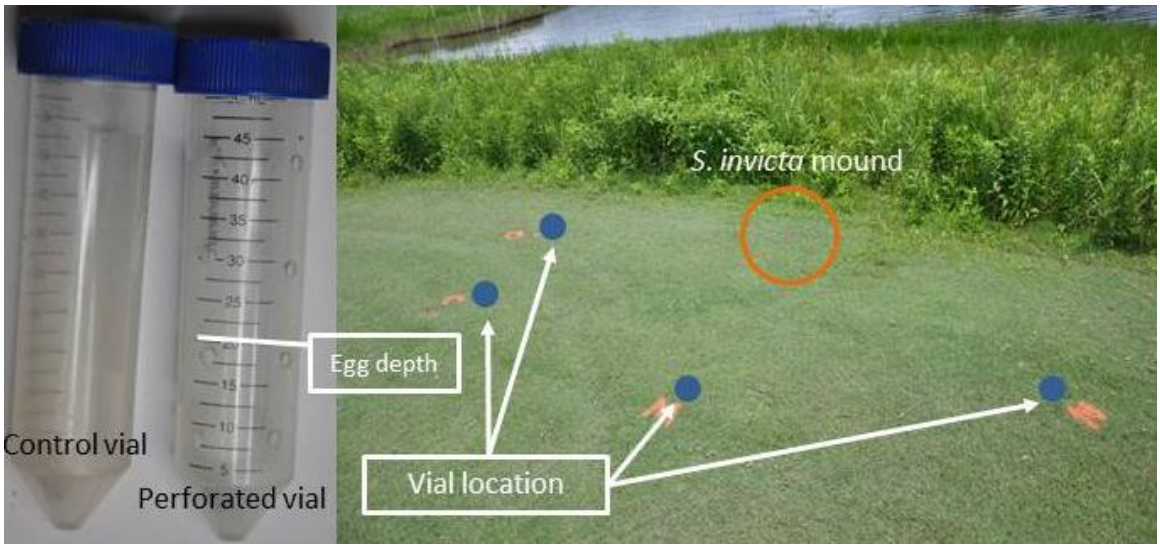

Figure 3.4. Left picture shows a non-perforated control vial compared to the perforated vial while also showing the depth at which *P. japonica* eggs were inserted with soil into each vial. The right figure shows the vial layout of two replicates, each containing a solid control and perforated vial.

This field site for this trial was the Links and Short Courses at Grand National. Mounds were located within hybrid bermudagrass rough and determined active using the potato chip test as before. Vials were inserted into a hole made with a soil probe 2 m from the center of an active mound to depth where the eggs were approximately 6 cm deep, a typical depth for scarab eggs in

the soil (Figure 3.4). Vials were collected at 24, 48, and 72 h and the soil in each was sampled in the field for presence of ants and remaining *P. japonica* eggs. After this, the soil was remoistened and the remaining eggs were replaced into the original vial and reinserted into the same location around the same *S. invicta* mound. The effect of the number of *P. japonica* eggs lost compared to the control over time was determined using analysis of variance (ANOVA) test ($P < 0.05$) followed by a Least Significant Difference (LSD) test among different time treatments (Statistix 8, Tallahassee, FL) (Zar 1999).

Manicuring test

In July 2010 two tests were created to determine the most natural experimental design to quantitatively analyze *S. invicta* soil foraging. The first trial used a standard golf course cup cutter to remove three 10 cm deep cores, 2 m from an active *S. invicta* mounds for four replicates. One hole was created as in the Egg Susceptibility Trial, the second was performed similarly to this one but, using a ball mark repair tool, had the walls of the core "manicured" for a better fit, and the final hole had the core destructed and the hole backfilled with the crumbled soil. A 9 g slice of hot dog (Bar-S Food Co., Phoenix, AZ) was placed as the bottom of each hole as an ant attractant and, after approximately 12 h of exposure, ant presence and absence was recorded. The objective of this study was to determine which of these three designs replicated natural foraging conditions most realistic and *P. japonica* eggs as a response variable.

The second test used the three aforementioned methods but used 10 *P. japonica* eggs as a response variable, measuring percent loss of eggs after 24 h. A control was included in this test to measure hatch of eggs so that a mean number of hatched eggs could be determined to ensure not all egg loss in treatments was due to larva crawling out of the dish. To determine which

method replicated the most natural foraging conditions the design with the least loss due to *S. invicta* predation.

Variable Soil Depth Foraging Trial

A trial to assess foraging depths of *S. invicta* was conducted. Exposure time was also a factor since we hypothesized that it may take longer for fire ants to forage lower in the soil. In this trial, 50 ml vials were modified so that five foraging "windows" were created at specific depths in each vial. The uppermost "window" restricted foraging to a soil depth of 1 to 2.5 cm when fully inserted. The middle "window" restricted foraging to 2.5 to 6.3 cm, and the lowest "window" was 6.3–10 cm deep when fully inserted (Figure 3.5). Each window represents a treatment with an intact vial on the surface used as a control.

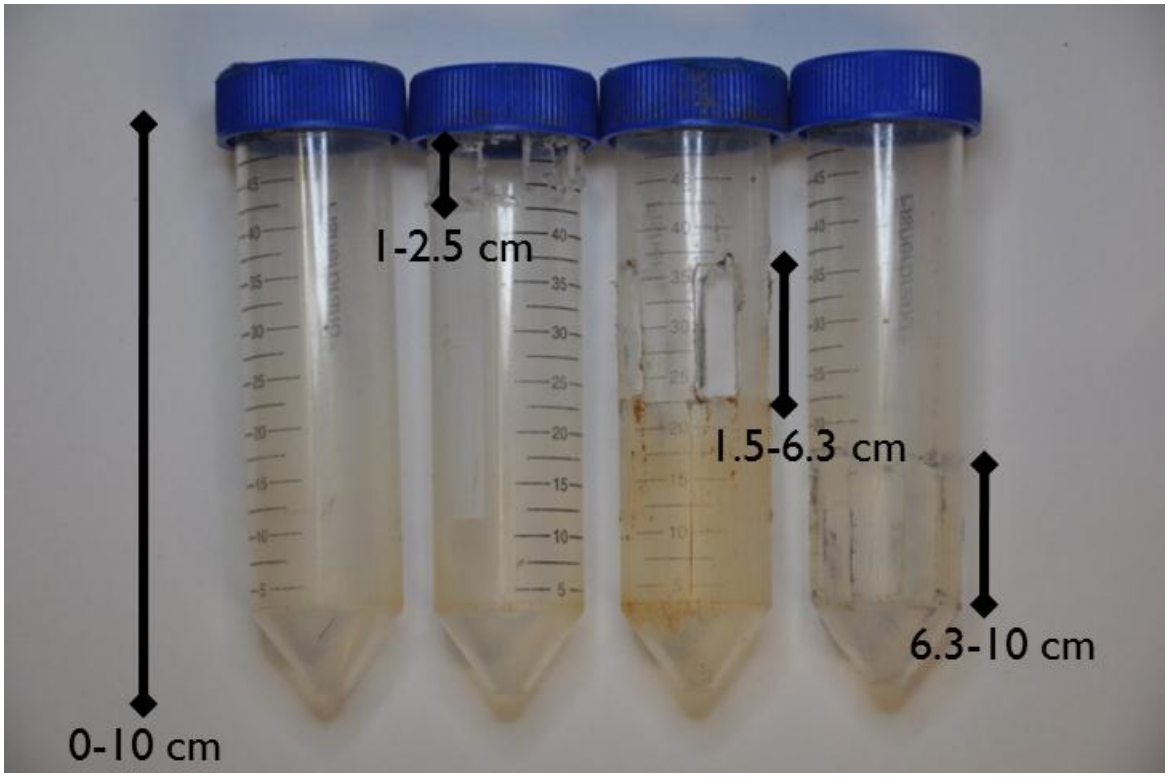

Figure 3.5. Fifty mL centrifuge vials showing the various foraging "windows" that restrict soil foraging of *S. invicta* to certain depths compared to the left-most, solid walled control.

This trial was conducted in the hybrid bermudagrass rough using visible *S. invicta* mounds determined active as before. Air and soil temperatures were recorded using a datalogger as previously described. Windowed vials were inserted into the soil 2 m from an active *S. invicta* mound, using a soil probe as before, baited with approx. 9 g of hot dog then capped. Vials to measure surface foraging *S. invicta* were intact, 50 mL centrifuge vials which were also inserted into the soil but left uncapped during hot dog baiting (Figure 3.6). All vials were placed >1 m apart. Vials were retrieved after 1, 24, 48, 72 h, or 8 d after baiting. A new surface control vial was used to correspond with each time period. Using the 24 h time treatment as an example, the surface control was baited at 23 h then both the surface control and the windowed vials

exposed for 24 h were harvested at the same time. This provided a temporal control for each foraging time. "Window" vials were removed from the soil with pliers and quickly placed into a Ziploc bag so that *S. invicta* could not escape. All vials were then transported in a cooler to the lab and frozen. Ants in each vial were washed from the vial with ethanol and counted. A total of eight replicates were placed around five mounds. Each replicate contained five time treatments, and within each time treatment three depth or "window" vials, with a corresponding surface control (Figure 3.6).

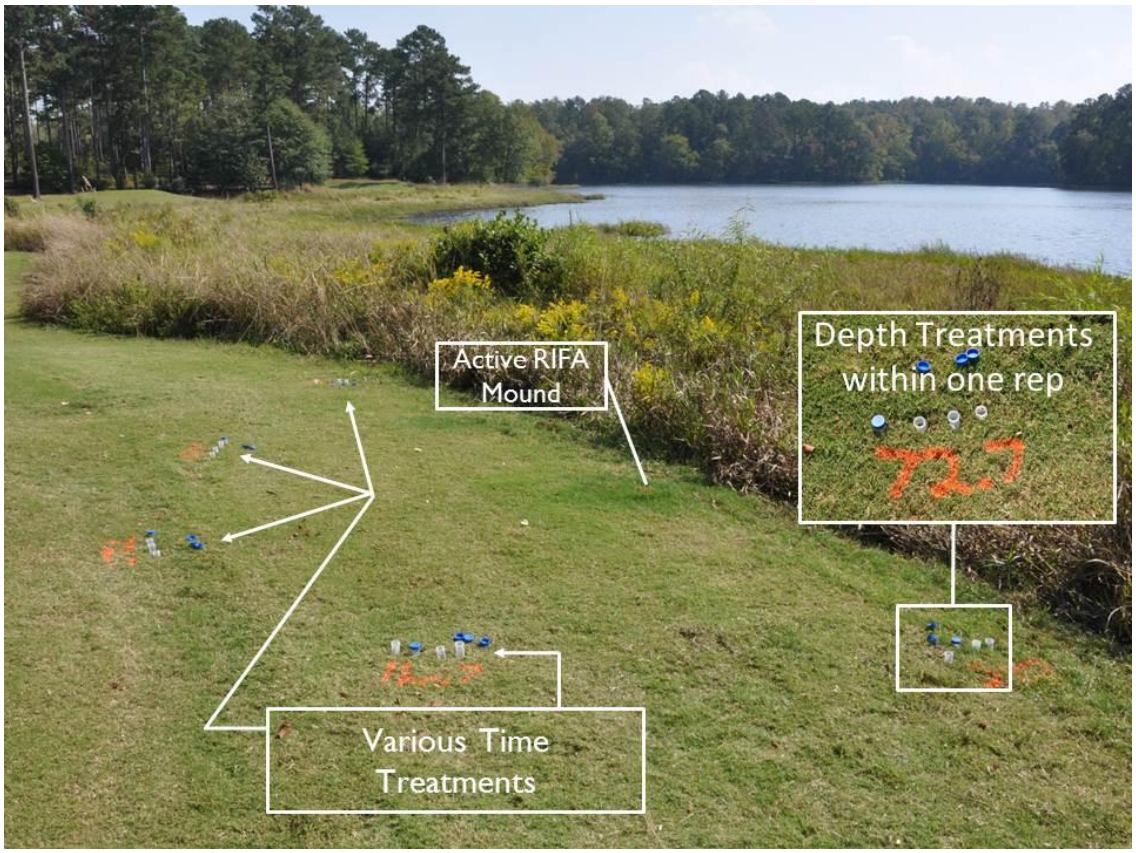

Figure 3.6. A single mound with five separate time treatments 2 m from mound center depicted in the bottom left box. The right hand enhancement shows the various depth treatments within a time treatment and the surface control capped on the left.

Disturbance Test

As noted previous trials and other published work (Zenger and Gibb 2001, White 1940), ants may swarm to an area in response to soil disturbance. This would create an artifact that could possibly confound results of our previous trials and some published studies. A test, therefore, was conducted to determine *S. invicta* response to soil disturbance and the residual effect of this disturbance on recruitment of ants to hot dog baits. This test was performed on the infield of the Auburn University Hutsell (Wilbur) Track in Auburn where the dominant turfgrass species was *Eremochloa ophiuroides* (Munro) Hack (centipedegrass) maintained at an approx. 6.5 cm. Air and soil temperatures were recorded using a datalogger as previously described.

In November 2010, 50 mL centrifuge vials, with the bottom foraging "window" (6.3 to 10 cm depth), were inserted 2 m around actively foraging *S. invicta* mounds using the soil probe as previously described. These mounds were determined active using the potato chip test (Jones et al. 1998). In this experiment, vials were placed >1 m apart. After all the vials were inserted into the soil, hot dogs were not added until 1, 3, or 6 h after insertion. A surface foraging control vial was used again (Figure 3.7). Surface control vials were not inserted into the soil as before but instead placed horizontally on the turf surface 4 m from the mound center. The vials were not inserted into the soil to avoid a new disturbance that may result in a swarming response by *S. invicta*. Control vials were shaded with parasol drink umbrellas (Figure 3.8).

Eight replicates using four active *S. invicta* mounds were used. Three time treatments, 1, 3 and 6 h created one replicate with two of these replicates per mound. A surface control vial was placed for each replicate. The timing of this vial placement was done as the previously described Variable Soil Depth Foraging Trial so that they were exposed for an hour and then collected with the corresponding soil vials.

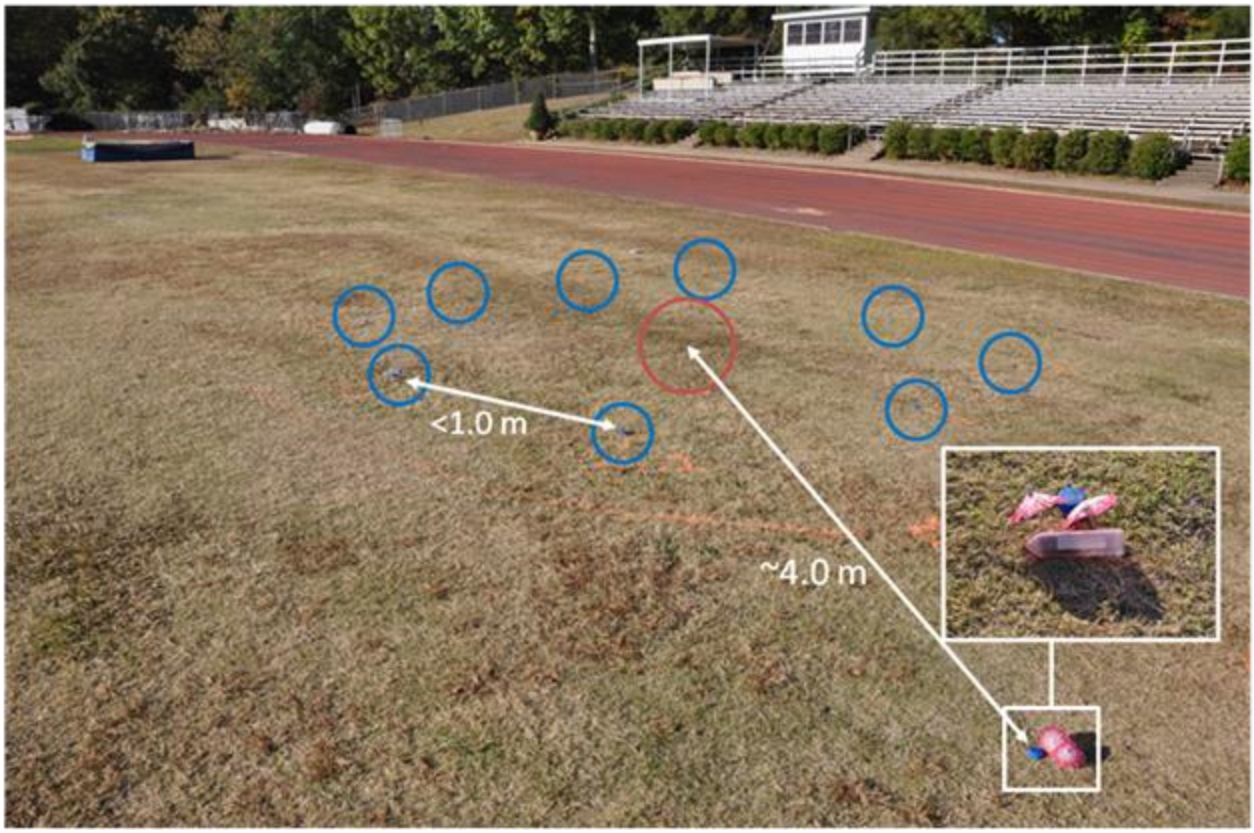

Figure 3.7. Layout of the disturbance test trial around an active *S. invicta* mounds. Blue circles depict where bottom "window" vials were inserted with a surface control vial 4 m from mound center.

Figure 3.8. Surface control vial being shaded with cocktail parasol drink umbrellas containing a 9 g slice of hot dog to collect surface foraging *S. invicta.*

When vials were collected and capped they were immediately placed in a Ziploc bag for collection, frozen, ants species identified, and counted. The point of which disturbing the soil prior to baiting was no longer considered a factor was determined by when no *S. invicta* were present in any "window" vials.

Soil Foraging Trial

A final experiment, incorporating the results of the previous experiments, was conducted in 2010 to determine if *S. invicta* will forage below the soil surface to hot dog baits. This trial was performed on the same location and used the same experimental conditions as the previously

described Disturbance Test except that vials were not baited with hot dog slices until >9 h after installation. Bottom "window" vials (6.3 to 10 cm windows) were inserted into the soil to restrict foraging to a depth of 6.3 to 10 cm. Vials were then capped and retrieved after 24, 48, and 72 h.

Five *S. invicta* mounds were used with nine vials in three groups of three, one for each retrieval time, arranged 2 m from each mound and 1 m between each vial. The numbers of ant around each of the three vials representing the same retrieval time were averaged together for each mound creating five true replicates for data analysis. Each replicate had a surface control vial placed 4 m from the active *S. invicta* mound center account for aggregation affect. This surface control vial was placed on the turf surface and covered with cocktail parasol drink umbrellas (Figure 3.8). Control vials were baited 1 h prior to collection as in the Disturbance Test and exposed for 1 h as before.

Surface control vials and "window" vials in the soil were retrieved from the soil with pliers and placed immediately into plastic Ziploc bags. Bags were taken to the lab, frozen, and ant species were identified and counted. The effect of the time treatment on *S. invicta* abundance was determined using randomized complete block design analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test ($P < 0.05$) (Statistix 8, Tallahassee, FL) (Zar 1999).

Results

Egg Susceptibility Trial

After the first experiment, 75% ± 8.91% SEM of implanted eggs were missing after 24 h. There was 5% ± 2.44% egg hatch in the control with significantly less (Randomized Complete

Block Design ANOVA $P < 0.05$) loss of eggs in the treatments ($F = 15.77$ $P = 0.0012$, df = 1,7). Similarly, the second test had similar predation rates, 70% ± 12.06% of eggs, and similar egg hatch 5% ± 1.67% hatch in controls (Figure 3.9) and significantly less (RCBD ANOVA $P < 0.05$) loss in the treatments than the control ($F = 26.75$ $P = 0.0001$ df = 1,7).

Larval Susceptibility Trial

After 24 hours of exposure 15% of the implanted white grubs were lost and only one replicate had *S. invicta* present in the core during sampling (Figure 3.9) (Table 3.1).

Adult Susceptibility Trial

Predation of the four scarab species implanted 2 m from *S. invicta* mounds ranged from 0 to 16% after 24 h. Predation was lowest for *E. humilis rugiceps* and *C. nitida* (Figure 3.9). Unusually high numbers of *S. invicta* were found in a single pipe of replicate six which had the highest predation loss recorded.

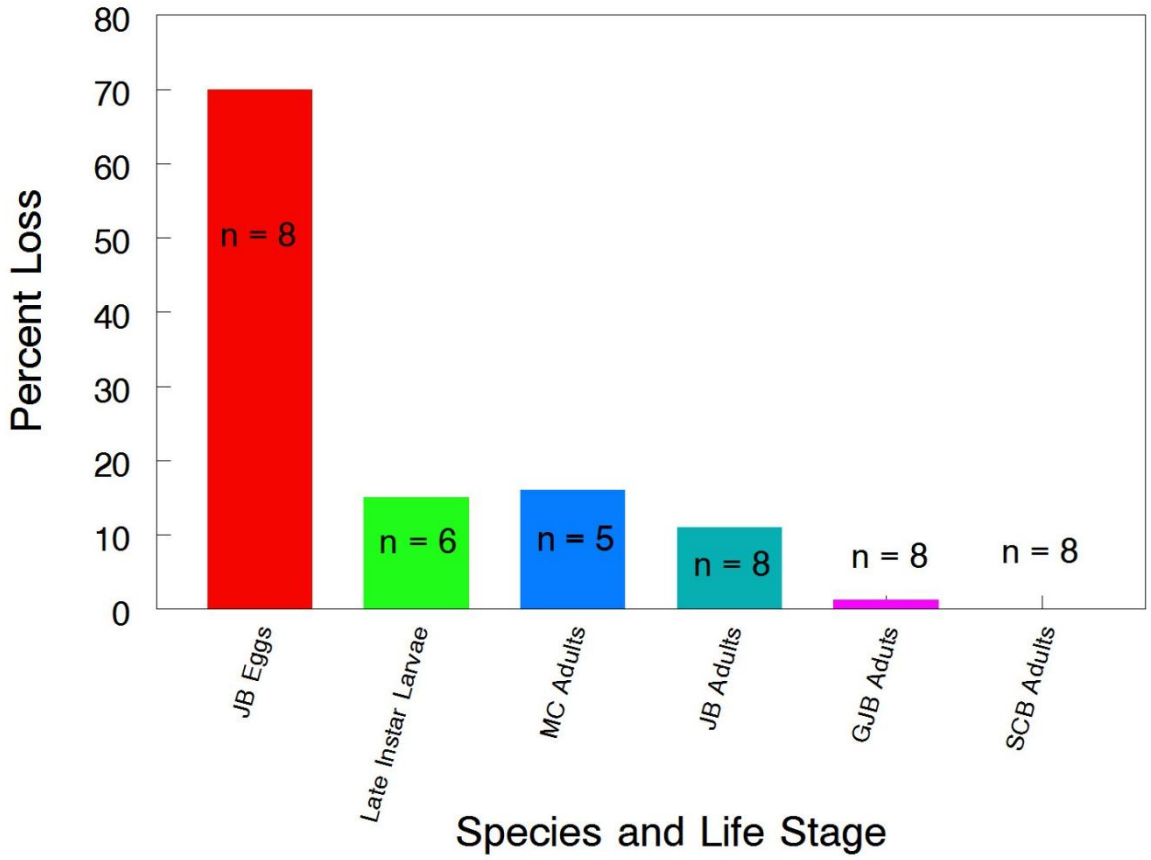

Figure 3.9. Percent loss of various life stage and species subjected to 24 h of *S. invicta* predation 2 m from active mounds.

Temporal Foraging Trial

There was no significant difference between the egg numbers at the beginning of the trial (Table 3.1) and egg numbers at 24, 48, and 72 h ($F = 2.88$, $P = 0.0895$, df = 2, 7 for original counts) ($F = 1.62$, $P = 0.2338$, df = 2, 7 for changed counts). No *S. invicta* were present in vials nor were foraging tunnels found during sampling.

Manicuring Test

The first presence and absence test determined that the original method used with the Egg Susceptibility Trial had *S. invicta* present in all replicates, the "manicured" method had *S. invicta* in half of the replicates, and the backfilled method had no *S. invicta* present in any replicates. *Popillia japonica* egg predation was greatest with the "manicured" method with 55% loss, the old and backfilled methods had 27 and 22% loss respectively. The control had no larva hatch in any replicates.

Variable Soil Depth Foraging Trial

All vials were collected at their appropriate times; however, all vials were full of *S. invicta*. For this reason, the collected ants were not counted and the experiment redesigned as the Disturbance Trial.

Disturbance Test

Vials spaced 1 m apart and baited 1 or 3 h after installation, were again overwhelmed by ants. However, those vials baited after 6 h had no *S. invicta* present in any window vials.

Soil Foraging Trial

A final experimental trial was conducted incorporating adjustments for potential artifacts in previous trials. In this trial, all vials in the soil had no *S. invicta* present while surface control vials had *S. invicta* present (Figure 3.10).

Table 3.1. 2010 temporal *S. invicta* foraging trial eggs numbers over 24, 48 and 72 h. Numbers in red depict miscounts that were later changed to match the 72 h counts.

		24 h Egg Count	48 h Egg Count	72 h Egg Count
Replicate 1	Control	15	14	15
	Perforated	15	15	12
Replicate 2	Control	15	15	15
	Perforated	15	15	14
Replicate 3	Control	15	14	15
	Perforated	14	14	14
Replicate 4	Control	15	14	15
	Perforated	15	15	15
Replicate 5	Control	15	14	15
	Perforated	15	15	15
Replicate 6	Control	15	15	15
	Perforated	15	15	7
Replicate 7	Control	15	14	14
	Perforated	15	14	15
Replicate 8	Control	15	15	14
	Perforated	15	14	15

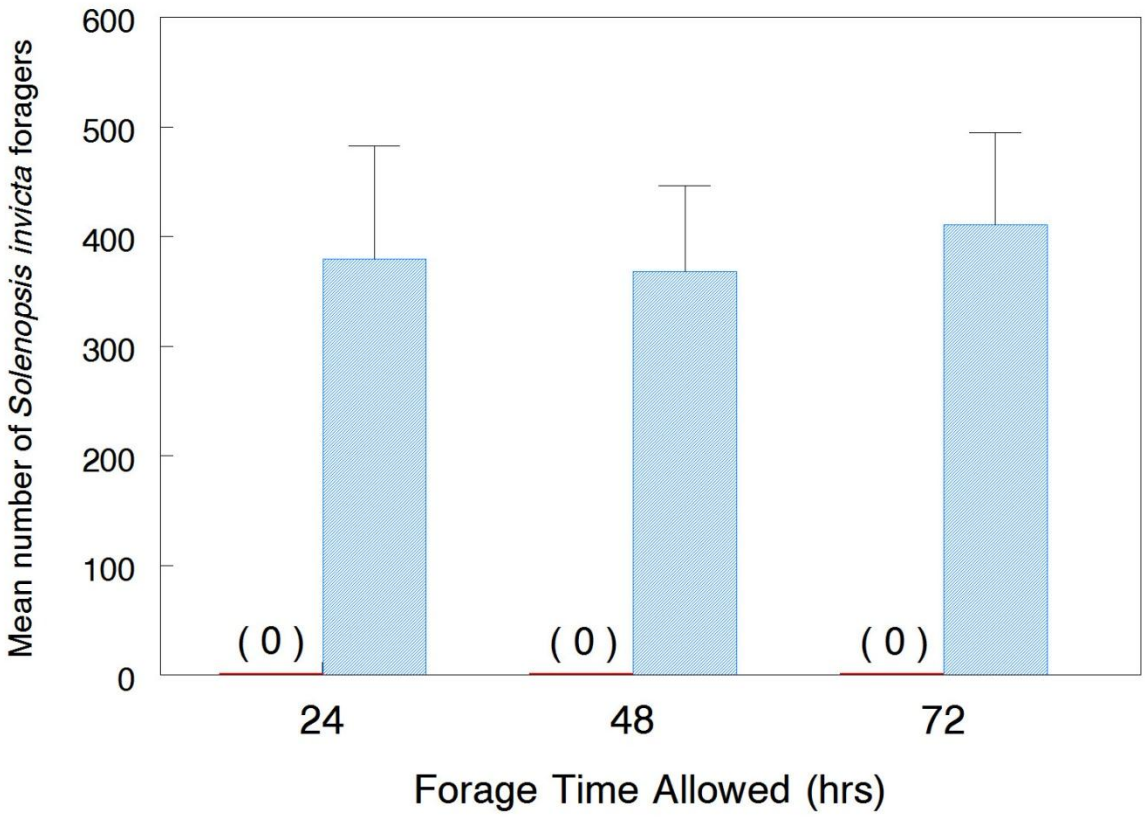

Figure 3.10. Soil and surface control vial records among the three sampling times with *S. invicta* surface means depicted in blue and soil ”windows” in red ($P < 0.0001$ df = 2, 4).

Discussion

As hypothesized, adults were not as susceptible to *S. invicta* predation as other life stages. This is most likely due to the adults' ability to escape predation via locomotion or flight and their hardened exoskeleton. White (1940) noted that a single *P. japonica* adult was observed fighting off attacks from *Formica fusca* var. *subsericea* Say for half an hour before ultimately succumbing to multiple ant attacks. Adult Susceptibility Trials may have had higher predation rates due to the consumption of dead adults that may often die on the top of the turf canopy

(*person. observ.*). No control was included to account for dead *P. japonica* so no calculations could be used to consider the mean number of dead adults.

Although scarab larvae are not as protected as adults it appears that their hypogeal habitat allows them to escape *S. invicta* predation. As with adult scarab trials, no control variables to observe larval death were recorded and, if included, could have explained why predation rates were around 16%. However, both adult and larval trials noted any *S. invicta* presence during sampling and those replicates that had *S. invicta* present were noted during data analysis and mentioned in the results section. More trials were planned for late summer and early fall 2010 using early instar larvae from various species collected in the black light but the eggs incubated into soil cores with tall fescue grass did not survive in usable numbers.

Unpublished reports have also shown that live scarab larval pests of turfgrass have been collected from the bottom of *S. invicta* mounds (J. Oliver, *personal comm.*). Furthermore, certain pests (termites and cutworm larvae) are reported cohabitating with ants inside active fire ant mounds (Hays and Hays 1958, Shelton et al. 1999). Although *S. invicta* can be significant predators, it is clear that certain pests once in the soil, may be able to 'hide in plain sight' from fire ants. The abundance of prey on the surface may make it less efficient for ants to forage in the soil. Also, *S. invicta* use vision and pheromones as part of their foraging strategy, which would be less effective in a soil versus above ground environment. Thus, certain insects coexist around or in active mounds with little threat of attack. In fact, most other insects found in ant mounds aren't attacked until the mound is disturbed and exposed (Hays and Hays 1958, Shelton et al. 1999).

Experiments conducted with eggs in the field indicted that *S. invicta* could be a significant predator of white grubs in managed turfgrass. These data are similar to results with

ants and prey in turfgrass (Zenger and Gibb 2001, López and Potter 2000). López and Potter had >50% loss of eggs after 20 h and Zenger and Gibb had 73% loss after 72 h using similar methods of implanted eggs into soil. This method is also common for evaluating recovery of ecosystem services following pesticide applications (e.g., Kunkel et al. 1999 and related studies). These data, however, are inconsistent with the results presented earlier (Chapter II) when selective removal of *S. invicta* with baits and soil insecticides had no impact on white grub populations compared to untreated controls. In these trials, we suggest that the replacement of the soil core causes an improper fit essentially creating surface foraging conditions through gaps in the soil core and the hole created by the golf cup cutter. To address this problem, the soil was manicured or backfilled around the hole and no fire ants were noted in those holes. Yet ants were abundant in holes made consistent with the original design. This "canyon" effect may have been a confounding factor in past experiments and should be accounted for in future experiments on ants in turfgrass. Because of this, our subsequent trials examining foraging of *S. invicta* in the soil used hot dog baits and numbers of ants as a response variable.

Three factors were found to significantly skew data from multiple soil foraging trials, "canyon", swarming, and disturbance effect. The 'canyon' effect was previously noted during the eggs trials so the other two factors will now be discussed in relation to experimental designs and the potential for altering test results.

Several of our trials showed that *S. invicta* data may be skewed by characteristic swarming tendencies to food sources by the release of their trail marking pheromones creating an aggregation effect. During the Variable Soil Depth Foraging Trial, multiple vials were located within a 250 cm^2 space. Because *S. invicta* lays a trail-marking pheromone for recruitment, *S. invicta* likely inundated close vials as an artifact of recruitment. After just 1 h, many of the

bottom “window” vial replicates, which were hypothesized to have *S. invicta* present at 72 h or 8 d after baiting, had *S. invicta* present which was inconsistent with the previous results. Because of this, data was not recorded and a new trial was designed to quantify *S. invicta* foraging.

The final and arguably most important and common effect found to influence *S. invicta* foraging tendencies was the disturbance effect. Zenger and Gibb (2001) noted, “Observations made during this study suggest that ant foraging behavior is increased following soil disturbance.” Furthermore, they noted that the first 4 to 6 h after soil disturbance “far more ants were observed foraging” (Zenger and Gibb 2001). This was observed in almost every trial we performed in 2009 and 2010 with the exception of the Temporal Foraging Trial. White (1940) also noted this in his *P. japonica* and ants species interactions when he stated “This pupa had been unmolested by the ants, although egg galleries and runways formed a network around it. As soon as it was disturbed, however, ants immediately attacked and carried it away.”

In conclusion, the results of multiple field studies indicate that *S. invicta* may not forage for prey within the soil. Despite several publications (Zenger and Gibb 2001, López and Potter 2000) showing ant species being beneficial predators of subterranean and surface inhabiting insects, it is hypothesized that above ground prey sources do not seem to warrant the energy expenditure that soil digging requires. Also, past experimental designs (Zenger and Gibb 2001, López and Potter 2000) have been shown to possibly significantly confound results by creating one or more of the three “canyon”, aggregation, and/or disturbance effect. Future studies into hypogeal foraging of *S. invicta* and other ant species must take these factors into account when creating experimental designs.

Chapter IV

ADDITIONAL EXPERIMENTS

Field Trial Precipitation Vials

A method was created to accurately record precipitation amounts in each plot, the entire summer, all while not interfering with regular golf course maintenance or play. The 50 mL centrifuge vials commonly used throughout this study were perfect cylinders except for the bottom 5 mL portion which was a conical shape. To easily read the collected precipitation weekly in all 16 plots, gulf wax was melted until 5 mL could be pipetted into each vial to fill the bottom conical shape to create a perfect cylinder.

To ensure our recorded rates matched actual rain amounts, four of these new vials were placed at the Auburn University Turfgrass Unit along with 3 rain gauge catch cans during an irrigation cycle. All vials and catch cans had the same reading of 1.25 cm proving this design a success.

Chapter V

FINAL CONCLUSION

The data presented from this study shows that the suppression of *Solenopsis invicta* Buren does not influence populations of white grubs (Coleoptera: Scarabaeidae) within managed turfgrass and that experimental designs examining soil foraging of various ant species can be greatly skewed by three discovered factors. Despite the foraging voracity of *S. invicta* in the southern United States, one possible hypothesis as to why *S. invicta* does not forage through the soil for prey is that above ground biomass resources are plentiful enough to not warrant soil foraging for prey.

Several characteristics allow *S. invicta* to be a successful arthropod in invading new niches and overcoming prey. The large size of young as well as developing colonies, *S. invicta* ability to recruit workers using trial marking pheromones, and recruiting pheromone allow *S. invicta* to search, locate, and utilize food sources with surprisingly little energy expenditure. The foraging tunnels, which are not truly tunnels used in search of food, but "protective access" highways as termed by Markin et al. (1975), serve as protection for foragers from unfavorable abiotic conditions as well as protection from prey. However, because these attributes are so successful to *S. invicta* colonies it allows populations of white grubs within the soil, and even directly under active mounds to remain undisturbed.

The most appropriate way to measure affects *S. invicta* has on turfgrass pest populations is to measure their influences on natural populations as done in Chapter II and White (1940).

These studies are difficult to perform in managed turfgrass because of the destructive sampling needed to locate grubs. Therefore, attempting to quantify soil foraging of various ant species is very difficult with artificially implanted response variables mainly due to the disturbance effects created with many experimental designs. Conclusions from studies based on these current experimental designs, stating that common turfgrass ant species are significant soil predators, are biased and need to be reassessed using methods similar to the ones described in Chapter II and White (1940).

This study provides insight into *S. invicta* and white grub interactions within turfgrass while also challenging traditional experimental designs. With this knowledge further studies can help understand what role, if any, *S. invicta* plays in hypogeal habitats as well as attempt to explain why white grub populations are perceived to be increasing in the southern Unites States.

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