

ABSTRACT

YOUNGS, KATHARINE M. Evaluation of Resistance to Southern Chinch Bugs, *Blissus insularis* Barber, in St. Augustinegrass *Stenotaphrum secundatum*, Germplasm. (Under the direction of Dr. Yasmin J. Cardoza.)

Work for this thesis dealt with identifying new sources of SCB resistance in a set plant introductions (PIs). Chapter one is a review of the literature dealing with SCB control methods for this pest, including host plant resistance and identifying new sources of St. Augustinegrass host plant resistance. SCB are the most economically important pest of St. Augustinegrass and their management is increasingly difficult due to their ability to develop resistance to current control methods. Host plant resistance is an effective alternative for SCB control due to decreased efficacy of insecticides, regulatory policies involving the use of chemicals, and consumer demand for sustainable production practices. SCB are still controlled for the most part with chemicals; however, a couple of resistant varieties are marketed for their resistance to SCB, NUF-76, marketed under the trade name 'Captiva', and 'Raleigh'.

Several varieties have been shown to suppress SCB populations; however the inevitability of resistance development in SCB, coupled with the aesthetic needs of the industry deem identifying new sources of host plant resistance necessary. Therefore, in Chapter 2 we focused on screening 18 St. Augustinegrass and two 'pembagrass' (a crossbreeding relative) plant introductions. Based on the results from this study, we determined some of the selected PIs exhibited antibiosis, based on low survival and slower development of SCB neonates, when compared to our susceptible reference varieties. Two of the PIs, PI 600734 and PI 647924, showing antibiosis are diploid which may facilitate transfer of resistance genes to commercial varieties. Adult preference, based on oviposition and fecal output was also significantly affected by plant genotype. Oviposition on seven PIs was similar to that obtained on the resistant reference varieties. Moreover, feeding, as assessed by fecal output, on six PIs was

equivalent or lower to that observed on the resistant reference varieties, indicating marked differences in insect preference for the various grass lines investigated herein. In addition to identifying lines with antibiosis we also determined that three of the tested PIs display tolerance to SCB feeding, as indicated by their high functional plant loss index, FPLI, even at the highest infestation level tested in this study.

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Evaluation of Resistance to Southern Chinch Bugs, *Blissus insularis* Barber, in St.
Augustinegrass *Stenotaphrum secundatum*, Germplasm

by
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DEDICATION

I am thankful for having such an inspirational family therefore, I dedicate this to my entire family. My parents raised me to work hard and to never give up. Despite my mother's intolerance for insects and my father's dislike for environmental studies, they both pushed me to follow my passion, even if it was to save bugs. I especially would like to thank my sister, Laura Youngs, who helped edit this thesis, and my fiancé, Matt Sidebottom, who volunteered countless hours to counting, planting, and providing moral support.

BIOGRAPHY

Katharine Youngs earned a B.S. degree in Environmental Technology from North Carolina State University in December, 2010. Prior to graduation she worked as a research technician in the Steve Frank Ornamental Entomology laboratory at North Carolina State University. During her time in the Frank lab she studied the effect of manual and airblast insecticide applications on natural enemy abundance and diversity. This research focused on the effectiveness of two sprayer methods and effects each had on insect predator populations. Katharine spent much of her time identifying insects to family from sticky cards, each housed in nurseries using different sprayer methods. Katharine also assisted M.S. student Sarah Wong researching black pearl pepper plants as a banker system for the biological control of thrips.

In January, 2011 Katharine started a Masters program in Entomology at North Carolina State University under adviser Yasmin Cardoza. Her M.S. research focuses on evaluating host plant resistance in turfgrass against the southern chinch bug. She conducted experiments to evaluate three main categories of resistance: antixenosis, antibiosis, and tolerance. To accomplish this, she maintained colonies chinch bugs to support her research needs. In addition she participated in other research in the Cardoza lab by maintaining tomatoes, cucumbers, *Arabidopsis*, radish, cabbage, and collards. She also learned to prepare artificial diets for, and reared, *Pieris rapae*, *Heliocoverpa zea*, and *Trichoplusia ni*.

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A big thank you goes to Sarah Wong for moral support, advice, and guidance. I can't tell you how much I learned from watching you, and it gave me the courage to dig in and never give in. And of course, I think almost every NCSU entomology student before me can say this, thank you, Gene Dupree, for all the small and big things that you do every day to make our work lives easier.

Finally I want to show my appreciation for the Entomology Department. Especially to all the faculty and staff that helped me through the years (that list is quite long) and have taught me the importance of collaboration, as well as, inspired my research throughout my degree. I especially want to thank the graduate program coordinator, Wes Watson. He helped me tremendously every step of the way, and I don't think a 'thank you' expresses my gratitude enough, but THANK YOU!!! I love this department, and I am sad to leave!!!

TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
CHAPTER 1: Literature Review and Research Goals.	1
1. Introduction.....	1
1.1 Popular Commercial Turfgrass Species	2
1.2 St. Augustinegrass.....	5
2. Chinch Bugs, <i>Blissus spp.</i>	7
2.1 Biology	7
2.2 Host preference and geographical range	9
2.4 Biological Control	11
2.5 Cultural Control.....	13
2.6 Host Plant Resistance	13
2.7 Research Goals	15
CHAPTER 2: Investigating St. Augustinegrass resistance against the southern chinch bug, <i>Blissus insularis</i> Barber	17
Abstract.....	17
1. Introduction.....	19
2. Materials and Methods.....	20
2.1 Plant material.....	20
2.2 Chinch rearing procedure	21
2.3 Germplasm damage ratings in response to SCB feeding (Choice)	22
2.4 Neonate performance experiment on selected St. Augustinegrass lines (No Choice)	23
2.5 Adult oviposition and fecal output on selected plant lines	24
2.6 Evaluation of Tolerance to SCB Feeding in Selected Germplasm.....	25
2.7 Statistical Analysis	26
1. Results.....	26
3.1 Germplasm damage ratings in response to SCB feeding (choice)	26
3.2 Neonate performance experiment on selected St. Augustinegrass lines (No Choice)	27
3.3 Adult oviposition and fecal output on selected plant lines	28
3.4 Evaluation of Tolerance to SCB Feeding in Selected Germplasm.....	30
4. Discussion	32
REFERENCES.....	39

APPENDIX	46
APPENDIX A – Grass Nodes for Planting	47
APPENDIX B – Grass Performance Across Experiments	48
APPENDIX C – Interior View of Antixenosis Arena	49
APPENDIX D – Antibiosis Arenas	50
APPENDIX E – Ovipositions and Fecal Arena	51
APPENDIX F – Final Damage Ratings	52
APPENDIX G – Antixenosis Results.....	53
APPENDIX H – Antibiosis Results	54
APPENDIX I – Oviposition Results	55
APPENDIX J – Feeding Results	56
APPENDIX K – Oviposition and Feeding Sex Ratios.....	57
APPENDIX L – Tolerance Results	58

LIST OF TABLES

- Table 1.** Performance across all experiments by genotype. S = susceptible, HR = highly resistant and MR = moderately resistant, N/A= germplasm not included in assay. These were designated based on statistical groupings from each analysis. **48**
- Table 2.** Mean number of immatures, adults, and total insects on each St. Augustinegrass genotype at the end of the germplasm damage ratings in response to SCB feeding (choice) **53**
- Table 3.** Mean number of eggs by genotype per week obtained from the adult oviposition and feeding preference experiment. Values represent mean±SE for 6 replicates per plant genotype. Values within columns followed by the same letter are not statistically different (Tukey-Kramer mean separation test $P \geq 0.5$). **55**
- Table 4.** Mean number of fecal spots per week per live adult obtained from the adult oviposition and feeding preference experiment.. Values represent mean fecal spots ±SE for 6 replicates per plant genotype. Values within columns followed by the same letter are not statistically different (Tukey-Kramer mean separation test $P \geq 0.5$). **56**
- Table 5.** Weekly percent surviving male and female southern chinch bug during the oviposition and feeding preference experiment. Values represent mean ± SE for 6 replicates per plant genotype. Values above columns followed by the same letter are not statistically different (Tukey's mean separation test $P \geq 0.5$). **57**
- Table 6.** Data from tolerance experiment. Damage rating, number of recovered insects, dry weight, and FPLI by infestation level. Values represent mean±SE for 5 replicates per plant genotype. Means for genotypes within infestation level followed by the same letter are not statistically different (Tukey's mean separation test $P \geq 0.5$). **58**

LIST OF FIGURES

- Figure 1.** Grass was collected by single stolons that were clipped with 3 nodes (13-17cm) and then rooted by submersing the proximal ends in a cup of water. Water was changed every other day until a tap root had developed (approx. 7-10 days). Once a tap root was established, the stolon was planted for experiment. 47
- Figure 2.** Antixenosis experimental set-up. Twenty-six St. Augustinegrass genotypes were randomly placed within a 1 m² cage. Each arena was infested with 260 mixed adult and fifth instar SCB. SCB were allowed to feed and reproduce for 4 weeks. Damage for each of the lines was recorded and compared. 49
- Figure 3.** Antibiosis, choice experiment set-up. Ten neonate nymphs (<48h) were confined to individual plants using organza mesh sleeves held upright over the plant canopy by an electrical wire loop. Insects were allowed to settle and feed on the plants for four weeks. At the end of the experiments, number of adults and nymphs on each of the lines were counted and recorded. 50
- Figure 4.** Oviposition experiment set-up. One male and one female were restricted to a single stolon for one week at a time for four consecutive weeks. Aluminum foil was used in the arena to facilitate recording of fecal spots. Excretory spots and eggs were counted weekly and averaged over the duration of the experiment. 51
- Figure 5.** Damage rating obtained after a 6 week period for each of the PIs and reference varieties in the germplasm damage ratings in response to SCB feeding (choice) experiment. SCB susceptible reference varieties are highlighted in white, SCB resistant reference varieties are in black and plant introductions are in gray. Bars represent means for n=8 and error bars=1SE. Bars headed by the same letter are not statistically different (Tukey's mean separation test $P \geq 0.5$). 52
- Figure 6.** Number immature (dark bars) and adult (light bars) recovered from experimental germplasm at the end of the 4 week period in the neonate performance experiment (No Choice). Susceptible reference varieties are Seville, Floratam, and Raleigh and the resistant reference varieties are Captiva, and FX10. Values represent means for n=9 and error bars=1SE. Bars headed by the same letter are not statistically different (Tukey's mean separation test $P \geq 0.5$). Capital letters indicate means separation for immatures, and lowercase for adult means separation. 54

CHAPTER 1: Literature Review and Research Goals.

1. Introduction

It is estimated that over 100 million acres of turfgrass is maintained in home lawns, golf courses, and recreational spaces, making turf grass an important crop throughout the United States (Milesi et al 2005; Romero and Dukes 2009). In a 2002 study, researchers reported that the U.S. turfgrass industry generated \$57.9 billion in revenue, employing 822,849 people, and of those, 28,860 jobs were in North Carolina (Haydu et al 2009). In fact, North Carolina ranks fifth in the nation in turfgrass production with approximately 14,500 acres in production (USDA 2010). In 2009 alone, \$25.5 million worth of sod, sprigs, and plugs were sold by North Carolina producers (USDA 2010).

Turfgrass provides erosion and dust control, low-cost recreational space, and can even boost property values (Romero and Dukes 2009; USDA 2010). There are several species of turfgrass, each differing in their adaptability to cold or warm weather, dry or wet climates and susceptible to an array of pathogen and insect pests. The transitional climate of North Carolina, characterized by mild temperatures with moderate winter rainfall and dry summers, is best suited to grow ‘bermudagrass’ (*Cynodon dactylon* L., Pers.), ‘centipedegrass’ (*Eremochloa ophiuroides* Munro, Hack.), ‘creeping bentgrass’ (*Agrostis stolonifera* L.), ‘tall fescue’ (*Festuca arundinacea* Schreber), ‘zoysiagrass’ (*Zoysia matrella* L. Merr.), and ‘St. Augustinegrass’ (*Stenotaphrum secundatum* Walt., Kuntze) sods (USDA 2010). A brief description of the characteristics of the most common turfgrass species within the industry is provided below.

1.1 Popular Commercial Turfgrass Species

There are a number of turfgrass species that are commercially available and vary significantly in their climatic adaptability, maintenance requirements and pest susceptibilities. The most predominantly used in the field are discussed below.

Bahiagrass (*Paspalum notatum* Flugge) is a warm-season species found in the southeast region of the US and known for rapid growth, drought tolerance, and soil adaptability (Reyno et al, 2012). Bahiagrass was introduced from Brazil in 1914 and used as a pasture grass on the poor sandy soils of the region (Romero and Dukes 2009). It is not a preferred turfgrass for recreational areas because of its light color, coarse texture, and open canopy (Reyno et al, 2012) results in poor coverage. Additionally, it is difficult to mow, due to its tough leaves and stems and it can be a very competitive and unsightly weed in highly manicured turf and yards planted to other species. It is intolerant to shade and particularly susceptible to mole crickets (Romero and Dukes 2009). Therefore this species is often used in areas needing erosion control (Pozzobon and Valls 1997).

Bermudagrass (*Cynodon dactylon*L., Pers.) is a medium- to fine-textured warm-season turfgrass known for heat, drought, and salt tolerance, but it does not perform well in shade (Brosnan and Deputy 2008), which makes it less appealing for homeowner adoption. As the name suggests, Bermudagrass is an introduced turfgrass from Africa, and is now the most widely used species on athletic fields and golf course fairways and tee boxes due to its high wear tolerance, and rapid recovery (Brosnan and Deputy 2008; Romero and Dukes 2009). On the other hand, it can be an invasive, hard to control weed in some turf settings and it is characterized by an undesirable wide range of leaf textures (Brosnan and Deputy

2008). Moreover, bermudagrass pest problems, such as armyworms (*Spodoptera frugiperda* Smith), cutworms (*Agrotis ipsilon* Hufnagel), sod webworms (*Herpetogramma phaeopteralis* Guenée), bermudagrass mites (*Eriophyes cynodoniensis* Say) and Rhodesgrass scale (*Antonina graminis* Maskell), and needs, therefore, higher level of pesticide grass species (Romero and Dukes 2009).

Buffalograss (*Buchloe dactyloides* (Nutt.)Engelm.)is a native, low-growing, perennial, warm-season turfgrass known for drought tolerance and fertilizer and watering requirements. Its tolerance to prolonged droughts and to both hot and cold extreme temperatures together with its high seed producing characteristics enables Buffalograss to survive a wide range of environmental conditions (Romero and Dukes 2009). It is considered one of the most uniform and attractive turf species. Buffalograss is found throughout the Great Plains (Duble, 2008a), though it has not historically been substantially planted in the Carolinas. However, Buffalograss has increased in popularity because it is drought tolerant and has fewer pest and disease problems compared to other counterparts (Heng-Moss et al. 2002), however the western chinch bug, *Blissus occiduus*, is a major pest of buffalograss (Parker 1920; Farstad 1951) and it is also not well adapted to shaded or heavily traveled sites (Heng-Moss et al. 2002).

Centipedegrass (*Eremochloa ophiuroides* Munro, Hack.) is a slow-growing, coarse-textured, warm-season turf that is adapted for use in areas receiving low maintenance. It was first introduced from southern China to the U.S. in 1916 (Brosnan and Deputy 2008). It is often referred to as "lazy man's grass" due to its need for infrequent mowing and fertilization (Haygood 1990). It has a light-green color and reproduces vegetatively by stolons (Romero

and Dukes 2009). It does not tolerate heavy traffic, compaction, high pH, excessive thatch build up, drought, or heavy shade (Romero and Dukes 2009). Centipedegrass can sometimes be confused with St. Augustinegrass as they both have leaves that form right angles with the stem, and both propagate through stolons (Haygood 1990). Centipedegrass is highly susceptible to damage from nematodes and is occasionally prone to root damage by immature Japanese beetles, *Popillia japonica* (Brosnan and Deputy 2008). It has a tendency to exhibit iron chlorosis and produces a heavy thatch if over fertilized (Haygood 1990). It does not tolerate traffic, compaction, high pH, high salinity, excessive thatch, drought, or heavy shade (Romero and Dukes 2009).

Creeping bentgrass (*Agrostis stolonifera* L.): was introduced from Western Europe in mid-19th century. The name creeping bentgrass, derives from the fast-growing creeping stolons that develop at the surface of the ground. It has minimal insect problems like the cutworm, *Agrotis ipsilon* Hufnagel, and immature European crane flies, *Tipula paludosa* Meigen. However, due to its poor drought tolerance and high fertilizer and watering requirements, bentgrass is not suitable for private home lawns, and is cultivated exclusively on golf courses (Romero and Dukes 2009).

Tall fescue (*Festuca arundinacea* Schreber) was introduced from Europe in the early 1800's and can be found from the Pacific Northwest to the southern states in low-lying pastures (Romero and Dukes 2009). It tolerates heat better than other cool-season species. Compared to bluegrass and perennial ryegrass, tall fescue tolerates shade conditions quite well, only being inferior to fine fescue (*Festuca tenuifolia* Sibthorp) in the shade (Kenna, 2006; Duple, 2008b). Tall fescue has a good wear tolerance for cool season grasses, but it is

not nearly as wear tolerant as bermudagrass (Romero and Dukes 2009). Due to this, its use in the southeast and North Carolina is limited (Schardl and Leuchtman 2005).

Zoysiagrass (*Zoysia japonica* Steudel) was introduced from Asia (Mohlenbrock 1967). It is warm-season grass that spreads by rhizomes and stolons to produce a very dense, wear-resistant turf (Mohlenbrock 1967). It is best adapted to the Piedmont and Coastal Plain regions of North Carolina, but some of the more cold tolerant cultivars can be grown in the western part of the state as well (Schardl and Leuchtman 2005). The requirements for maintaining Zoysiagrass are similar to other turfgrasses, but it does not tolerate complete shade and heavy compaction (Mohlenbrock 1967). It is also susceptible to damage by Japanese beetles, mole crickets (*Scapteriscus borellii* and *S. vicinus*) and the hunting billbug (*Sphenophorus venatus vestitu*) (Schardl and Leuchtman 2005).

The focus of our research is St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, which will be discussed in greater detail in section 1.2.

1.2 *St. Augustinegrass*

St. Augustinegrass is a warm-season grass believed to have originated in Africa (Kenna, 2006) or from both, the Gulf of Mexico and the Mediterranean (McCarty and Cisar, 1997). It is popular in tropical and subtropical climatic regions (Sauer 1972) and it grows vigorously in the southern U.S. during the warm (80 to 95 °F) months of late spring, summer, and early fall. In fact, it is called a seashore pioneer plant due to its expansive nature (Trenholm et al 2005). St. Augustinegrass is increasing in popularity due to its texture, aesthetically pleasing appearance and shade tolerance (Winstead and Ward 1974; Trenholm and Nagata 2005).

Like the other warm-season grasses, it goes dormant and turns brown in the winter (McCarty

and Cisar, 1997). It is very susceptible to winter injury and cannot be grown as far north as bermudagrass and zoysiagrass (Romero and Dukes 2009). Due to its susceptibility to cold temperatures, St. Augustinegrass' range is currently limited to the coast of North Carolina. However, increasing temperature trends due to the global warming effects are allowing range expansion of this subtropical plant further inland and north (Thompson et al 1998; McCarty 2002; Visser 2008; Romero and Dukes 2009). This phenomenon has the potential to make warm-season turfgrasses more appealing to a continually-growing U.S. market. Moreover, the NCSU turfgrass breeding program, headed by Dr. Susana Milla-Lewis, has an active research program focused on identification of St. Augustine germplasm adapted to cooler climates, which include all lines selected for this research project. Therefore, St Augustinegrass has potential to be widely grown in the piedmont region of NC in a not-so distant future (Romero and Dukes 2009) and possibly other temperate areas. On the other hand, St. Augustinegrass is fast growing which makes it prone to thatch (layer of dead plant material found between the green and the soil) accumulation (Horn et al. 1973), which is conducive to insect and disease problems (Haygood 1990). Among insect pests, the southern chinch bug (SCB), *Blissus insularis* Barber is particularly injurious. Thatch provides a cover for pest species to remain undetected, allowing damage build-up and forcing land owners to take curative actions (Horn et al. 1973). Even further, the thatch acts like a shelter to shield pest species from insecticides, which decreases the effectiveness of the chemical treatments, and permits low-dose exposure and that makes a resistance to chemicals a possible outcome (Haygood 1990).

The SCB displays a dietary preference for St. Augustinegrass, making this insect pest the second most expensive plant feeding arthropod in Florida (Cherry and Nagata 1997, Kerr 1966), and a serious economic threat to the entire southeastern United States. In fact, SCB caused damage estimated at \$1,500,000 of turfgrass in Georgia alone in 2006, (Oetting et al 2006). Chemical applications often fail to reach their target due to the cryptic habitat of SCB, and constant insecticide applications also result in suppression of natural enemy populations (Potter 1994). Moreover, SCB are notoriously adept at evolving counter-adaptations to chemical controls (Nagata and Cherry 1999).

2. Chinch Bugs, *Blissus* spp.

2.1 Biology

The common name of chinch bugs is used for a complex of 15 *Blissus* species in the Blissidae family within the order Heteroptera. There are four species of chinch bugs commonly associated with turfgrass in North America: the true or common chinch bug (*Blissus leucopterus leucopterus* Say); the hairy chinch bug (*Blissus leucopterus hirtus* Montandon), the western or buffalograss chinch bug (*Blissus occiduus* Barber) and the southern chinch bug (*Blissus insularis* Barber). These four species are difficult to distinguish morphologically in the field and require microscopic examination for proper identification. SCB, referred in historical text as *Blissus leucopterus* var. *insularis*, are recognized from other *Blissus leucopterus* subspecies as being both shorter and narrower with “shorter terminal antennal segment, pronotal color and villosity and color differences in the hemelytral veins and apical spots of the corium” (Barber 1918).

Overall physical characteristics for the three species are similar. Adult chinch bugs are roughly 3 to 5 mm in length and black with white markings on the wings. All species of chinch bugs have wings resting flat dorsally and with a black spot between the wings (Kerr 1966). Adults are quick, and may be either long- or short-winged, which is determined by heritability, population density and/or host plant condition (Komblas 1962; Cherry 2001).

Females have a pre-ovipositional period of 5 to 13 days (Wilson 1929). Adult fecundity has been estimated to vary from 105 to 250 eggs per female (Beyer 1924, Kerr 1966). Eggs are approximately 1 mm and oval-shaped with a blunt end from which four small projections extend (Stacy 2001). Eggs are first pale white in color, subsequently turn amber, and eventually red before hatching (Kerr 1966). Chinch bugs undergo five nymphal instars, ranging in size from 1 to 3 mm. Chinch bug nymphs take approximately 28 to 30 days to mature (Wilson 1929; Kuitert and Nutter 1952; Eden and Self 1960). The generation time has been estimated to be 45 to 56 d (Wilson 1929; Kuitert and Nutter 1952; Eden and Self 1960). The first two nymphal stages are red, with a white band across on the dorsal side of the abdominal region, while the third and fourth stages are dark orange and turn dark brown with age. The fourth nymphal stage has visible wing pads (Komblas 1962). The fifth nymphal stage is black with well-developed wing pads (Kerr 1966).

Chinch bugs have piercing-sucking mouthparts and feed on the phloem of grass plants within meristem tissue (Painter 1928). In doing so, chinch bugs deposit their salivary sheaths in the plant tissue at the site of feeding (Backus 1988). They normally reside in the thatch area of the turfgrass stand and prefer to feed on the lower leaf sheaths and crown area of the plant (Anderson et al. 2006).

2.2 Host preference and geographical range

Blissus spp. are widely dispersed in North America, and have overlapping geographic distributions. The true chinch bug or common chinch bug (*Blissus leucopterus leucopterus* Say, 1832), feeds on plants belonging to the grass family; both wild and cultivated, such as wheat, rye, barley, oats, and corn. The common chinch bug is widely distributed across the east coast and western plains of the United States and south in Mexico (Gulsen et al 2004).

The hairy chinch bug (*Blissus leucopterus hirtus*) feed on most cool-season turfgrasses, such as Kentucky bluegrass, crabgrass, and non-endophytic perennial ryegrass, bentgrasses, and fescues (Dahm et al 1936). The hairy chinch bug can be an occasional pest of zoysiagrass and St. Augustinegrass (Vittum et al. 1999). It is the most common species of chinch bugs in the northeastern US. Its geographical range includes southern regions of the eastern Canadian provinces, parts of the Midwest to Minnesota, and into the Mid-Atlantic states as far south as Virginia (Leonard 1968) in the US.

The Western chinch bug (*Blissus occiduus* Barber), also occasionally referred to as the buffalograss chinch bug, feeds preferentially on buffalograss but has recently been deemed as a pest on zoysiagrass areas of southeastern Nebraska (Vittum et al. 1999; Eickhoff et al. 2006). This species was first detected infesting a buffalograss lawn in Lincoln, Nebraska, in 1989 (Baxendale et al. 1999), and has since spread throughout the great American prairie region including Arizona, California, Colorado, Kansas, Montana, Nebraska, New Mexico, and Oklahoma, as well as, regions of Canada (Baxendale et al. 1999).

The southern chinch bug SCB (*Blissus insularis* Barber) exhibits high preference for St. Augustinegrass, but can be also pose problems on centipedegrass, zoysiagrass, bahiagrass, torpedograss, pangolagrass, bermudagrass, and sudangrass (Brandenburg and Villani 1995; Sweet 2000). SCB is found throughout the Gulf States, from Texas and Eastern region from Florida to North Carolina (Henry and Froeschner 1988; Sweet 2000).

2.3 Chemical Control

Application of chemical pesticides is the most prevalent form of pest control in conventional agricultural production and is often a cost efficient way of providing arthropod suppression in these systems (Kerr 1956; Reinert 1982; Cherry and Nagata 2005). Management for the SCB has historically relied on insecticides such as tobacco dust, calcium cyanide, nicotine sulfate, DDT, parathion, dieldrin, aldrin, chlordane, chlorpyrifos, and diazinon (Watson and Bratley 1929; Kelsheimer 1952; Wolfenbarger 1953; Kerr 1956). Pyrethroids, such as bifenthrin, cyfluthrin, lambda-cyhalothrin and permethrin, are currently the most common insecticide for *B. insularis* control for home owners and turfgrass professionals. A heavy reliance on chemicals combined with much of the *B. insularis* populations living in mild climates has led to resistance to organophosphates, neonicotinoids and organochlorines (Kerr 1958; 1961; Reinert and Niemczyk 1982; Reinert 1982; Reinert and Portier 1983) and to bifenthrin in Florida (Cherry and Nagata 2005). Lack of rotation with other chemical formulations has also been a major contributor to *B. insularis* developing resistance to chemical pesticides (Potter 1994). Furthermore, warmer climates in the southeast are conducive to faster development, yielding higher SCB generations per year, and in Florida, where there is no insect winter kill, SCB have the opportunity to develop and proliferate

resistant populations. It is estimated that there are approximately 3 generations each year in the upper regions of the southeast, including Georgia, South Carolina and North Carolina (Buss 2010). Environmental conditions in the gulf coast states, including Texas, Alabama, Louisiana, and Mississippi, allow 3-5 generations (Potter 1991), while 7 to 10 generations can develop in southern Florida (Buss 2010). This gives southerly SCB populations more opportunities to develop and spread resistance to chemical management alternatives. Biological control, host-plant resistance, and cultural control provide some alternatives to chemicals management of SCB.

2.4 Biological Control

Biological control is a method of controlling pests using other living organisms (Murdoch et al 1985; Rosenheim et al 1995). Classical biological control relies on arthropod predators for suppression of pest populations (Huffaker and Messenger 1976; Murdoch et al 1985); however, in recent years development of commercial microorganisms, cultural controls, and breeding for host plant resistance have become successful alternatives to chemical applications.

I. Natural Enemies

Southern chinch bugs are prey items for a number of predatory insects (Kerr 1966; Reinert 1978) such as the big-eyed bug, parasitic wasps, such as the egg parasitoid *Eumicrosoma benefica* Gahan, the larval stage of a tachinid, *Phoranthia occidentis* Walker, the striped earwig, *Labidura riparia* Pallas, minute pirate bugs, *Orius* spp., spiders, coccinellids, lacewing, *Chrysopa plorabunda*, ants, *Lasius flavusnearcticus*, *Lasiusniger* (L.), *Formica fusca subsericea* Say, and two species of anthocorids: *Xylocoris vicarius* Reuter and

Lasiochilus pallidulus Reuter (Leonard 1968; Reinert 1978). Big-eyed bugs (*Geocoris* spp.) in the family Lygaeidae are the most abundant and have been named the most effective predator of SCB (Reinert 1978). However because of the extremely low human tolerance for pest damage on turfgrass, chemical pesticides are the first line of defense against turfgrass pests. This heavy reliance on chemicals against SCB unfortunately suppresses their natural enemy populations (Potter 1994), severely limiting their potential contributions to SCB suppression.

II. Microorganisms

Microbial pesticides contain pathogenic microbes (e.g., a bacteria, fungi, viruses or protozoans) as the active ingredient (EPA 2012). Some formulations are commercially available to treat for chinch bug infestations and other turf pests. For example, *Beauveria bassiana*, *Sporotrichum globuliferum*, and *Empusa aphidis* are species of entomopathogenic fungi capable of causing mortality in many insect species, including SCB (Leonard 1968; Krueger et al 1992). *Beauveria bassiana* is a naturally occurring entomopathogen that thrives under humid conditions, and leads to increased reproduction rates of the fungus (Ramosky 1984). *Beauveria bassiana* has been shown to be an effective control method under laboratory conditions (Ramosky 1984); however, commercial formulations, such as Mycotrol®, Botanigard®22WP, and Naturalis®TNO, have been reported to have a variable chinch bug control rates due to their feeding habits (Ramoska and Todd 1985). Research indicates that *Beauveria bassiana* formulations reduce mole cricket, another soil bound turf pest, activity and damage (Thompson and Brandenburg 2005). However, mole crickets have chewing mouthparts, whereas chinch bugs have piercing-

sucking mouthparts (Anderson et al. 2006), which can make foliar applications much less effective, and could explain the lack of field efficacy of these and other microbial formulations (Wright 1962).

2.5 Cultural Control

Cultural control is the practice of modifying the environment and plant physiology to reduce the prevalence of unwanted pests by exploiting pests vulnerabilities (Fife and Graham 1966; Cohen and Berlinger 1986). Cultural practices can have both physiological and mechanical effects on turfgrass. For example, high levels of fertilization, over-watering, and poor mowing practices can cause grasses to build a thick thatch layer which is favorable to SCB outbreaks (Trenholm et al. 2000). Thatch accumulation can foster chinch bug population build up by providing a physical barrier against natural elements and pesticides (Merchant and Mott 2006). Keeping thatch to a minimum is, therefore, the first step in controlling SCBs as it also maximizes efficacy of other management tactics. Proper irrigation and limited sunlight exposure promotes establishment and proliferation of fungal insect pathogens. Adoption and deployment of resistant turfgrass varieties is another cultural practice that can substantially reduce SCB outbreaks and will be discussed in detail in the following section.

2.6 Host Plant Resistance

Host plant resistance includes a range of adaptations evolved by plants which improve their survival and reproduction by reducing the impact of pests and diseases (Hayes 1935; Fleschner 1952; Price 1986). There are three clearly delineated categories of host plant resistance: antixenosis, antibiosis, and tolerance. Antixenosis includes any plant

characteristic that leads to the pest's non-preference for a resistant plant when compared to a susceptible plant (Painter 1951; Kogan and Ortman 1978). Antibiosis are chemical plant properties that can negatively affect the life history of the pest, leading to increased mortality, decreased fecundity, or reduced longevity (Painter 1951). Resistant grass exhibiting tolerance is an acceptable and adequate host for the pest but it can withstand large infestations and can compensate for any damage caused by the noxious organism (Painter 1958).

Deploying new varieties with resistance to the SCB is the most feasible and environmentally friendly management strategy. In fact, deployment of host plant resistance has been proven an effective management tactic for the SCB, which was maintained under control since 'Floritam' St. Augustinegrass was released in 1973 (Horn et al. 1973) and subsequently planted throughout the Southern US (Reinert and Dudeck 1974; Crocker et al. 1982, 1989; Busey and Zaenker 1992). This grass exhibited a high level of insect toxicity (antibiosis) (Reinert and Dudeck, 1974). However, reports of resistance-breaking SCB populations in Floritam fields in Florida (Busey and Center 1987) has renewed interest in identifying additional sources of SCB in St. Augustine germplasm.

Diploid grasses have two sets of chromosomes (one from each parent) and the ability to reproduce under the right circumstances (Phillely et al. 1996). Genotypes with 27 or more chromosomes are referred to as polyploids and are incapable of normal sexual reproduction and the production of viable offspring (Phillely et al. 1996). While both diploid and polyploid St. Augustinegrass germplasm is available, most diploid cultivars are commercially favored because they have narrow leaf blades, which give them a much finer and more aesthetically pleasing texture (Phillely et al. 1996). This has practical significance because future gene

transfer into established cultivars is more efficient if the resistant gene originates from another diploid St. Augustinegrass line (Rangasamy 2008). Transfer of desirable traits from polyploidy germplasm into diploid cultivars can be a difficult, long term process due to hybridization/reproductive constraints (Phillely et al. 1996). Even further, polyploid grasses are generally more expensive due to the process of sterility manipulation and verification of the polyploid chromosome number (Phillely et al. 1996). Therefore, identification of resistance to SCB especially in diploid St. Augustinegrass germplasm is crucial. Doing so, may allow turf breeders to expedite the development of commercially-appealing pest resistant turf lines that can be made available in NC and throughout the Southeastern region. Furthermore, the germplasm selected for this project has already been screened for resistance to the gray leaf spot pathogen, *Pyricularia grisea*, at NC State which will increase the potential for identifying resistance against two of the most devastating pests of St. Augustinegrass in the Southeast.

2.7 Research Goals

The purpose of this project was first to evaluate potential germplasm resistance to SCB in St Augustinegrass. To accomplish this, an experimental group containing 18 plant introductions (PIs) of St. Augustinegrass, along with two PIs (410357 and 36031) of its closely related, cross-breeding species, pombagrass, *S. dimidiatum* (L.) Brongn, (Table 1) was screened for damage responses to SCB feeding. Six reference varieties, three resistant, FX-10, Captiva and ‘Floralawn’, and three susceptible, ‘Seville’, Raleigh (Crocker et al. 1989) and Floratam (Busey and Center 1987)(Table 1), were also included to facilitate damage rating ascription for the test genotypes. The second objective was to determine if germplasm showing a low

damage rating in objective 1 exhibit antibiosis against SCB, which would result in higher mortality and/or slower development of insects. Therefore, antibiosis effects were evaluated by confining ten neonate SCB to individual grass lines to assess survival and development of the insects. The third objective was to determine potential tolerance to SCB feeding in lines exhibiting low damage yet having relatively high number of insects in objective 1. Tolerance was evaluated by subjecting selected PIs and two reference varieties: Seville (susceptible) and Raleigh (resistant/susceptible) according to literature reports, to three (0, 10 and 30) SCB infestation levels for 4 weeks under no-choice scenarios. The fourth and final objective in this study was to identify adult feeding or oviposition deterrence. Adult SCB pairs (male + female) were confined to individual stolons of a single plant line. Survival, oviposition and fecal production were documented weekly and insects were moved to a new stolon of the same host plant for four consecutive weeks.

CHAPTER 2: Investigating St. Augustinegrass resistance against the southern chinch bug, *Blissus insularis* Barber

Abstract

St. Augustinegrass is a warm-season turfgrass with increasing adaptability range in the southeast. However, the southeast has environmental conditions suitable for the southern chinch bug (SCB) outbreaks. This insect is reported as the most destructive pest of St. Augustinegrass. The SCB is very adept at evolving counter-adaptations to chemical controls. The cryptic habitat and fast adaptation – combined with unreliable natural control – renders conventional controls ineffective. Host plant resistance has historically been an effective management tool for SCB. Since 1973, the Floratam St. Augustinegrass variety effectively controlled SCB in the Southeast. However, Floridian and Texan insect populations have now circumvented this resistance. However, deploying new varieties with resistance to the SCB may still be the most feasible and environmentally-friendly management strategy. Yet, the number of cultivars with resistance against SCB is very limited, and their efficacy, climatic adaptability, and aesthetic characters are highly variable. The main focus of this study is the identification of alternative sources of resistance to SCB in previously uncharacterized St. Augustinegrass plant introductions (PIs). PIs exhibited a wide range of responses to SCB feeding, as indicated by damage ratings. Damage ratings for seven PIs grouped with our resistant reference varieties. Moreover, nine PIs exhibited antibiosis, based on low survival and slower development of SCB neonates, when compared to our susceptible reference varieties. In addition, we also determined that three of the tested PIs display tolerance to SCB feeding, as indicated by their high functional plant loss index (FPLI), a formula used to measure tolerance that is based on the plant dry weight loss and damaged ratings due to

insect feeding, even at the highest infestation level tested in this study. Altogether our study has produced strong support to indicate these St. Augustinegrass germplasm are good candidates for future SCB resistant breeding in this species of turfgrass. However further studies need to be performed to map the resistance genes to further expedite transfer of desirable traits to commercially appealing varieties.

1. Introduction

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, performs better under a wider range of soil conditions than most warm-season grasses. This is especially true in sandy coastal areas where Zoysia and Bermuda grass perform poorly due to infestations by sting nematodes. Among warm-season turfgrasses, St Augustinegrass performs well under shade, a valuable trait for use in lawns, particularly in smaller residential landscapes where trees are dominant, as is the case in many neighborhoods throughout North Carolina. Furthermore, St. Augustinegrass has good salt, heat and, to a moderate extent, drought tolerance. All these characteristics, in conjunction with its relatively low input requirements make this species a very desirable turfgrass for the Southeastern US. However, because St. Augustinegrass is fast growing, and prone to thatch accumulation (Horn et al. 1973), which can exacerbate insect and disease problems (Haygood 1990). Among these, the southern chinch bug (SCB), *Blissus insularis* Barber is predominant.

The SCB is arguably the most destructive pest of St. Augustinegrass in the Southern US (Kerr 1966; Vittum et al. 1999) from the East Coast, including North Carolina, to the West Coast and it is a pest wherever this grass is grown worldwide. This insect is considered the single most important pest of St. Augustinegrass, which is its preferred host, but it will also feed on species such as torpedograss, bermudagrass, bahiagrass, centipedegrass, and zoysiagrass (Vittum et al. 1999). Chinch bugs feed by sucking the plant phloem while they inject salivary toxins causing the grass to turn yellow, then brown until the plant dies (Kerr 1966; Reinert 1978).

Historically, numerous insecticides have been used for killing chinch bugs, but the insect has managed to adapt by developing resistance. Additionally, growing concerns over the persistent use of chemicals and the potential negative side environmental effects has spiked interest in the development of alternative management tactics, including the use of SCB resistant germplasm. Host plant resistance has been proven an effective management alternative for the SCB, which was maintained at bay since Floratam St. Augustinegrass was released in 1973 and subsequently planted throughout the Southern US (Reinert and Dudeck 1974; Crocker et al. 1989; Busey and Zaenker 1992). This grass variety exhibited a high level of insect toxicity (Reinert and Dudeck 1974, Crocker et al. 1989). However, decreased resistance in Floratam to FL and TX SCB populations has renewed interest in screening germplasm for breeding resistance to the SCB in this turfgrass species (Busey and Center 1987). Recent research efforts have identified new sources of St. Augustinegrass resistant to *B. insularis*, including FX-10, which is thought to be resistant to both *B. insularis* populations (Busey 1990, 1993).

2. Materials and Methods

2.1 Plant material

St. Augustinegrass germplasm used in this study included three varieties with documented varying resistance levels to SCB: FX-10, Captiva, and Floralawn and three SCB-susceptible varieties: Floratam, Raleigh, and Seville. These varieties were chosen to serve as points of reference for ascribing resistance/susceptibility scores to the new germplasm lines included in this study. The experimental germplasm group included the above-mentioned reference

varieties along with 20 PIs, 18 *S. secundatum* and 2 *S. dimidiatum*. St. Augustinegrass reference varieties were obtained from the North Carolina State University (NCSU Raleigh, NC) turfgrass breeding program's germplasm collection. All plant introductions (PIs) were originally obtained from the USDA Plant Germplasm System (Griffin, GA).

Individual grass lines were planted using vegetative material (stolons) from established mother plants, maintained under the same conditions experimental plants were grown (described below). Single stolons were clipped to contain 3 nodes each (13-17cm long) (Fig 1) and were stimulated to produce roots by submersing the distal ends in a cup of water. Water was changed every other day until a tap root had developed (approx. 7-10 days). Once a tap root was established, the stolon was transplanted as described for each of the experiments below. Grass lines were trimmed to a height of 8 cm approximately every two weeks, to simulate mowing conditions. All grass was grown in Fafard Growing Mix no. 2 (Conrad Farfard Inc., Agawam, MA, USA). Fertilizer used in throughout this study was southern turf builder lawn fertilizer (2% iron, 32-0 -10 N-P-K; Scotts Company Marysville, OH). All plant material and experiments were maintained under greenhouse conditions in Raleigh, North Carolina ($28\pm 5^{\circ}\text{C}$), and under natural light with an approximate 14:10 L:D cycle.

2.2 Chinch rearing procedure

All SCB used for experiments were offspring from adult SCB collected from infested residential properties in Wilmington, NC. The SCB colony was maintained by infesting new St. Augustinegrass (Seville) flats (15.25 cm x 20.32 cm) with 40-70 adults transferred from older infested flats every four weeks. Experimental colonies were kept under greenhouse

conditions as described in the previous section. To maintain genetic diversity, approximately 300-400 field-collected late instar and adult SCB were added to the greenhouse colonies 3 to 4 times each summer.

2.3 Germplasm damage ratings in response to SCB feeding (Choice)

The purpose of this experiment was to compare plant damage when exposed to SCB feeding under a free choice-scenario for six weeks. This also served as a proxy for assessing possible negative impacts on insect feeding preference (antixenosis), based on damage ratings exhibited by each plant line. Test PIs and corresponding reference susceptible and resistant varieties were planted individually into nine cm-diameter pots and grown for six weeks before being used for the experiment. Growing conditions and maintenance throughout the experiment were as described above in section 2.1. One pot of each plant genotype was randomly assigned a place within a 1-m² PVC frame arena covered with a chiffon mesh sleeve. These arenas were used to prevent accidental infestations of experimental plants prior to experiment setup and to contain SCB to test plants after infestation. Pots within each arena were placed so rims were in contact with each other to facilitate SCB movement among grass germplasm (Fig 2). A total of 260 SCB (10/plant line) mixed 5th instar and adults were collected from greenhouse colonies into a 50 ml conical centrifuge tube. The tube containing the insects was affixed to the terminal end of a 22.8 cm long bamboo stake inserted into the soil of a pot in the center of the cage and above plant canopies (Fig 2). Insects were allowed to exit the tube, settle and feed freely on the plants for four consecutive weeks. On week six, each of the plant genotypes was rated on a scale of one to five, as described by Heng-Moss (2002). Damage was assessed twice within this week, with a three-day period between each

damage assessment. Values obtained for each line were averaged to obtain a mean damage rating per plant line and these values were used for data analysis. In addition, number of immature, adult, and total (immature + adult) insects remaining on each of the lines at the end of the experiment were recorded. To accomplish this, plant foliage and a surface layer of 3.81 cm of soil were collected and placed into individual paper bags (14 × 21 × 45 cm W × L × H). Pieces of fresh sweet corn ears (8-10 cm long), obtained from a local grocery store, were placed within each bag to lure insects away from plant material. Insects on the corn pieces were collected every two days and fresh corn pieces were provided over a two-week period, when no more insects were recovered. The experiment was set up as a complete random block design with 8 replicates over 3 trials containing either 3 or 2 blocks each, conducted from August of 2011 to September of 2012.

2.4 Neonate performance experiment on selected St. Augustinegrass lines (No Choice)

This experiment was designed to assess performance by neonate SCB on selected PIs under no-choice conditions to test for potential antibiosis factors. In total, 18 genotypes (of the original 26) were selected for this experiment, including five reference varieties (Seville, Raleigh, Floratam, Captiva, and FX-10) and 13 experimental PIs. The experimental PIs were selected based on their low damage ratings in the previous experiment. Terracotta pots (16 cm diameter) were planted with four stolons from individual germplasm lines, as described above. Planting, growing conditions and maintenance throughout the experiment were as described above in section 2.1. Individual lines were individually infested at four weeks with 10 neonates (<48 h) SCB under greenhouse conditions. Insects were allowed to feed and develop on each line for four weeks. Each pot was enveloped with an organza sleeve, which

was tied at the top and fitted snugly around the pot with a large rubber band (Fig 3). At the end of the experiment, insects remaining on each of the lines were collected by aspiration, counted, sexed, and segregated by developmental stage to assess their performance among the tested germplasm. Ten replicates of this experiment were deployed in a complete randomized block design over 5 trials of 2 replicates each, obtained from June 2012 through March 2013.

2.5 Adult oviposition and fecal output on selected plant lines

This experiment was designed to determine feeding and oviposition by adult SCB under no-choice scenarios. The same St. Augustinegrass genotypes used in the neonate performance experiment were used for this experiment. Eight stolons of each genotype were planted into 16 cm pots and maintained as described in section 2.1. Methods described by Rangasamy (2008) were used to obtain adults of similar age. Briefly, fifth instar nymphs were selected from the experimental colony and then provided with fresh Seville grass clippings. Insects that molted to the adult stage within a five-day period were selected for the experiment. Insect couples were confined to individual stolons using a petri dish cage (Fig 4). After each weekly interval, insect survival was recorded and live insects were transferred gently using a mouth aspirator to a new stolon on the same plant. This procedure was repeated for four consecutive weeks. The experiment was set up in singlet or duplicates and repeated over time to obtain a total of 6 replicates from July 2012 until May 2013. Male and female survival, number of eggs and number of fecal spots were recorded weekly to compare across genotypes.

2.6 Evaluation of Tolerance to SCB Feeding in Selected Germplasm

This experiment was designed to evaluate the performance of selected PIs in response to feeding by varying densities of SCB. Seven genotypes were screened for tolerance in the spring of 2013 (Seville, Raleigh, PI 212293, PI 410360, PI 410361, PI 5090338, and PI 509039). The five PIs were selected based on the comparatively high number of insects recovered from them and low damage rating observed in the first experiment. Four rooted stolons were planted per pot (16 cm diameter), with each stolon containing precisely three nodes and trimmed to approximately 8 cm every 2 weeks to standardize aerial plant tissue available to the insects, and to mimic standard growing conditions. Growing conditions and maintenance throughout the experiment were as described above in section 2.1. Three pots of each genotype were planted per replicate to be exposed to 0, 10, or 30 adult SCB for four weeks. At the end of the four weeks, damage ratings and survival of males and females insects were recorded to compare across grass lines. Plants were unearthed, collected, thoroughly washed to remove soil from roots, air dried for 3 h, and then placed within paper bags into an oven at 75°C for 60 h, to obtain dry weights. Functional Plant Loss Index (FPLI) was estimated for each grass genotype as described by Panda and Heinrichs (1983). The experiment was a complete randomized block design with 7 replicates occurring over 3 trials of two replicates each and one with a single replicate obtained from February to April of 2013.

2.7 Statistical Analysis

To test the effect of block, replicate and plant genotype on damage rating, number of immatures, number of adults and total number of insects, data for germplasm damage ratings in response to SCB feeding were analyzed using PROC Glimmix (SAS Institute, 2009) followed by Tukey-Kramer test for multiple comparisons, at a significance level of $P \geq 0.05$. Data for the neonate performance experiment (no-choice) were also analyzed using Proc Glimmix as above. The effect of block, replicate and plant genotype for the neonate performance experiment on selected St. Augustinegrass lines on number of immatures, gender, and total number of insects were evaluated. Analysis for the adult feeding and oviposition experiment was conducted using a repeated measures analysis of variance (ANOVA) using PROC GLM (SAS Institute, 2009) to test for the effects of plant genotype on number of eggs, number of fecal spots, and surviving male and female over the four weeks. Data for the evaluation of tolerance was analyzed with ANOVA using PROC GLM (SAS Institute, 2009) to test for the effects of plant genotype, infestation level and their interaction on damage rating, number of recovered insects, dry weight of plant material, and FPLI.

1. Results

3.1 Germplasm damage ratings in response to SCB feeding (choice)

Effects of block and replicate on mean damage rating, number of immature, number of adult and total number of insects were not found significant. On the other hand, plant genotype had a significant effect on damage rating ($F = 16.43$; $df = 24, 78$; $P < 0.0001$) (Fig 5). All PIs tested showed varying degrees of damage and insect numbers by the end of the study (Figure

5). These results indicate that no antixenosis is present in the experimental germplasm tested. Interestingly, damage ratings for PI 509039, PI 365031, PI 300130, PI 300129, PI 509038, PI 291594, and PI 290888 statistically grouped with the resistant reference varieties. Two of the resistant plant introductions were diploid (PI 509038 and PI 509039).

Plant genotype was also found to significantly impact on the number of immatures ($F=33.13$; $df = 25, 175$; $P < 0.0001$), number of adults ($F =14.53$; $df = 25, 175$; $P < 0.0001$), and total number of insects ($F =14.88$; $df = 25, 175$; $P < 0.0001$) remaining on each of the lines at the end of the experiments (Table 1). Number of immatures, adults, and total insects on all PIs was significantly lower than those on our susceptible reference variety, Seville (table 1). Not surprisingly, Seville yielded significantly higher numbers of immature and total insects compared to all other genotypes (Table 1), starkly contrasting with numbers yielded by FX10, our most resistant reference variety. Interestingly, number of adults did not differ significantly among Seville and PI 410361 yet, number of immatures on eight PIs were statistically similar to FX10 (Table 1).

The seven identified resistant PIs, based on these results, were selected for further antibiosis resistance characterization.

3.2 Neonate performance experiment on selected St. Augustinegrass lines (No Choice)

Effects of block and replicate on gender, number of immatures, and total surviving insects were not found significant. However, plant genotype (PID) had a significant effect on number of surviving insects ($F = 12.87$; $df = 25, 175$; $p < 0.0001$) and immatures ($F =7.94$; $df = 11, 86$; $P < 0.0001$) (Fig 6), but not gender (data not shown $F:M = 0.82: 0.78$). Insect performance was null on 4 experimental PIs, PI 289729, PI 291594, PI 300129 and PI

365031 (Fig 6), which was comparable to results obtained from our most resistant reference variety, FX10. Moreover, five additional PIs, PI 290888, PI 300130, PI 600734, PI 647924 and PI 647925, suppressed SCB development to levels equivalent to our second most resistant reference variety, Captiva (Fig 6). These nine PIs are therefore considered to possess antibiosis resistance factors. Two of these, PI 600734 and PI 647924, are diploid, which makes them desirable for commercial development, if deemed acceptable, or for future resistance breeding efforts.

3.3 Adult oviposition and fecal output on selected plant lines

Number of eggs was significantly affected by plant genotype on weeks 1 ($F=22.19$; $df=17$, 90; $p<0.0001$), 2 ($F=27.2$; $df=17$, 90; $p<0.0001$), 3 ($F=31.39$; $df=17$, 90; $p<0.0001$), and 4 ($F=25.86$; $df=17$, 90; $p<0.0001$) (Table 2). Our susceptible reference variety, Seville, had significantly higher number of eggs compared to all other genotypes over the four weeks (Table 2). Insects oviposited on all plant lines and varieties tested, albeit to varying degrees starting on week one and throughout the experiment (Table 2). Eight of the test PIs showed oviposition levels comparable to those of the resistant reference varieties FX10 and Captiva (Table 2). Four additional PIs showed intermediate oviposition levels comparable to those of Raleigh (Table 2). On week one, insects oviposited on all genotypes with the highest number of eggs on Seville, and the lowest number of eggs on PI 365031, which was not significantly different from the resistant reference lines, FX-10 and Captiva (Table 2). The number of eggs oviposited across all genotypes during week 2 tended to drop for most genotypes compared to week 1, except for Seville, PI 410361, PI 509038, 509039, and Raleigh (Table 2). Egg numbers maintained a similar pattern for week 3 (Table 2). By the final week, only Seville

and PI 410361 continued to yield high numbers of eggs compared to other germplasm (Table 2). FX-10, PI 291594, PI 300129, PI 300130, PI 365031, PI 600734, PI 647924, Floratam and Captiva initially received low number of eggs but by week four experienced no oviposition at all (Table 2).

Fecal output (a proxy for insect feeding) was observed on all tested genotypes, which indicates insects probed and ingested plant phloem. However, number of fecal spots was significantly affected by plant genotype for week 1 ($F = 66.12$; $df = 17, 90$; $p < 0.0001$), week 2 ($F = 34.98$; $df = 17, 90$; $p < 0.0001$), week 3 ($F = 52.02$; $df = 17, 90$; $p < 0.0001$), and week 4 ($F = 32.53$; $df = 17, 90$; $p < 0.0001$) (Table 2). During week one, feeding was significantly suppressed in all but two of the experimental PIs, PI 410361 and PI 509038, when compared to our most susceptible reference variety, Seville (Table 2). There was a significant reduction in feeding among seven experimental PIs, PI 289729, PI 291594, PI 300129, PI 300130, PI 325031, PI 647924 and PI 647925, to levels below or equivalent to our most resistant reference varieties, FX 10 and Captiva (Table 2). Similar to the first week, during week two SCB fecal output on PIs 410361 and 509038 were not significantly different from Seville (Table 2). Remarkably, four PIs, PI 289729, PI 300130, PI 325031, and PI 647924, had levels equivalent or below to FX 10 and Captiva. In addition to the latter PIs, SCB fecal output for PI 212293 also grouped with that of Seville (Table 2). Four PIs, PI 212293, PI 410361, PI 509038, and PI 509039 consistently displayed feeding performances equivalent to those of susceptible Seville (Table 2). Two PIs, PI 289729 and PI 365031, showed feeding performances equivalent to those of FX 10 and Captiva throughout the four weeks (Table 2), confirming them as unsuitable hosts for adult SCB.

Male percent survival did not differ across plant genotype during the first two weeks, however, on week 3 Seville as well as PI 212293, PI 290888, PI 410361, PI 509038, PI 509039, and PI 600734, had significantly higher survival compared to PI 365031 and FX10 (Table 3) . On week 4, Seville, PI 212293, a PI 290888, PI 410361, PI 509038, and PI 509039 had significantly higher males percent survival compared to FX10, 365031, and 289729 (Table 3). Similar to males, surviving female percent did not differ significantly across genotypes for weeks 1 and 2, but on week 3, FX10 and PI 291594 showed significantly lower female percent survival compared to Seville, PI 290888, PI 410361, PI 509038 and PI 509039 (Table 3). On week 4, Seville, PI 509038, PI 290888, PI 410361, PI 509039 had significantly higher percent female survival compared to FX10, Captiva, PI 300129, PI 291594, PI 365031, and PI 289729 (Table 3).

3.4 Evaluation of Tolerance to SCB Feeding in Selected Germplasm

Damage rating was significantly impacted by infestation level ($F=149.41$; $df=2, 84$; $p<0.0001$), plant genotype ($F=32.52$; $df=6, 84$; $p<0.0001$), and by the interaction between infestation level and plant genotype ($F=6.82$; $df=12, 84$; $p<0.0001$) (Table 4). Damage ratings for susceptible the known susceptible, Seville, PI 410360 and PI 410361 increased with increasing SCB infestation level (Table 4). On the other hand, damage ratings for all other PIs and Raleigh were only slightly or moderately increased with our highest infestation level of 30 SCB (Table 4). At infestation level 10, damage ratings for all tested germplasm were significantly lower than that of the susceptible Seville (Table 4). The lowest damage rating at infestation level 10 were found in 212293, 509038, 509039, and Raleigh (Table 4). At infestation level 30, only PI 410361 had a damage rating that was equivalent to Seville,

while all others were significantly lower (Table 4), yielding damage ratings equivalent to, or lower than the resistant Raleigh (Table 4).

Number of insects recovered from plants at the end of the experiments was significantly impacted by infestation level ($F=9220.24$; $df=2, 84$; $p<0.0001$), plant genotype ($F=143.60$; $df=6,84$; $p<0.0001$), and their interaction ($F= 82.31$; $df= 12,84$; $p< 0.0001$) (Table 4). At infestation level 10, the number of insects recovered from all plant lines was all equivalent to those from Seville, except for PI 212293 and Raleigh (Table 4). When infestation level was increased to 30 insects per plant, all germplasm had high numbers insects, comparable to Seville, except for Raleigh (Table 4).

Dry weight was significantly impacted by infestation level ($F=52.86$; $df= 2,84$; $p< 0.0001$), plant genotype ($F= 14.79$; $df= 6,84$; $p< 0.0001$), and by the interaction between infestation level and plant genotype ($F= 4.05$; $df= 12,84$; $p< 0.0001$). Under non-infested conditions (Infestation level 0), dry weights for PI 212293 and Seville were equivalent to each other, and significantly higher than those of all other genotypes (Table 4). However once infested, dry weight mass was negatively impacted, albeit only slightly noticeable at infestation level 10, but quite markedly for Seville, PI 410360, and PI 410361 (Table 4). On the other hand, dry weight for PI 212293, PI 509038 and PI 509039 were not as negatively affected even at infestation levels of 30 SCB/plant, which may indicate tolerance to insect damage on these lines. Raleigh's dry weight was not as reduced by insect infestation level as that of Seville, but we believe this to be due to lower insect survival on the former plant variety.

Functional Plant Loss Index (FPLI) was significantly impacted by infestation level ($F= 128.84$; $df= 1, 84$; $p< 0.0001$) and plant genotype ($F= 32.44$; $df= 6, 84$; $p< 0.0001$) (Table 4), but not by the interaction between infestation level and plant genotype. FPLI values increased in accordance with infestation level for all genotypes tested (Table 4). At infestation level 10, Seville yielded the highest FPLI values compared to all other plant genotypes, followed by PI 410360, PI 410361 and Raleigh. The lowest FPLI values were obtained for PI 509039, PI 509038 and PI 212293. Results for FPLI values at infestation level 30 followed a similar trend except values for PI 410361 were no longer statistically different from Seville and were significantly higher than those for all other genotypes (Table 4). The low FPLI values obtained for PI 509039, PI 509038 and PI 212293 are indicative of tolerance to SCB feeding in these lines (Table 4).

4. Discussion

Host plant resistance has been proven an effective management approach against crop insect pests, including turf pests. This approach is not only economical, but is also environmentally safe, as it significantly reduces reliance on chemical pesticides for pest suppression. Indeed, SCB populations on St. Augustinegrass were kept in check after the release and wide adoption of Floratam in 1973 (Horn et al. 1973, Reinert and Dudeck 1974; Crocker et al. 1982, 1989; Busey and Zaenker 1992). That is, until new SCB population with counter-adaptations to Floratam's resistance emerged in Florida by 1987, which have continued to spread to date (Busey and Zaenker 1992).

To our knowledge, the PIs evaluated herewith, except for PI 410357 (Crocker et al., 1989) and PI 290888, PI 300130, and PI 365031 (*S. dimidiatum*) (Busey 1990), have not

been previously tested for SCB resistance. However, these plant introductions are currently being screened for cold tolerance and resistance to the gray leaf spot pathogen by the NCSU turf breeder responsible for supplying the plant materials for this project, Dr. Susana Milla-Lewis. This collaborative effort has the potential for identifying St. Augustinegrass germplasm that is not only better adapted to northern SE climatic conditions, but may have multiple pest resistance as well. Overall the St. Augustinegrass germplasm evaluated in this study shows great potential as a source of resistance against SCB, a severe pest of turfgrass in the SE US.

Antixenosis is a mechanism of host plant resistance which affects insect preference by possessing characteristics making the plant undesirable or undetectable as a food source for insect pests. In the present study the experimental PIS exhibited a wide range of responses to SCB feeding, as indicated by their damage ratings. In fact, all of these lines yielded damage ratings above 1, which indicates insect feeding; therefore, no antixenosis was detected in this assay. Nonetheless, damage ratings for seven PIs grouped with our resistant reference varieties, which indicates these lines either not as preferred as hosts or were more tolerant to SCB feeding. Thus, these PIs were selected to evaluate immature performance under no-choice scenarios (antibiosis).

Antibiosis is another mechanism of resistance, which affects the development, reproduction, or life span of the insect. Antibiosis was the most significant mechanism of resistant in our tested turfgrass germplasm. Earlier studies have shown Floratam to exhibit antibiosis against SCB (Reinert and Dudeck 1974; Crocker et al. 1989). However, Busey and Center (1987) reported SCB overcoming Floratam resistance in Florida. Results for this

variety in our study yielded moderate damage rating in the choice experiment and the moderate survivorship in the antibiosis experiment, suggesting this variety is suitable to host NC SCB, its susceptibility isn't as high as Seville's. Raleigh has previously been reported as having antibiosis (Anderson et al. 2006) and tolerance (Chong et al. 1990). During our study, this variety was found to display low levels of damage in the antixenosis experiment and results from the antibiosis assay showed this variety to allow moderate immature survival and development (antibiosis). Yet, insect mortality on this variety in the tolerance assay was high, and fecal output and oviposition were significantly reduced. Put together, these results support antibiosis as the major mechanisms of resistance in Raleigh; however, the varying outcomes observed for Raleigh from the various experiments may indicate that the factors conferring antibiosis in this variety may be more effective against adults. Nine experimental PIs, PI 289729, PI 290888, PI 291594, PI 300129, PI 300130, PI 365031 (*S. dimidiatum*), PI 600734, PI 647924 and PI 647925, also exhibited antibiosis, based on low survival and slower development of SCB neonates, when compared to our susceptible reference varieties. Two of these, PI 600734 and PI 647924, showing antibiosis are diploid which may facilitate transfer of resistance genes to commercial varieties.

St. Augustinegrasses germplasm inhibiting or limiting adult feeding and oviposition can be helpful by slowing infestations from growing exponentially over time, effectively reducing pest population size. Adult preference, based on oviposition and fecal output was also significantly affected by plant genotype. Interestingly, NC SCB oviposition on Floratam was markedly low, which contrasts with findings by Rangasamy (2006), who found SCB produced 11 and 5 times more eggs on this variety compared to FX-10 and Captiva,

respectively. Yet, adult oviposition and feeding preference in this study on Floratam were significantly reduced compared to Seville. This concurs with findings by Chong et al. (2009), who conducted a similar SCB oviposition experiment in using chinch bugs from South Carolina (SC). In this experiment, the author released ten SCB adults into a cage arena that isolated a single stolon of each cultivar, which is similar to our experimental design. Similar to our findings, Chong et al. (2009) reports that Floratam significantly suppressed SCB oviposition when compared to Raleigh. Based on our results, we conclude that Floratam has moderate resistance against more northern SCB populations, such as those in NC and SC. Unlike Florida and Texas, Floratam is not widely planted in North Carolina, therefore, the SCB used in this study have not been under the same selection pressure and have not yet developed counter-adaptations to this variety. This performance disparity may suggest genetic differences between Floridian and NC SCB populations due to selective pressure experienced by insects in these two regions. However, this is something that needs to be determined in future research. Oviposition on six PIs, PI 291594, PI 300129, PI 300130, PI 365031 (*S. dimidiatum*), PI 600734, PI 647924, was similar to that obtained on the resistant reference varieties. Busey (1990) also reported significantly suppressed oviposition by both standard and resistance-breaking SCB on 3000130 (*S. secundatum*) and 365031 (*S. dimidiatum*). Moreover, feeding, as assessed by fecal output, on six PIs was equivalent or lower to that observed on the resistant reference varieties, indicating markedly low consumption rates on the various grass lines investigated herein. It is interesting to note that although adult SCB readily fed on most PIs, some even to levels equivalent to those of Seville, oviposition selection was more astringent. Moreover, mortality of females was

higher on some of our test germplasm, indicating gender-biased lethality on some of the lines tested herein.

Tolerant St. Augustinegrass germplasm offers the potential for maintaining green turfgrass throughout the year, in spite of SCB presence, which could greatly reduce pesticide applications. Although Raleigh has previously been shown to exhibit antibiosis (Anderson et al. 2006), it was included in this study because it allowed moderate levels of immature development during the antibiosis experiment, yet had low damage ratings during the choice experiment. During the tolerance experiment, Raleigh had low damage ratings and low FPLI values; however, these can be explained by the high mortality observed at both infestation levels in our tolerance assay. This confirms Raleigh's mechanism of resistance is antibiosis, rather than tolerance, and which appears to be effective against mature SCB stages. It is worth noting that in spite of our results and those of others, previous studies have found Raleigh to be susceptible to SCB (Crocker 1989; Chong et al. 2009; Reinert et al. 2011); however, those assays were conducted using insect populations from Texas. Texas has a history of SCB populations with counter-adaptations to host plant resistance, and this may explain these discrepancies. Chong et al. (2009) inoculated different St. Augustinegrass cultivars with ten SCB nymphs and found Raleigh to allow moderate levels of SCB development yet, yielded lower levels of plant tissue damage. We also determined that three of the tested PIs, PI 212293, PI 509038, and PI 509039, display tolerance to SCB feeding, as indicated by their high FPLI even at the highest infestation level tested in this study. All three are diploid genotypes, which also showed moderate levels of antibiosis during the two different antibiosis experiments. It is important to note that Raleigh had the lowest number of

recovered insects at the end of the experiment, so although its damage ratings, dry weight and FPLIs were not as affected by insect infestation levels, this could be due to insect mortality, which points to antibiosis, and not tolerance, being the mechanism of resistance in this genotype.

Previous research conducted in Florida categorized FX10 and Captiva as having high levels of antibiosis against SCB (Rangasamy et al. 2006). Here we report that these two varieties displayed high levels of antibiosis against NC SCB populations as well. Our results also suggest that North Carolina, may not yet host SCB populations capable of counteracting resistance in commercial varieties such as Raleigh and Floratam, as both these varieties displayed moderate levels of antibiosis resistance against SCB in our study.

Of the 20 plant introductions tested here, nine exhibited antibiosis, three provided evidence of tolerance, and the remainder were suitable SCB hosts. In all, this research identified 12 genotypes displaying some form of resistance, five of which are *S. secundatum* diploids, 212293, 509038, 509039, 600734, 647924, which will be valuable for future turf breeding programs. The resistance mechanisms in PI 600734 and PI 647924 was determined to be antibiosis affecting both immature and adult performance; whereas tolerance to insect feeding was found in PI 212293, PI 509038, and PI 509039. In addition, two polyploid *S. dimidiatum* lines, PI 289729 and PI 365031, were found to have antibiosis activity against both immature and adults as were *S. secundatum* polyploid PIs, PI 291594, PI 300129 and PI 300130.

Results obtained from this study open the door to future more in-depth research to determine the direct market feasibility or breeding potential for the SCB resistant lines. Additional research will be needed to determine the potential for resistance genes from diploid genotypes identified in this study to be transferred directly into commercial varieties using conventional breeding. Prior development of molecular resistance markers will greatly facilitate these breeding efforts. Moreover, resistance genes from polyploid genotypes could be isolated and inserted into commercial varieties using modern molecular techniques.

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APPENDIX

APPENDIX A – Grass Nodes for Planting



Figure 1. Grass was collected by single stolons that were clipped with 3 nodes (13-17cm) and then rooted by submersing the proximal ends in a cup of water. Water was changed every other day until a tap root had developed (approx. 7-10 days). Once a tap root was established, the stolon was planted for experiment.

APPENDIX B – Grass Performance Across Experiments

Species	PID	Ploidy	Choice	Antibiosis	Oviposition/Fecal	Tolerance
<i>S. dimidiatum</i>	212293	2X	MR	MR	MR	HR
<i>S. secundatum</i>	289729	4X	MR	HR	HR	N/A
<i>S. secundatum</i>	290888	4X	HR	HR	HR	N/A
<i>S. secundatum</i>	291594	4X	HR	HR	HR	N/A
<i>S. secundatum</i>	300129	6X	HR	HR	HR	N/A
<i>S. secundatum</i>	300130	4X	HR	HR	HR	N/A
<i>S. dimidiatum</i>	365031	6X	HR	HR	HR	N/A
<i>S. secundatum</i>	410353	2X	S	N/A	N/A	N/A
<i>S. secundatum</i>	410355	2X	S	N/A	N/A	N/A
<i>S. secundatum</i>	410357	2X	S	N/A	N/A	N/A
<i>S. secundatum</i>	410360	2X	S	N/A	N/A	S
<i>S. secundatum</i>	410361	2X	S	S	MR	S
<i>S. secundatum</i>	410363	2X	S	N/A	N/A	N/A
<i>S. secundatum</i>	410364	2X	S	N/A	N/A	N/A
<i>S. secundatum</i>	414079	2X	S	N/A	N/A	N/A
<i>S. secundatum</i>	509038	2X	HR	MR	MR	HR
<i>S. secundatum</i>	509039	2X	HR	MR	MR	HR
<i>S. secundatum</i>	600734	2X	MR	MR	MR	N/A
<i>S. secundatum</i>	647924	2X	MR	HR	HR	N/A
<i>S. secundatum</i>	647925	2X	MR	HR	HR	N/A
<i>S. secundatum</i>	Floralawn	4X	MR	N/A	N/A	N/A
<i>S. secundatum</i>	Floratam	4X	MR	MR	MR	N/A
<i>S. secundatum</i>	FX10	4X	HR	HR	HR	N/A
<i>S. secundatum</i>	Captiva	2X	HR	HR	HR	N/A
<i>S. secundatum</i>	Raleigh	2X	MR	S	MR	MR*
<i>S. secundatum</i>	Seville	2X	S	S	S	S

Table 1. Performance across all experiments by genotype. S = susceptible, HR = highly resistant and MR = moderately resistant, N/A= germplasm not included in assay. These were designated based on statistical groupings from each analysis.

*Although Raleigh’s performance in the tolerance assay suggests tolerance, it is attributed to low damage ratings due to high insect mortality, indicating antibiosis.

APPENDIX C – Interior View of Antixenosis Arena

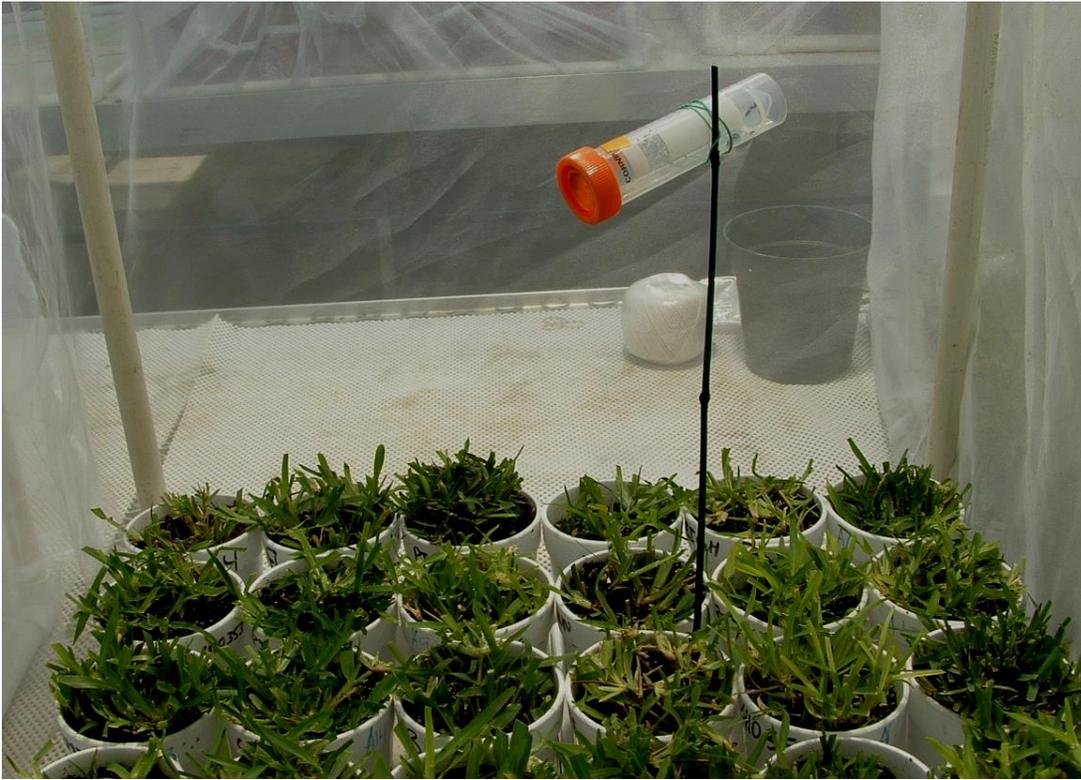


Figure 2. Antixenosis experimental set-up. Twenty-six St. Augustinegrass genotypes were randomly placed within a 1 m² cage. Each arena was infested with 260 mixed adult and fifth instar SCB. SCB were allowed to feed and reproduce for 4 weeks. Damage for each of the lines was recorded and compared.

APPENDIX D – Antibiosis Arenas



Figure 3. Antibiosis, choice experiment set-up. Ten neonate nymphs (<48h) were confined to individual plants using organza mesh sleeves held upright over the plant canopy by an electrical wire loop. Insects were allowed to settle and feed on the plants for four weeks. At the end of the experiments, number of adults and nymphs on each of the lines were counted and recorded.

APPENDIX E – Ovipositions and Fecal Arena



Figure 4. Oviposition experiment set-up. One male and one female were restricted to a single stolon for one week at a time for four consecutive weeks. Aluminum foil was used in the arena to facilitate recording of fecal spots. Excretory spots and eggs were counted weekly and averaged over the duration of the experiment.

APPENDIX F – Final Damage Ratings

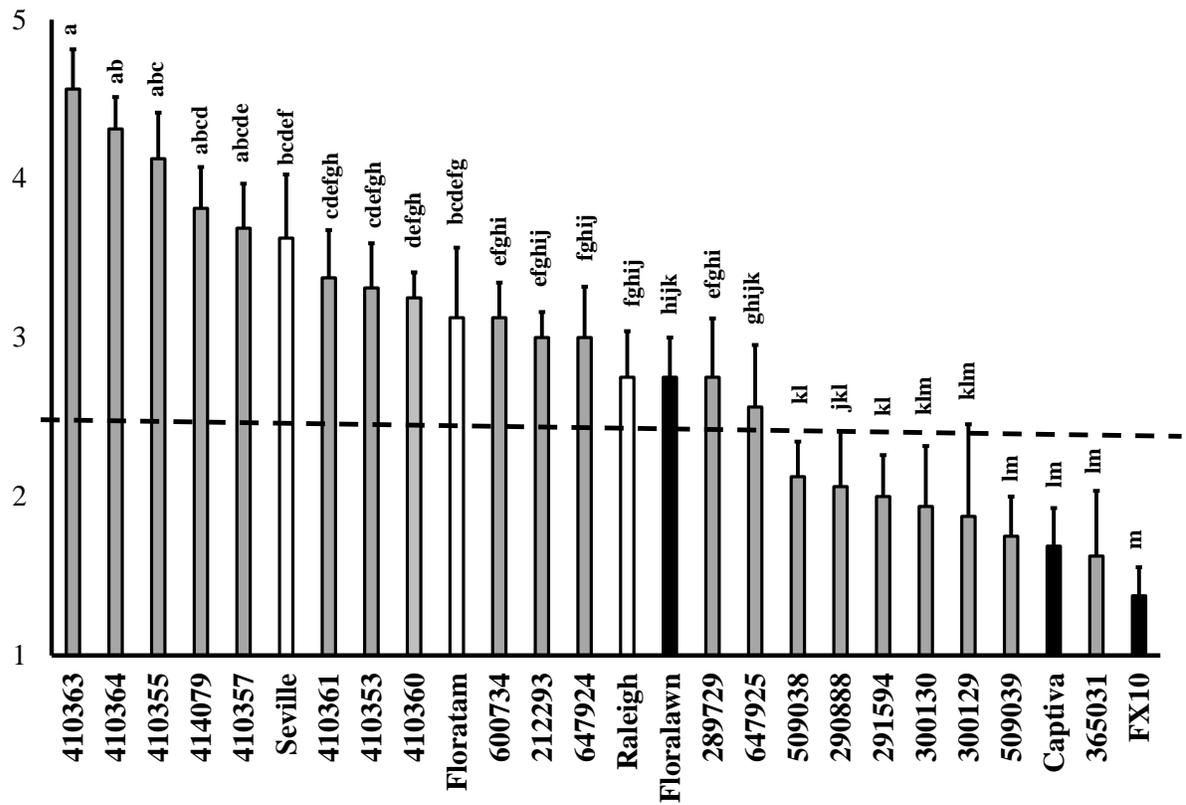


Figure 5. Damage rating obtained after a 6 week period for each of the PIs and reference varieties in the germplasm damage ratings in response to SCB feeding (choice) experiment. SCB susceptible reference varieties are highlighted in white, SCB resistant reference varieties are in black and plant introductions are in gray. Bars represent means for n=8 and error bars=1SE. Bars headed by the same letter are not statistically different (Tukey’s mean separation test $P \geq 0.5$).

APPENDIX G – Antixenosis Results

PID	Immatures	Adults	Total
212293	16.1 ± 5.60 bcd	3.7 ± 1.06 bcdef	19.8 ± 6.44 abcdef
289729	3.3 ± 1.61 ijklm	0.8 ± 0.51 efg	4.2 ± 1.91 defg
290888	1.8 ± 0.54 jklm	1.0 ± 0.32 efg	2.8 ± 0.71 defg
291594	0.6 ± 0.49 m	0.5 ± 0.18 g	1.1 ± 0.58 g
300129	1.3 ± 0.98 jklm	0.7 ± 0.36 fg	2.1 ± 1.28 efg
300130	1.3 ± 0.53 jklm	0.5 ± 0.26 g	1.8 ± 0.61 efg
365031	1.1 ± 0.98 klm	0.5 ± 0.26 g	1.6 ± 1.20 fg
410353	7.5 ± 1.34 fghi	4.3 ± 1.36 bcd	11.8 ± 2.31 bcdefg
410355	16.1 ± 1.96 bcd	7.8 ± 1.74 ab	23.8 ± 3.15 abc
410357	13.2 ± 2.22 bcdef	4.8 ± 1.27 bcd	18.1 ± 2.59 abcdefg
410360	13.7 ± 3.25 bcde	6.2 ± 2.42 bc	20.0 ± 5.13 abcde
410361	18.3 ± 4.01ab	7.7 ± 2.69 ab	26.1 ± 3.92 ab
410363	10.7 ± 2.87 def	4.1 ± 0.93 bcde	14.8 ± 3.02 abcdefg
410364	13.0 ± 2.60 bcdef	5.8 ± 1.55 bc	18.8 ± 3.14 abcdefg
414079	9.7 ± 3.05 defg	2.2 ± 0.90 cdefg	12.0 ± 3.53 bcdefg
509038	4.7 ± 2.55 ghij	3.1 ± 1.89 cdefg	7.8 ± 3.16 bcdefg
509039	16.8 ± 9.21 bc	4.0 ± 1.62 bcde	20.8 ± 9.81 abcd
600734	0.7 ± 0.52 lm	1.3 ± 0.37 defg	2.1 ± 0.69 efg
647924	3.5 ± 1.62 ijklm	2.5 ± 1.08 cdefg	6.0 ± 1.37 cdefg
647925	4.0 ± 1.78 ijk	2.3 ± 0.53 cdefg	6.3 ± 1.91 cdefg
Floralawn	3.8 ± 1.34 ijkl	3.1 ± 1.36 cdefg	7.0 ± 2.20 cdefg
Floratam	4.5 ± 1.53 hijk	2.3 ± 0.73 cdefg	6.8 ± 2.17 cdefg
FX10	0.5 ± 0.37 m	0.5 ± 0.26 g	1.0 ± 0.59 g
Captiva	4.6 ± 3.11 ghij	0.5 ± 0.18 g	5.1 ± 3.06 defg
Raleigh	9.2 ± 1.14 fgh	4.8 ± 1.05 bcd	14.1 ± 1.65 abcdefg
Seville	26.8 ± 5.35 a	12.7 ± 3.50 a	31.3 ± 5.57 a

Table 2. Mean number of immatures, adults, and total insects on each St. Augustinegrass genotype at the end of the germplasm damage ratings in response to SCB feeding (choice) experiment. The susceptible reference varieties are Seville, Floratam, and Raleigh and the resistant reference varieties are Floralawn, Captiva, and FX10. Values represent means ±SE for 8 replicates per plant genotype. Values within columns followed by the same letter are not statistically different (Tukey’s mean separation test $P \geq 0.5$).

APPENDIX H – Antibiosis Results

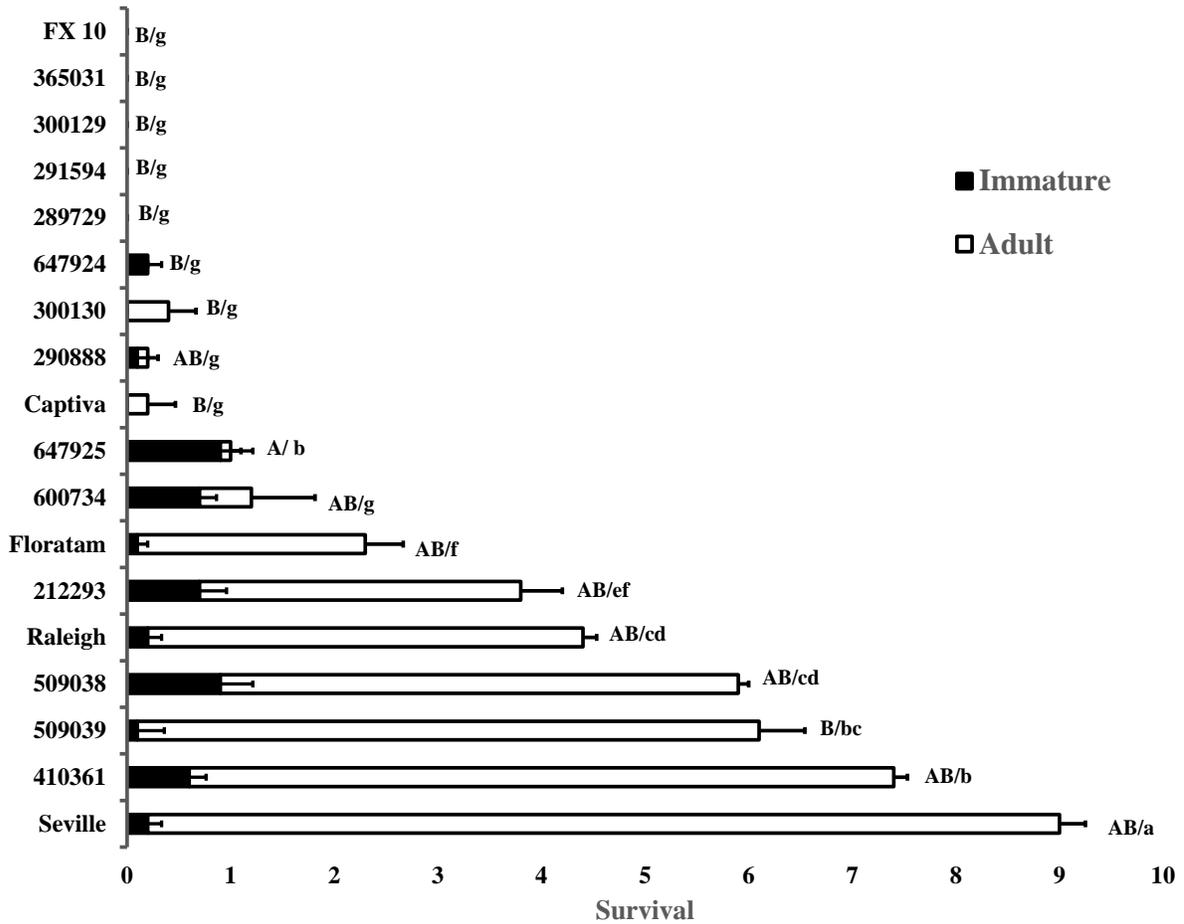


Figure 6. Number immature (dark bars) and adult (light bars) recovered from experimental germplasm at the end of the 4 week period in the neonate performance experiment (No Choice). Susceptible reference varieties are Seville, Floratam, and Raleigh and the resistant reference varieties are Captiva, and FX10. Values represent means for n=9 and error bars=1SE. Bars headed by the same letter are not statistically different (Tukey’s mean separation test $P \geq 0.5$). Capital letters indicate means separation for immatures, and lowercase for adult means separation.

APPENDIX I – Oviposition Results

Table 4. Mean number of eggs by genotype per week obtained from the adult oviposition

Variable	PID	Week1	Week 2	Week 3	Week 4
No. eggs	212293	8.3 ± 1.52 cde	4.5 ± 0.99 def	8.3 ± 1.20 de	8.0 ± 1.34 bc
	289729	4.0 ± 0.57 efg	0.3 ± 0.21 f	1.0 ± 0.44 f	0.0 ± 0.00 e
	290888	3.8 ± 0.87 efg	2.1 ± 0.83 ef	5.0 ± 0.57 def	2.6 ± 0.88 cde
	291594	1.3 ± 0.61 fg	0.0 ± 0.00 f	0.1 ± 0.16 f	0.0 ± 0.00 e
	300129	1.0 ± 0.51 fg	0.0 ± 0.00 f	0.3 ± 0.33 f	0.0 ± 0.00 e
	300130	3.3 ± 0.49 efg	0.6 ± 0.21 ef	1.6 ± 0.66 ef	0.6 ± 0.33 de
	365031	0.6 ± 0.49 g	0.1 ± 0.16 f	0.0 ± 0.00 f	0.0 ± 0.00 e
	410361	16.1 ± 2.02 b	17.6 ± 3.17 b	17.6 ± 2.82 b	14.1 ± 1.92 b
	509038	7.0 ± 1.29 cdef	7.5 ± 1.14 cde	9.8 ± 1.04 cd	12.1 ± 1.49 b
	509039	11.1 ± 1.57 bc	13.1 ± 2.05 bc	15.6 ± 1.31 bc	14.0 ± 1.94 b
	600734	6.0 ± 0.77 cdefg	2.5 ± 0.56 ef	2.5 ± 0.67 ef	0.8 ± 0.40 de
	647924	1.3 ± 0.55 fg	0.0 ± 0.00 f	0.3 ± 0.33 f	0.0 ± 0.00 e
	647925	6.6 ± 1.11 cdefg	3.0 ± 0.25 ef	3.6 ± 0.76 def	8.0 ± 2.00 bc
	Floritam	4.8 ± 0.65 defg	2.3 ± 0.42 ef	3.5 ± 0.76 def	0.8 ± 0.54 de
	FX10	2.0 ± 0.57 fg	0.3 ± 0.21 f	0.0 ± 0.00 f	0.0 ± 0.00 e
	Captiva	5.1 ± 0.94 cdefg	2.8 ± 0.47 ef	1.6 ± 0.76 ef	0.1 ± 0.16 e
	Raleigh	10.6 ± 1.25bcd	11.0 ± 2.16 bcd	9.8 ± 2.05 cd	7.3 ± 2.67 bcd
	Seville	22.5 ± 2.91a	26.3 ± 3.55 a	29.1 ± 3.97 a	23.1 ± 2.95 a

Table 3. Mean number of eggs by genotype per week obtained from the adult oviposition and feeding preference experiment. Values represent mean±SE for 6 replicates per plant genotype. Values within columns followed by the same letter are not statistically different (Tukey-Kramer mean separation test $P \geq 0.5$).

APPENDIX J – Feeding Results

Variable	PID	Week1	Week 2	Week 3	Week 4
No. fecal spots	212293	23.3 ± 0.76 b	24.1 ± 0.74 ab	27.0 ± 1.21 ab	24.0 ± 1.39 ab
	289729	10.5 ± 0.76 fgh	8.1 ± 0.47 fgh	7.8 ± 0.91 fg	5.8 ± 2.42 def
	290888	16.1 ± 0.54 de	15.8 ± 1.32 cd	13.5 ± 0.84 def	17.1 ± 0.70 bc
	291594	9.1 ± 0.40 h	12.0 ± 1.03 defg	10.0 ± 0.63 ef	4.0 ± 1.69 def
	300129	11.0 ± 0.44 fgh	13.3 ± 1.08 def	10.6 ± 0.49 def	3.8 ± 1.01 def
	300130	13.8 ± 0.47 efg	10.6 ± 0.66 efg	13.6 ± 0.76 def	5.5 ± 1.85 def
	365031	9.0 ± 0.57 h	6.0 ± 0.44 h	2.8 ± 0.47 g	1.3 ± 0.80 f
	410361	26.1 ± 0.87 ab	24.0 ± 1.29 ab	27.5 ± 0.84 ab	28.3 ± 0.71 a
	509038	24.6 ± 1.47 ab	19.8 ± 1.11 bc	26.3 ± 1.31 ab	25.3 ± 1.17 ab
	509039	22.8 ± 0.79 ab	21.1 ± 1.30 bc	22.0 ± 0.73 bc	25.1 ± 0.91 ab
	600734	14.6 ± 1.20 def	11.6 ± 0.55 defg	16.1 ± 1.04 cd	10.1 ± 2.15 cde
	647924	8.0 ± 0.73 h	8.3 ± 0.61 fgh	10.5 ± 1.58 def	9.1 ± 1.88 cdef
	647925	11.0 ± 0.81 fgh	14.1 ± 0.94 de	11.0 ± 2.04 def	11.5 ± 2.39 cd
	Floritam	18.8 ± 0.70 cd	17.1 ± 1.70 cd	13.6 ± 2.31 def	11.0 ± 3.51 cde
	FX10	9.8 ± 0.60 gh	7.0 ± 0.73 gh	2.1 ± 0.54 g	0.6 ± 0.49 f
	Captiva	10.8 ± 0.79 fgh	13.6 ± 1.05 def	7.8 ± 1.01 fg	2.6 ± 0.61 ef
	Raleigh	18.3 ± 0.84 d	20.3 ± 1.66 bc	15.6 ± 1.20 de	11.5 ± 2.75 cd
	Seville	28.5 ± 1.17 a	28.1 ± 1.62 a	30.6 ± 1.25 a	30.3 ± 0.95 a

Table 5. Mean number of fecal spots per week per live adult obtained from the adult oviposition and feeding preference experiment.. Values represent mean fecal spots ±SE for 6 replicates per plant genotype. Values within columns followed by the same letter are not statistically different (Tukey-Kramer mean separation test $P \geq 0.5$).

APPENDIX K – Oviposition and Feeding Sex Ratios

Survivors	PID	Week1	Week 2	Week 3	Week 4	
Males	212293	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
	289729	100.0 ± 0.00 a	83.3 ± 16.67 a	50.0 ± 22.36 ab	00.0 ± 0.00 c	
	290888	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
	291594	100.0 ± 0.00 a	83.3 ± 16.67 a	66.6 ± 21.08 ab	66.6 ± 21.08 abc	
	300129	100.0 ± 0.00 a	100.0 ± 0.00 a	83.3 ± 16.67 ab	16.6 ± 16.66 bc	
	300130	100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	33.3 ± 21.08 abc	
	365031	100.0 ± 0.00 a	50.0 ± 22.36 a	16.6 ± 16.66 b	00.0 ± 0.00 c	
	410361	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
	509038	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
	509039	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	83.3 ± 16.67 ab	
	600734	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 abc	
	647924	100.0 ± 0.00 a	100.0 ± 0.00 a	50.0 ± 22.36 ab	50.0 ± 22.36 abc	
	647925	83.3 ± 16.67 ab	66.6 ± 51.64 a	66.6 ± 21.08 ab	66.6 ± 21.08 abc	
	Floratam	100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	50.0 ± 22.36 abc	
	FX10	66.6 ± 21.08 b	66.6 ± 21.08 a	16.6 ± 16.66 b	00.0 ± 0.00 c	
	Captiva	100.0 ± 0.00 a	83.3 ± 16.67 a	50.0 ± 22.36 ab	33.3 ± 21.08 abc	
	Raleigh	100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	50.0 ± 22.36 abc	
	Seville	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
	Females	212293	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	83.3 ± 16.67 ab
		289729	100.0 ± 0.00 a	100.0 ± 0.00 a	83.3 ± 16.67 ab	16.6 ± 16.66 b
290888		100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
291594		83.3 ± 16.67 a	83.3 ± 16.67 a	33.3 ± 21.08 b	33.3 ± 21.08 abc	
300129		100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	33.3 ± 21.08 abc	
300130		100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	66.6 ± 21.08 ab	
365031		100.0 ± 0.00 a	100.0 ± 0.00 a	50.0 ± 22.36ab	16.6 ± 16.66 b	
410361		100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
509038		100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
509039		100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	
600734		100.0 ± 0.00 a	100.0 ± 0.00 a	83.3 ± 16.67 ab	66.6 ± 21.08 ab	
647924		100.0 ± 0.00 a	83.3 ± 16.67 a	83.3 ± 16.67 ab	50.0 ± 22.36 abc	
647925		100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	66.6 ± 21.08 ab	
Floratam		100.0 ± 0.00 a	100.0 ± 0.00 a	83.3 ± 16.67 ab	50.0 ± 22.36 abc	
FX10		100.0 ± 0.00 a	83.3 ± 16.67 a	33.3 ± 21.08b	16.6 ± 16.66 b	
Captiva		100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	16.6 ± 16.66 b	
Raleigh		100.0 ± 0.00 a	100.0 ± 0.00 a	66.6 ± 21.08 ab	50.0 ± 22.36 abc	
Seville		100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	

Table 6. Weekly percent surviving male and female southern chinch bug during the oviposition and feeding preference experiment. Values represent mean ± SE for 6 replicates per plant genotype. Values above columns followed by the same letter are not statistically different (Tukey’s mean separation test $P \geq 0.5$).

APPENDIX L – Tolerance Results

Infestation Level	PID	Damage Rating	Recovered Insects	Dry Weight	FPLI
0	212293	1.0 ± 0.00 a	n/a	4.0 ± 0.45 ab	n/a
	410360	1.0 ± 0.00 a	n/a	2.9 ± 0.30 b	n/a
	410361	1.0 ± 0.00 a	n/a	2.8 ± 0.34 b	n/a
	509038	1.0 ± 0.00 a	n/a	3.4 ± 0.36 b	n/a
	509039	1.0 ± 0.00 a	n/a	3.0 ± 0.28 b	n/a
	Raleigh	1.0 ± 0.00 a	n/a	5.1 ± 0.20 a	n/a
	Seville	1.0 ± 0.00 a	n/a	5.0 ± 0.22 a	n/a
10	212293	1.0 ± 0.00 c	8.4 ± 0.24 b	3.6 ± 0.40 ab	27.8 ± 1.84 c
	410360	2.0 ± 0.00 b	9.8 ± 0.20 a	2.3 ± 0.27 b	51.2 ± 3.48 b
	410361	2.0 ± 0.00 b	9.8 ± 0.20 a	2.3 ± 0.43 b	52.3 ± 4.85 b
	509038	1.0 ± 0.00 c	9.2 ± 0.20 ab	3.0 ± 0.35 ab	28.6 ± 1.57 c
	509039	1.4 ± 0.24 bc	9.2 ± 0.20 ab	2.9 ± 0.35 ab	30.3 ± 5.80 c
	Raleigh	1.2 ± 0.20 c	6.0 ± 0.44 c	4.2 ± 0.35 a	36.4 ± 7.12 bc
	Seville	3.2 ± 0.20 a	9.6 ± 0.24 ab	2.9 ± 0.34 ab	79.7 ± 2.41 a
30	212293	2.4 ± 0.24 c	27 ± 0.31 a	3.1 ± 0.48 a	59.1 ± 5.60 c
	410360	3.1 ± 0.37 bc	29 ± 0.31 a	1.2 ± 0.14 bc	80.8 ± 3.72 ab
	410361	3.8 ± 0.20 ab	29.4 ± 0.60 a	0.7 ± 0.13 c	92.9 ± 2.78 a
	509038	2.2 ± 0.20 c	28.0 ± 0.54 a	2.6 ± 0.34 a	56.2 ± 4.28 c
	509039	2.2 ± 0.20 c	27.4 ± 0.81 a	2.5 ± 0.26 ab	52.5 ± 4.64 c
	Raleigh	1.6 ± 0.40 c	13.8 ± 1.42 b	2.8 ± 0.34 a	61.4 ± 8.12 bc
	Seville	4.4 ± 0.24 a	29 ± 0.31 a	0.8 ± 0.19 c	97.5 ± 1.08 a

Table 7. Data from tolerance experiment. Damage rating, number of recovered insects, dry weight, and FPLI by infestation level. Values represent mean±SE for 5 replicates per plant genotype. Means for genotypes within infestation level followed by the same letter are not statistically different (Tukey’s mean separation test $P \geq 0.5$).