

MECHANISMS OF RESISTANCE TO SOUTHERN CHINCH BUG, *Blissus insularis*
BARBER (HEMIPTERA: BLISSIDAE), IN ST. AUGUSTINEGRASS

BY

MURUGESAN RANGASAMY

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2008

© 2008 Murugesan Rangasamy

To my beloved mother, Thirumathi K. Kaliammal, and father, Thiru C. Rangasamy Gounder

ACKNOWLEDGMENTS

I pay my sublime obeisance to my esteemed parents, wife Priya, and daughter Sudhiksha whose love, sacrifice and moral support helped me to put this tireless venture to a fruitful result.

To express the feelings in my heart, I put on record my deepest gratitude and profoundest indebtedness to my advisors, Dr. Ron Cherry and Dr. Heather McAuslane, for their guidance and making my dissertation worthy of presentation. Their constant attention, patient helping, sense of dedication and search for perfection are underlying factors which will be part of my memory forever. I consider myself fortunate to be their disciple. I am obliged to the members of my advisory committee, Dr. Bala Rathinasabapathi, Dr. Eric Schemlz, Dr. Russell Nagatta, and Dr. Elaine Backus for their support, guidance and valuable suggestions which led to a successful completion of my research and compilation of this dissertation.

I give special thanks to Ms. Debbie Boyd Mr. Alwin Wilson and Ms. Shan Wu, our biological scientists for their technical assistance. I sincerely thank Dr. Marty Marshall for use of his spectrophotometer, Ms. Karen Kelly and Dr. Sharron Mathews for helping with electron microscope, Dr. Louis Jack for his help with the EPG workshop. I duly acknowledge my lab mates, Karla Adesso, Amit Sethi, and Jennifer Mayor, for their critical comments and suggestions on my research and presentations. No appropriate word could be traced in the lexicon to avouch my friends Bhuvana, Chandramohan, James, Karthik, Ravi, Sabari, Subbu, Sureshbabu and others who helped me both concentrate and relax during my PhD program. All may not be mentioned but none is forgotten.

TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS	4
LIST OF TABLES	8
LIST OF FIGURES	9
ABSTRACT	11
 CHAPTER	
1 LITERATURE REVIEW	13
Taxonomy of the Chinch Bugs	13
Host Plants and Feeding Preferences	15
Feeding Habit and Nature of Damage	16
Oviposition and Development	17
Seasonal Wing Polymorphism and Movement	18
Management of Southern Chinch Bugs	19
Cultural Methods	20
Biological Control Agents	20
Chemical Control Methods	21
<i>St. Augustinegrass and Host Plant Resistance against Southern Chinch Bugs</i>	23
Breeding Resistant Varieties against Chinch Bugs, Continuing Missions	26
Refining the Screening Techniques	29
Other Species of Chinch Bugs and Host Plant Resistance	29
Characterization of Resistance against Chinch Bugs in Buffalograss	30
<i>Chinch Bug Resistance in Sorghum</i>	31
Investigating the Mechanisms of Southern Chinch Bug Resistance in <i>St.</i> <i>Augustinegrasses</i>	31
2 CATEGORIES OF RESISTANCE IN <i>ST. AUGUSTINEGRASS</i> LINES TO SOUTHERN CHINCH BUG (HEMIPTERA: BLISSIDAE)	33
Introduction	33
Materials and Methods	35
Plants and Insects	35
Choice Tests	36
No-Choice Tests	37
Ovipositional Studies	38
Developmental Studies	38
Statistical Analysis	39
Results	39
Choice Tests	39
No-Choice Tests	40

	Ovipositional Studies.....	41
	Developmental Studies.....	41
	Discussion.....	42
3	OXIDATIVE RESPONSES OF ST. AUGUSTINEGRASSES CHALLENGED BY SOUTHERN CHINCH BUG, <i>Blissus Insularis</i> BARBER (HEMIPTERA: BLISSIDAE).....	47
	Introduction.....	47
	Materials and Methods	48
	Plants, Insects, Sample Collection.....	48
	Sample Preparation.....	49
	Estimation of Total Protein	50
	Isozyme Profile Studies.....	51
	Statistical Analysis	52
	Results.....	53
	Total Protein Content	53
	Peroxidase Activities.....	53
	Polyphenol Oxidase Activities.....	53
	Lipoxygenase Activities.....	54
	Catalase Activities.....	54
	Isozyme Profile Studies.....	54
	Discussion.....	55
4	QUANTITATIVE ANALYSIS OF FEEDING BEHAVIOR OF SOUTHERN CHINCH BUG, <i>Blissus insularis</i> BARBER (HEMIPTERA: BLISSIDAE), ON RESISTANT AND SUSCEPTIBLE ST. AUGUSTINEGRASSES.....	68
	Introduction.....	68
	Materials and Methods	70
	Plants	70
	Insects.....	70
	Wiring and Acclimating of the Insects.....	70
	EPG Equipment and Data Acquisition.....	71
	Waveform Categories.....	72
	Definition of Non-Sequential Parameters	73
	Statistical Analysis	73
	Results.....	74
	Cohort Level.....	74
	Event Level.....	75
	Probe Level.....	75
	Insect Level	76
	Discussion.....	77

5	ROLE OF LEAF SHEATH LIGNIFICATION AND ANATOMY IN RESISTANCE AGAINST SOUTHERN CHINCH BUG, <i>Blissus insularis</i> BARBER (HEMIPTERA: BLISSIDAE) IN ST. AUGUSTINEGRASS LINES.....	87
	Introduction.....	87
	Materials and Methods	89
	Staining for Salivary Sheaths	90
	Lignin Quantification	91
	Staining for Lignin	91
	Transmission Electron Microscopy	92
	Experimental Design and Statistical Analysis.....	92
	Results.....	93
	Discussion.....	94
6	SUMMARY.....	108
	LIST OF REFERENCES.....	115
	BIOGRAPHICAL SKETCH	134

LIST OF TABLES

<u>Table</u>	<u>page</u>
2-1	Distribution (mean proportion \pm SEM) of adult southern chinch bugs among five St. Augustinegrass lines in a choice situation at varying time intervals after release *44
2-2	Mean number (\pm SEM) of excretory spots deposited per living chinch bug on St. Augustinegrass lines in a no-choice situation at varying time intervals after release45
2-3	Mean number (\pm SEM) of eggs produced per female alive on St. Augustinegrass lines at weekly intervals after release46
4-1	Durations and frequencies of probing parameters of EPG data recorded on four southern chinch bug resistant and susceptible St. Augustinegrass lines.....82
4-2	Total number of major waveform events (TNWE) on each of four southern chinch bug-resistant or -susceptible St. Augustinegrass varieties (n = 22).82
4-3	Mean waveform durations per event (WDE) for major waveforms recorded for southern chinch bug feeding on resistant and susceptible St. Augustinegrasses.83
4-4	Mean duration of major waveforms per probe (WDP) on each of four southern chinch bug-resistant or -susceptible St. Augustinegrasses.83
4-5	Mean number of major waveform events per insect (NWEI) on each of four southern chinch bug-resistant or -susceptible St. Augustinegrass varieties.83

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
3-1 Total protein content in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding.	60
3-2 Levels of peroxidase (POX) specific activity levels in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding.	61
3-3 Levels of polyphenoloxidase (PPO) specific activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding.	62
3-4 Levels of lipoxygenase (LOX) specific activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding.	63
3-5 Levels of catalase (CAT) specific activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding.	64
3-6 Native gels stained for POX activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding.	65
3-7 Native gels stained for PPO activity in St. Augustinegrass lines 1, 3, 5, and 8 d after challenge by southern chinch bug.	66
3-8 Native gels stained for LOX activity in St. Augustinegrass lines 1, 3, 5, and 8 d after challenge by southern chinch bug.	67
4-1 EPG waveforms produced by southern chinch bug feeding on St. Augustinegrass, recorded using an AC/DC EPG monitor.	84
4-2 Total percentage of time spent on total wave form duration (TWD) by southern chinch bugs on resistant and susceptible St. Augustinegrasses in different waveform categories.	85
4-3 Waveform duration per insect (WDI) for major waveforms on resistant and susceptible St. Augustinegrasses.	86
5-1 St. Augustinegrass stolon A. with the axillary shoot (as); B. Parts of axillary shoot,	98
5-2 Transverse section of outermost St. Augustinegrass leaf sheath showing southern chinch bug salivary sheath stained with McBride’s stain (400X).	99
5-3 Number of southern chinch bug salivary sheaths found on the outermost leaf sheath of axillary shoots.	100

5-4	Number of southern chinch bug branched salivary sheaths on the outermost leaf sheath of axillary shoots from southern chinch bug-resistant and -susceptible St. Augustinegrasses.....	101
5-5	Total lignin content of axillary shoot in southern chinch bug-resistant and -susceptible St. Augustinegrasses.	102
5-6	Transverse sections of outermost leaf sheath vascular bundles stained for lignin in southern chinch bug-resistant and -susceptible St. Augustinegrasses (400X).....	103
5-7	Transverse sections of the epidermal layer of the outermost leaf sheath in southern chinch bug-resistant and -susceptible St. Augustinegrasses (400X)	104
5-8	Thickness of epidermal layer of outermost leaf sheath in southern chinch bug-resistant and -susceptible St. Augustinegrasses.	105
5-9	Transmission electron micrographs (7000X) of the sclerenchyma cells of the outermost leaf sheath of St. Augustinegrasses resistant and susceptible to southern chinch bug.....	106
5-10	Percentage of cell wall area relative to total cell area of sclerenchyma of the outermost leaf sheath of southern chinch bug-resistant and -susceptible St. Augustinegrasses.....	107

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

MECHANISMS OF RESISTANCE TO SOUTHERN CHINCH BUG, *Blissus insularis*
BARBER (HEMIPTERA: BLISSIDAE) IN ST. AUGUSTINEGRASS

By

Murugesan Rangasamy

May 2008

Chair: Ronald H. Cherry
Cochair: Heather J. McAuslane
Major: Entomology and Nematology

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is the most commonly grown lawn grass in Florida. The southern chinch bug (SCB), *Blissus insularis* Barber (Hemiptera: Blissidae), is the most serious insect pest of St. Augustinegrass. Host plant resistance has long been one of most successful management tools to control the SCB. Resistance to SCB has been identified in the St. Augustinegrass lines 'Floritam', 'FX-10' and 'NUF-76'. Floritam was once resistant but is now susceptible. This research investigated the mechanisms of SCB resistance in FX-10 and NUF-76. Choice and no-choice tests and ovipositional and developmental studies identified strong antixenosis in FX-10 and NUF-76 and antibiosis in NUF-76. FX-10 and NUF-76 had significantly higher peroxidase activity and NUF-76 had significantly higher polyphenol oxidase activity 5 and 8 d after infestation compared to uninfested controls. FX-10 had significantly higher lipoxygenase activity 3, 5 and 8 d after infestation compared to uninfested controls. Native gels stained for peroxidase indicated that some isozymes in FX-10 and NUF-76 were induced 5 and 8 d after infestation. Electrical penetration graph studies showed that the SCB made more frequent probes, spent longer time on pathway related activities on FX-10 and NUF-76, compared to the susceptible Floritam and

Palmetto. Significantly higher number of salivary sheaths was observed in the outermost leaf sheath in FX-10 and NUF-76. The total lignin content of the axillary shoot and the thickness of the epidermal layer in SCB resistant and susceptible St. Augustinegrasses did not differ significantly. TEM studies on the leaf sheath showed sclerenchyma cells with thicker cell walls in NUF-76 and FX-10 than in Floratam and Palmetto suggesting that the sclerenchyma cells around the vascular bundle in FX-10 and NUF-76 could serve as an impediment to the SCB stylet penetration. From this research, I conclude that the strong antixenosis found in FX-10 and NUF-76 and antibiosis in NUF-76 could be due to a combination of induction of oxidative enzymes, thicker sclerenchyma cells around the vascular bundle and possible presence of feeding deterrents or toxins in phloem sap or sieve tube blocking mechanisms.

CHAPTER 1 LITERATURE REVIEW

Chinch bugs are in the family Blissidae and are specialized to feed on plant sap. In the United States, there are four economically important species and subspecies, i.e. *Blissus insularis* Barber, *B. leucopteros leucopteros* (Say), *B. leucopteros hirtus* Montandon and *B. occiduus* Barber feeding on grasses and related crop plants such as corn, sorghum and wheat. In Florida, the southern chinch bug, *B. insularis*, is a serious insect pest of St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. The taxonomy of the pest chinch bugs has been difficult and will be reviewed before concentrating on *B. insularis*, the subject of this dissertation.

Taxonomy of the Chinch Bugs

Blissus leucopteros was first described in the United States by Say in 1831 from a single specimen collected from the coast of Virginia. Although chinch bugs were collected from New York and Massachusetts, no economic damage was noticed (Fitch 1856). The first report of damage in the northeastern United States surfaced when Linter (1883) noticed chinch bug damage in a meadow of timothy grass at Hammond, New York. The name *B. leucopteros* was commonly applied to all chinch bugs collected in the United States until the early 1890s when Montandon (1893) described *Blissus hirtus* based on a single specimen collected from Hazelton, Pennsylvania. *Blissus hirtus* was certainly the form referred to as *B. leucopteros* by Fitch and Linter.

Although Montandon (1893) gave species status to *hirtus*, Barber (1918) did not consider *hirtus* as a separate species. Based on geographical location, body form and wing polymorphism, Barber (1918) classified *Blissus leucopteros* into three varieties, i.e. 1. *Blissus leucopteros* var. *hirtus* Mont., which he stated was common to the highlands of United States

and Canada, characterized by the presence of macropterous and brachypterous forms. 2. *Blissus leucopteros* var. *arenarius* Barber, found in Sandy Hook, New Jersey on a species of sand grass, characterized by being narrower and longer with only macropterous forms and, 3. *Blissus leucopteros* var. *insularis* Barber commonly found in Punta Gorda, Florida, characterized by a shorter and narrower body form than the typical *B. leucopteros*. The three varieties mentioned by Barber were subsequently called subspecies, races or synonymies by many authors, which led to confusion among researchers and with Barber himself.

Based on biological, morphological and cytological evidence Leonard (1966) considered *B. leucopteros*, *insularis* and *arenarius* as distinct species but retained *hirtus* at the subspecies level. He described *arenarius* and *maritimus* as subspecies of *B. arenarius*, and *hirtus* and *leucopteros* as subspecies of *B. leucopteros*.

Barber (1918) also described *B. occiduus* from specimens collected from Ft. Collins, Colorado, and Germino, New Mexico. The reported distribution of *B. occiduus* includes California, Colorado, Montana, and New Mexico in the United States and Alberta, British Columbia, Manitoba and Saskatchewan in Canada (Bird and Mitchner 1950, Slater 1964). *Blissus occiduus* is a relatively smaller chinch bug having conspicuous wing polymorphism and is an important insect pest of buffalograss, *Buchloe dactyloides* (Nuttall) Engelman (Baxendale et al. 1999).

Blissus insularis Barber is referred to here by its Entomological Society of America approved common name, southern chinch bug (Bosik 1997). The ESA approved common names of the other economically important species of chinch bugs include hairy chinch bug, chinch bug and western chinch bug for *B. leucopteros hirtus* Montandon, *B. leucopteros leucopteros* (Say), and *Blissus occiduus* Barber, respectively (Bosik 1997).

Host Plants and Feeding Preferences

Newell and Berger (1922) recorded the chinch bug for the first time in Florida on St. Augustinegrass. They noticed the insect at the base of the grass surrounding brown areas of lawns and determined it to be *B. leucopteros*, the same species that attacks cereals. Beyer (1924) considered *B. leucopteros* to be a serious pest in Florida because it was feeding on St. Augustinegrass, the grass grown generally as lawngrass over the entire state. However, Howard (1888) and Webster (1907) did not consider St. Augustinegrass as a host among the grasses damaged by the chinch bug.

Wilson (1929) reported that chinch bug caused extensive damage to St. Augustinegrass in the southeastern United States. He observed that no other cultivated plants were infested where *B. leucopteros* seriously damaged St. Augustinegrass. Hamilton (1935) reported the chinch bug *B. leucopteros* as a pest of lawns and golf courses in the eastern United States.

Waldon (1936) found *B. leucopteros hirtus* damaging lawns in Connecticut. Maxwell and MacLeod (1936) also reported that the same species attacked 19 different varieties of lawngrass. Kuitert and Nutter (1952) described for the first time the chinch bugs in St. Augustinegrass as *B. leucopteros insularis* and Kerr (1966), in a short note, mentioned that *insularis* and *leucopteros* differed morphologically. Kerr (1966) also believed that the chinch bug damaging lawns in Florida could be elevated to species status because *B. insularis* had distinct morphological features and it was not found on corn and other cereals. Kelsheimer and Kerr (1957) found *B. leucopteros insularis* severely damaging torpedo grass (*Panicum repens* L.), occurring occasionally on centipede grass [*Eremochloa ophiuroides* (Munro)], and rarely on Bermuda grass [*Cynodon dactylon* (L.) Pers.]. *Blissus leucopteros insularis* was considered to be the second most expensive plant-feeding arthropod in Florida (Kerr 1966). In Florida, St.

Augustinegrass was the only grass to suffer severe damage by chinch bugs although the bugs feed also on zoysia grass (*Zoysia* spp.), and bahia grass (*Paspalum notatum* Fluegg). However most of the damage on these other grass species occurred when they were grown near St. Augustinegrass with large bug populations (Kerr 1966).

Cherry and Nagata (1997) studied the ovipositional preference of southern chinch bug with four cultivars of St. Augustinegrass ('Bitter Blue', Floratam, FX-10, and 'Seville') and four other lawn grasses (*P. notatum*, *C. dactylon*, *E. ophiuroides* and *Z. japonica*). They concluded that southern chinch bug resided in and oviposited on St. Augustinegrass cultivars more than on other lawn grasses. Three cultivars of St. Augustinegrass, Bitter Blue, Seville and Floratam, favored high adult survival rates and the lowest survival rates were recorded on *P. notatum*, *C. dactylon*, and *E. ophiuroides*.

Feeding Habit and Nature of Damage

Beyer (1924) found that chinch bugs pushed themselves into the laterals of the close-fitting leaves of St. Augustinegrass and feeding caused a reddish stain on the damaged plant parts. Affected plants had reduced growth and became dwarf. Watson (1925) found that infested lawngrass turned brown in patches and then died out completely. He also reported that the plants around the brown patch area exhibited yellowing symptoms and were infested with chinch bugs sucking plant sap. Wilson (1929) designated the injury as "dieback" because of the progressive nature of the damage. The gregarious feeding habit of the bugs caused the plant to wither. The bugs abandoned the withered plants and moved into neighboring healthy plants. Lawns on dry sandy soils were found more vulnerable to insect attack probably because of reduced vigor of the plants and rapid multiplication of bugs. Kerr (1966) found the bugs aggregated in scattered patches rather than distributed evenly. Before moving into a new area, the bugs killed the plants

in the infested area completely. Short et al. (1997) reported that the injury first occurs in water stressed areas or in areas of full sunlight and infested lawns display circular discolored patches. Cherry (2001) reported that southern chinch bugs were extremely aggregated in yellow damaged St. Augustinegrass within areas of light and moderate infestations whereas only a few southern chinch bugs were present in the grass 5 m or more away from an infestation.

Oviposition and Development

Beyer (1924) reported that the female chinch bugs laid their eggs near the roots of the grass. The eggs were pushed into protected places, often into crevices at grass nodes or almost out of sight between leaf blades where they come together at their base. Adults continued to oviposit for many consecutive weeks (Wilson 1929, Kerr 1966).

Fecundity was estimated by Beyer (1924) to vary from 105 to 250 eggs or to average 250 eggs per female by Kerr (1966). Eggs are small and oval-shaped with a blunt end from which four small projections extend (Stacy 2001). Eggs are first pale white in color but turn amber and eventually red before hatching. The egg period varies widely with estimates of 9 to 13 days (Wilson 1929), 7 to 11 days (Kuitert and Nutter 1952), 2 weeks (Eden and Self 1960), 11 to 33 days (Komblas 1962), and 1½ weeks in summer but a month or more in winter (Kerr 1966). The first nymphal instar has yellow body color which changes to red with a faint band across the abdomen. The later instars are black with a narrow white band (Stacy 2001).

Nymphs take an average of 28 to 30 days (Wilson 1929, Kuitert and Nutter 1952, Eden and Self 1960) to reach maturity. Wilson (1929) reported a pre-oviposition period of 5 to 13 days with the average of 8 days while Eden and Self (1960) reported 7 to 10 days.

Males survive for an average of 42 days and the females for 70 days. The generation time has been estimated variously as 45 to 56 (Wilson 1929, Kuitert and Nutter 1952, Eden and Self

1960). Beyer (1924) described six nymphal stages and two generations with an overlapping third generation during the summer in Florida while Kerr (1966) reported only five nymphal instars. Wilson (1929) found mixed populations of adults and all stages of nymphs in spring because of the extended period of oviposition. The adults mated in spring after a hibernation period and started laying eggs (Eden and Self 1960).

Seasonal Wing Polymorphism and Movement

Polymorphism in chinch bug wings was reported by Van Duzee (1886). He noticed that the shortwinged (brachypterous) form ordinarily predominated except under hot and dry summer conditions, when the longwinged (macropterous) form predominated. This led him to believe that the chinch was a tropical longwinged species. Webster (1907) reported that short- and longwinged forms were found intermixed along seacoasts and northern sections of the United States. He insisted that this condition must have been brought about by disuse of wings for a period of time but not by temperature of the habitat. Wilson (1929) reported that both macropterous and brachypterous southern chinch bugs occur in Florida and the relative number of these two forms varied with season. Komblas (1962) concluded that population density could be a reason for wing polymorphism because nymphs grown under crowded conditions were more likely to develop into macropterous adults than were uncrowded nymphs. Numbers of macropterous adults of southern chinch bug in south Florida were higher in summer and fall, when population densities were also higher than they were in winter and spring seasons (Cherry 2001). However these data did not explain the exact cause for macroptery. Cherry (2001) believed that various factors like heritability, population density and host plant condition might determine wing polymorphism based on the reports that macroptery in the oriental chinch bug, *Cavelerius saccharivorus* Okajima was density dependant (Denno and Peterson 2000) and was

strongly influenced by seasonal factors like day length and temperature (Fujisaki 2000). Leonard (1966) found migration to be an influencing factor in wing polymorphism. Reinert and Kerr (1973) noted that although both macropterous and brachypterous forms are found in the southern chinch bug, the latter was predominant. Cherry and Wilson (2003) reported that the macropterous adults have reduced fecundity and egg laying rates compared to brachypterous females. Kerr (1966) reported that the major difference between *B. leucopterous* in northern states and *B. insularis* in Florida was the absence of large spring flights of *B. insularis*. Although some of the longwinged forms took flight, nothing equaled the mass flight of *B. leucopterous* from overwintering quarters. Kerr (1966) noted that *B. insularis* moved from one lawn to another mainly by walking and streams of bugs could be seen moving out from heavily infested areas. The bugs were able to cover several hundred feet in a half-hour period of time.

Management of Southern Chinch Bugs

Southern chinch bugs can easily be managed if populations are detected early in a season's population growth. Infestation can be detected easily by parting the grass near yellowed areas and examining the base of the turf and the soil surface for chinch bugs. Heavily infested areas would have large number of bugs migrating across sidewalks and driveways. Flotation and vacuum techniques can also be used to identify areas of infestation (Congdon and Buss 2002). There are preventive and curative methods available to control southern chinch bug. Preventive methods such as cultural methods will avoid occurrence of insect problems and curative methods will control the insects once their infestation is identified.

Cultural Methods

Cultural control is manipulation of crop production and management techniques to reduce or avoid pest damage to plants. Cultural control manipulations are based on habitat management and require a thorough understanding of the ecosystem in which the pest thrives.

Many insect pests of lawns live in thatch. Thatch is the layer of dead plant material found between the green tops of the grass plant and soil. Proper mowing helps to prevent the thatch from building up. St. Augustinegrass lawns should be mowed to a height of 3 to 4 inches (Congdon and Buss 2002). The lawns should be mowed weekly during the growing season and not more than 35 to 45 percent of the leaf blade should be removed at the time of mowing. When the thatch is thicker than 1 cm, vertical mowing of the lawn could help. Good aeration combined with top-dressing could increase microbial decay of dead grasses thus reducing the thickness of thatch (Congdon and Buss 2002).

Southern chinch bugs prefer dry hot environments thus prolonged drought or water stress can encourage problems. Water stress and dry weather increase the chances of survival for nymphs and adult chinch bugs by reducing the occurrence of insect diseases. On the other hand, excessive watering reduces microbial activity thus increasing the thickness of the thatch. Regular inspection for dry or water stressed areas during summer is necessary (Congdon and Buss 2002). Irrigating the lawn to a depth of 6 inches at a time is advised because frequent watering induces shallow rooting and in turn higher chinch bug damage (Merchant and Crocker 1998).

Biological Control Agents

Reinert (1978b) reported on natural enemies of the southern chinch bug in Florida. His list included a scelionid egg parasitoid, *Eumicrosoma benefica* Gahan, the only parasitoid found,

lygaeid predators (big-eyed bugs) *Geocoris uliginosus* (Say) and *G. bullatus* Say, anabid, *Pagasa pallipes* Stal., anthocorids *Xylocoris vicarius* (Reuter) and *Lasiochilus pallidulus* Reuter, a dermapteran, *Labidura riparia* Pallas, and a reduvid, *Sinea* spp. He has also found ant predators, primarily *Solenopsis geminata* (F.), a spider, *Lycosa* sp., and a fungus, *Beauveria bassiana* (Balsomo) Vuillemin. Reinert (1978b) concluded that the main biological agents capable of keeping the chinch bug population under control were *E. benefica*, *G. uliginosus*, *X. vicarius*, and *L. riparia*. Big-eyed bugs and anthocorids are similar to chinch bugs in both size and shape. *Eumicrosoma benefica* is capable of parasitizing up to 50 percent of the chinch bug population (Hellman and Mathias 2002). The effectiveness of the predators mainly depends on the number and dosage of insecticidal sprays. Although various species of ants were reported to be predators of chinch bugs (Wilson 1929, Kerr 1966, Reinert 1978b), the red imported fire ant (*Solenopsis invicta* Buren) was most often associated with southern chinch bug infestations in Florida (Cherry 2001). Beneficial nematodes were unable to give consistent results when tested against chinch bugs (Merchant and Crocker 1998).

Chemical Control Methods

Although fertility and water management, thatch control and use of resistant varieties give considerable reduction in incidence of chinch bugs, heavy damage accompanied by high insect populations necessitates the use of chemical control. It is essential to locate the problematic areas. If 20 to 25 chinch bugs per square foot area are detected, insecticide application is recommended (Congdon and Buss 2002). Spot treatment is enough in case of minimal infestation but treating the whole area is necessary if the infestation is heavy (Congdon and Buss 2002). It is also important to visit the treated areas to see whether the bugs are controlled or not. Repeated sprays may be required because the eggs are not killed by most of the available

insecticides (Nagata and Cherry 2003). Pesticides available for professional use include acephate, chlorpyrifos, diazinon, carbaryl, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, lambda-cyhalothrin, permethrin and imidacloprid.

Repeated use of insecticides increases possible development of insecticide resistance (Reinert and Portier 1983). The first report of chinch bug resistance to chlordane came in 1953 when Wolfenbarger (1953) in Miami found that chlordane failed to control the population of chinch bugs. Afterwards reports continued to surface on the resistance of chinch bugs against DDT in Sarasota (Kerr and Robinson 1958), and in Miami (Kerr 1958), and against parathion in Fort Lauderdale (Kerr 1960) as well as other organophosphates like chlorpyrifos and diazinon (Reinert and Niemczyk 1976). Reinert (1982) reviewed the resistance to other chlorinated hydrocarbons and organophosphates. In 1983, Reinert and Portier investigated the distribution and level of southern chinch bug resistance against organophosphates and carbamates. They found that the level of resistance against chlorpyrifos was 3.4-fold to 3.19×10^8 -fold along a 70 km coastal area of Broward and Palm Beach counties in Florida. A 9.25-fold level of resistance to propoxur, a carbamate insecticide, was also demonstrated.

Cherry and Nagata (2005) reported for the first time that the southern chinch bug had developed resistance to bifenthrin, a commonly used synthetic pyrethroid to control chinch bugs in Florida. In recent years, increased use of insecticides further aggravated the problem. Cherry and Nagata (2007) collected southern chinch bugs from 10 different urban areas in Central and South Florida and tested their resistance to different classes of insecticides. They found varying level of southern chinch bug resistance to deltamethrin, imidacloprid, and lambda-cyhalothrin and, for the first time also confirmed the resistance to bifenthrin. Cherry and Nagata (2007) also noted that the level of insecticide resistance varies depending on the location. Most populations

in Florida are still susceptible to these insecticides, however, the situation is alarming and further underlines the importance of improving natural host plant resistance.

St. Augustinegrass and Host Plant Resistance against Southern Chinch Bugs

St. Augustinegrass is grown extensively in southern coastal states and California (Crocker et al. 1982). The world's first record of planting of St. Augustinegrass was on 11th November 1880, as turf bordering an avenue at A.M Reed's Mulberry Grove plantation, at Yukon, Orange Park, Florida (Works Progress Administration 1939).

After the Second World War, the market for St. Augustinegrass grass increased dramatically and during the late 1960s varieties like 'Floratine', 'Florida Common' and 'Roselawn' were commercially available for lawns. McGregor (1976) estimated that the area under turfgrass in Florida was more than 3, 68,700 ha, of which St. Augustinegrass accounted for more than 46 percent of the home lawns. Carter and Duple (1976) estimated that 56 percent of the lawns in Texas were in St. Augustinegrass but 96 percent in the Gulf coastal areas. The area in St. Augustinegrass sod production was 3,100 hectares in 1974 (Florida Department of Agriculture and Consumer Services 1976) and 13,400 hectares in 1991 (Hodges et al. 1994). St. Augustinegrass is estimated to cover more than half a million hectares in Florida (Hodges et al. 1994) and accounts for 85% of the sod industry in Florida with a value of \$262 million annually (Haydu 2005).

The leaves of St. Augustinegrass have round-tipped leaf blades of 5 to 14 mm width that are distichously arranged (Busey 2003). St. Augustinegrass is the most versatile of the warm season grasses and is able to survive well in acid or alkaline soils, soils with low organic matter, and soils with poor or good drainage. It is salt tolerant and has few disease and nematode

problems requiring little maintenance. It is also able to perform well in full sunlight and moderate shade (Crocker 1993).

St. Augustinegrass expanded rapidly into warm coastal regions as a lawngrass mainly because of human migration. However a new mosaic virus called St. Augustine Mosaic Disease (SAD), which was first reported in 1966, in Texas, challenged the popularity of St. Augustinegrass. The disease was caused by the St. Augustine decline strain of Panicum Mosaic Virus (SAD-PMV) (Lee and Toler 1973, Niblett et al. 1977) and the virus spread throughout the St. Augustinegrass growing regions of Texas (Toler and Walla 1976) and Louisiana (Holcomb et al. 1972). Horn et al. (1973) estimated a \$100 million loss due to the disease and in the same year, almost 90 percent of the St. Augustinegrass in Corpus Christi, Texas was affected.

St. Augustinegrass researchers from Florida and Texas realized that the virus could not be controlled therapeutically because of the ability of the virus to spread through vegetative propagation and mechanically through lawn mowers. Although the virus was not found in Florida, imminent spread from the neighboring states was anticipated. Accessions of St. Augustinegrass were screened for virus resistance under greenhouse conditions and the Floratam variety was found resistant to the virus in 1968 (Horn et al. 1973). Floratam displayed a high level of resistance under greenhouse conditions and for 24 consecutive months under field conditions as well.

Reinert and Kerr (1973) reported that southern chinch bugs were able to damage seriously or kill whole lawns and were considered to be the most serious insect pest of St. Augustinegrass. Kerr (1966) rated the southern chinch bug as one of the most economically important arthropod in Florida second only to citrus mite, in terms of the cost of control measure (Strobel 1971). Because of the destructive nature of this pest, experiments were started to find resistant varieties

of St. Augustinegrass. In 1972, Reinert reported that nine accessions of St. Augustinegrass had seasonal mean populations of 1.6 or fewer bugs per 0.1 m². In 1974, Reinert and Dudeck identified three St. Augustinegrass accessions resistant to southern chinch bug. They screened 11 promising accessions along with the available commercial cultivars using confined tests and reported that accessions FA-108, Floratam and FA-118 were highly resistant. The resistance was characterized as antibiosis because adults and nymphs suffered 50 percent mortality and surviving adults had reduced fecundity. One of these three accessions, Floratam was released jointly by the University of Florida and Texas A&M University (Horn et al. 1973). The resistance to SAD virus and chinch bugs, superior color, ability to cover the ground fast and perform well under the conditions of Florida and Texas made Floratam more preferred than all other commercial varieties.

Because of the coarse-textured leaves of Floratam, the researchers continued their search for fine-textured St. Augustinegrass lines (Reinert 1978a) and also for lines having combined resistance to SAD and southern chinch bug (Bruton et al. 1979, Reinert et al. 1980, Crocker et al. 1982). Despite a decade of research, the only line advanced and released was Floralawn (FA-108) which is fast-growing with fine-textured leaves and combined resistance to SAD and southern chinch bug (Dudeck et al. 1986).

Breakdown of Floratam Resistance

After its release in 1973, Floratam quickly expanded in commercial sod production and accounted for 77 percent of St. Augustinegrass sod production in Florida (Busey 1986). Many investigations since the germplasm release confirmed its resistance to the southern chinch bug (Reinert et al. 1980, Crocker et al. 1982, Reinert et al. 1986) and because of its vegetative reproduction and poor seed production, genetic breakdown of the resistance was considered to be unlikely to occur (Busey 1979). Nevertheless, by 1987, Busey and Center discovered a

widespread population of chinch bugs that had overcome the resistance of Floratam and Floralawn St. Augustinegrasses. Because of the association of polyploidy and host resistance in St. Augustinegrass and the ability of the chinch bugs to overcome the host plant resistance, the population that overcame Floratam resistance was dubbed the polyploid damaging population (PDP). Busey and Center (1987) tested a local population of standard southern chinch bug and the PDP population with these resistant varieties and found that the PDP adults had higher longevity and oviposition rate than the standard adults when confined on Floratam and Floralawn varieties. They concluded that the phenomenon observed was due to variability in the insect and not the grass. Busey and Coy (1988) conducted a survey among sod producers and county agents in Florida and found that Floratam-killing southern chinch bugs were widespread. The county agents considered the southern chinch bug as the most serious insect pest of St. Augustinegrass while the sod producers thought that caterpillars were more serious than chinch bugs. Busey and Coy (1988) concluded that chinch bug continued to be the most limiting factor in St. Augustinegrass-established areas, because the southern chinch bug was damaging in most counties. Nagata and Cherry (2003) collected southern chinch bug populations from nine cities or their suburbs in Florida and found all populations capable of overcoming the resistance in Floratam.

Breeding Resistant Varieties against Chinch Bugs, Continuing Missions

Crocker et al. (1989) realized the necessity of continuing the effort to develop more resistant varieties of St. Augustinegrass in Texas after seeing the reports of the ability of the PDP of southern chinch bug to attack Floratam in Florida (Busey and Center 1987). Crocker et al. (1989) tested varieties and accessions for resistance against Texas strains of southern chinch bug and SAD-PMV. The results showed that Floratam still was strongly antibiotic to southern

chinch bug. Thus it was concluded that the breakdown of Floratam antibiosis in Florida was absent or not widespread in Texas. They concluded that the populations of southern chinch bugs from Texas and Florida might not respond uniformly to future varieties.

The susceptibility of Floratam was a major concern to the turfgrass industry in Florida at the time it was reported (Busey and Center 1987) but there was no information on how the PDP adapted to Floratam antibiosis. Busey (1990a) found that host adaptability in PDP was heritable and retained in subsequent generations irrespective of the previous host. The populations from various regions differing in ability to attack hosts were one potentially interbreeding population and capable of exchanging a particular genetic trait. The other important discovery was that oviposition rates of PDP on Floratam were much greater than previously reported (Busey and Center 1987) suggesting an improvement in host adaptation when the colony was maintained on the resistant host. Vegetative propagation (genetically homogenous in subsequent generations) in St. Augustinegrass offered a steady selection pressure for southern chinch bug adaptation. Also the long distance transport of sod increased the gene exchange between the PDP and standard populations thus host adaptability was expected to occur (Busey 1990a).

Busey (1990b) continued his quest for resistant varieties against the PDP of southern chinch bug and reported that PDP chinch bugs grown on FX-2, FX-10, and FX-33 exhibited shorter adult longevity and fewer cumulative lifetime eggs than insects grown on Floratam. Both laboratory and field PDP exhibited high mortality on FX-33. In addition to confined tests, Busey (1990b) also used the production of excrement as an indicator of feeding activity. As a result of this research, a southern chinch bug resistant and coarse-textured breeding line, FX-10, the only recognizable St. Augustinegrass cultivar with pubescence was registered (Busey 1993). This variety performed well under seasonal drought, was notably resistant to PDP and standard

populations of southern chinch bug, and had moderate resistance to *Pyricularia* leaf spot. Because of the coarse texture and other negative characteristics such as slow ground coverage, susceptibility to weeds, FX-10 was never extensively grown in Florida (Nagata and Cherry 2003). The other problem with FX-10 was difficulty in transferring its resistance to southern chinch bug into other desirable lines because of non-viable seeds. All commercially available St. Augustinegrass cultivars with resistance to southern chinch bugs like Floratam, Floralawn and FX-10 are polyploids and have coarse appearance. Although diploid ($2n=18$) lines from St. Augustinegrass produce viable seeds and have fine-textured leaves they have never been accepted widely because of their poor resistance against chinch bugs (Nagata and Cherry 2003).

Nagata and Cherry (2003) made a breakthrough in their quest for the southern chinch bug resistance when they reported that NUF-76, a diploid St. Augustinegrass selection, and NUF-216 both had resistance against southern chinch bug. Of the fourteen lines inspected for resistance, the mortality of the bugs was significantly higher on NUF-216 and NUF-76 lines than on any other line. Although the mortality on NUF-216 and NUF-76 was not significantly different from FX-10, the mortality on these three lines was higher than that on any other line. Interestingly, high variation in mortality between different locations was observed on some St. Augustinegrass lines and this variation was attributed to the irregular response of the chinch bugs within Florida to both host plant resistance as reported by Busey and Center (1987) and insecticide resistance reported by Reinert and Portier (1983). After finding a diploid St. Augustinegrass resistant to southern chinch bug, Nagata and Cherry (2003) expect that the transfer of the desirable characters with chinch bug resistance into other lines will be easier in the near future.

Refining the Screening Techniques

Chinch bug resistance in varieties like Floratam and Floralawn was termed antibiosis because these cultivars produced higher mortality in confined tests. However, the exact mechanism of the resistance could not be explained by the confined tests. Busey and Center (1987) complained that the mortality data on the susceptible 'Florida Common' were inconsistent when the experiments were conducted for different periods of time on the same host. The crowding of chinch bugs released on sprigs of experimental grass and the variation among the field and laboratory populations of the chinch were the reasonable causes discussed. Busey (1990b) used visual rating of the excreta as a criterion for screening the germplasm. However, realizing that mere visual examination of excreta could not be an accurate selection criterion, Busey and Zaenker (1992) postulated they could weigh the excreta. Because the excreta adhered to surfaces like sidewalls of the glass culture tubes, antistatic film and aluminum foil (Busey 1990b), it was determined that the collection and weighing of the excreta was possible. The antistatic film was rolled inside and toward the top of each culture tube and the tubes were inclined. They observed that more than 99 percent of the excreta was deposited on the film and not on the glass or the grass. The films were removed and dried at 24°C for several days before weighing. They reported that the excreta weight was highly correlated with oviposition rate and crowding of up to 20 bugs per sprig did not influence excreta production.

Other Species of Chinch Bugs and Host Plant Resistance

Host plant resistance to control chinch bugs has been explored in other crops such as sorghum and in other turfgrasses such as buffalograss. Buffalograss is a perennial warm season grass species native to the American Great Plains (Wenger 1943, Beard 1973). It is a fine textured low growing turfgrass, which spreads vegetatively by stolons (Nuland et al. 1981,

Falkenberg-Borland and Butler 1982, Riordan et al. 1996). In recent years, buffalograss earned popularity as an alternative turfgrass because it is drought tolerant and has few pest and disease problems (Heng-Moss et al. 2002). One of the reported insect pests of buffalograss is *B. occiduus* whose hosts include barley, *Hordeum* sp., corn, *Zea mays* L., oats, *Avena sativa* L., sugarcane, *Saccharum officinarum* L., wheat, *Triticum aestivum* L., bromegrass, *Bromus* spp., and various native grasses including buffalograss (Ferris 1920, Parker 1920, Farstad et al. 1951). Currently very few control measures are available for chinch bugs and other insect pests in buffalograss (Baxendale et al. 1999). Extensive differences exist among buffalograss selections in their resistance to mealy-bugs, *Tridiscus sporboli* and *Trionymus* sp., suggesting the possibility of also breeding chinch bug resistance in buffalograss (Johnson-Cicalese et al. 1998). Based on greenhouse and field evaluations of eleven lines, NE91-118, ‘Tatanka’, ‘Bonnie Brae’ and ‘Cody’ had high to moderate resistance to *B. occiduus* whereas NE84-45-3 and 378 were highly susceptible (Heng-Moss et al. 2002).

Characterization of Resistance against Chinch Bugs in Buffalograss

The basis of resistance in the four chinch bug resistant buffalograsses was categorized (Heng-Moss et al. 2002). Among the cultivars investigated, they found NE91-118, ‘Cody’ and ‘Tatanka’ exhibiting moderate-to-high levels of tolerance and ‘Bonnie Brae’ exhibiting moderate-to-low levels of tolerance to *B. occiduus* based on insect damaging ratings and functional plant loss indices. They suggested studying parameters such as height of regrowth, dry weight and yield clippings, root length, and tillerings that all represent plant aesthetics, an important aspect in assessing the tolerance of turfgrass to chinch bugs. The antibiosis tests showed no differences among the resistant and susceptible cultivars of buffalograss thus ruling out the possibility of any influence on the growth and development of *B. occiduus* by the

resistant cultivars. This is inconsistent with earlier discoveries reporting that resistance against southern chinch bug was antibiosis (Reinert and Dudeck 1974, Reinert et al. 1980). However, cultivars like NE91-118 and 609 were not preferred by chinch bugs in choice tests suggesting the presence of antixenosis. These results supported the earlier discovery of antixenosis resistance in NE-609 to the mealybugs *T. sporoboli* and *Trionymus* sp. SEM studies showed differences in trichomes densities among resistant and susceptible lines. Leaf blades of the resistant lines like NE91-118, NE-120, 378 and 609 had few trichomes and were characterized as glabrous (Johnson-Cicalese et al. 1998).

Chinch Bug Resistance in Sorghum

Chinch bugs that damage sorghum and small grains were recorded as *B. leucopteros* *leucopteros* (Webster 1909). The concept of host plant resistance as an important tool for managing chinch bugs in sorghum plants became evident when Dahms (1948) reported the “milos” sorghum were very susceptible to chinch bugs, the “feteritas” were susceptible and the “kafirs” and “sorgos” were resistant. During the early 1980s resistance to chinch bugs in sorghum lines and hybrids under field conditions was reported. However, Meehan and Wilde (1989) realized the importance of screening the sorghum lines under greenhouse or growth chamber conditions during the periods of low, natural field infestations. Under greenhouse conditions the commercial sorghum hybrids and lines were subjected to nymphs and adults at the seedling stage. Plant mortality for hybrids or lines was inconsistent between choice and no-choice tests. However, the days from infestation to death of plants in studies done in growth chambers were in agreement with the results from earlier field tests.

Investigating the Mechanisms of Southern Chinch Bug Resistance in St. Augustinegrasses

Thus far, studies on the mechanisms of resistance in St. Augustinegrass to southern chinch bug have been confined to those done on Floratam. Reinert and Dudeck (1974) and Reinert

(1978) reported that Floratam and 10 other St. Augustinegrass accessions exhibited antibiosis to southern chinch bug based on adult and nymphal mortalities. Reinert et al. (1980) and Crocker et al. (1989) used the term ‘antibiosis’ to describe the resistance to southern chinch bug in St. Augustinegrass lines. Unfortunately, none of the above-mentioned reports explained the exact cause of the nymphal and adult mortality. Busey and Zaenker (1992) quantified the feeding response of southern chinch bug on St. Augustinegrass as weight of excreta produced and frequency of confined bugs on the grass as opposed to off the grass. They found significant differences among hosts in excreta weight within 24 h after releasing the insects and a strong correlation between excreta weight and oviposition rate. Although they proposed a new resistance bioassay technique they did not determine the exact resistance mechanism operating in St. Augustinegrass against southern chinch bug.

The goal of this study is to determine more precisely the mechanisms of southern chinch bug in both polyploid (‘FX-10’) and diploid (NUF-76) St. Augustinegrass lines. This goal will be achieved by the following objectives:

- **Objective 1.** To determine the modality of resistance to southern chinch bug in St. Augustinegrass.
- **Objective 2.** To compare oxidative responses of southern chinch bug resistant and susceptible St. Augustinegrass lines to *B. insularis* infestation.
- **Objective 3.** To study precise feeding behavior of southern chinch bugs on susceptible and resistant St. Augustinegrass lines using electrical penetration graph technique (EPG)
- **Objective 4.** To determine the role of lignification and leaf sheath anatomy in southern chinch bug resistance in St. Augustinegrass.

CHAPTER 2
CATEGORIES OF RESISTANCE IN ST. AUGUSTINEGRASS LINES TO SOUTHERN
CHINCH BUG (HEMIPTERA: BLISSIDAE)

Introduction

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze is the most widely used lawn grass in tropical and sub-tropical climatic regions (Sauer 1972). It is the primary turfgrass in Florida and is grown on approximately 70% of all lawns (Busey 2003). The southern chinch bug, *Blissus insularis* Barber is the most serious insect pest of St. Augustinegrass (Crocker 1993). Southern chinch bugs suck the sap from the grass until it withers and dies, turning from a healthy green color to brown-yellow. Infestation typically occurs first in water stressed areas and along edges of the lawn or areas in which grass is growing in full sunlight (Short et al. 1995). The annual cost of southern chinch bug management and losses in Florida alone was estimated at \$5 million in 1983 (Hamer 1985) and is undoubtedly much more today. The economic importance of this insect has increased as it has developed resistance against several insecticides (Reinert and Portier 1983, Cherry and Nagata 2005).

‘Floritam’, a polyploid variety of St. Augustinegrass with resistance to southern chinch bug, has been available in Florida and Gulf Coast areas since its release in 1973 (Horn et al. 1973). Reinert and Dudeck (1974) classified the resistance to southern chinch bug found in ‘Floritam’ as antibiosis because insects on ‘Floritam’ exhibited more than 50 percent adult and nymphal mortalities and surviving insects had reduced fecundity compared to insects reared on susceptible varieties of St. Augustinegrass. However, Busey and Center (1987) reported instances of southern chinch bugs overcoming ‘Floritam’ resistance in Florida, beginning in 1985. They named this resistance-breaking population of the southern chinch bug, the polyploid-damaging population. Nagata and Cherry (2003) collected southern chinch bug populations from nine cities or their suburbs in Florida and determined the distribution of the

polyploid-damaging population to be widespread. This problem seems to be confined to Florida however because, in 1989, Crocker et al. (1989) reported that there was no evidence of the polyploid-damaging population of southern chinch bug in Texas.

The lessened resistance in 'Floritam' prompted plant breeders to continue their search for resistance to the southern chinch bug. 'FX-10', a polyploid St. Augustinegrass, was registered in 1993 (Busey 1993) with resistance against the southern chinch bug, but because of its very coarse texture, toughness and poor ground coverage, it was never extensively grown (Nagata and Cherry 2003). Nagata and Cherry (2003) reported no significant difference in southern chinch bug mortality among 'FX-10', NUF-216 and NUF-76. The latter two lines are products of the University of Florida's turf-breeding program. Mortality on all three lines was significantly higher than that on 'Floritam' and 'Floratine'. This research was significant because Nagata and Cherry (2003) identified, for the first time, a diploid St. Augustinegrass line, NUF-76, with resistance to the southern chinch bug; all previous resistant lines were polyploids. Diploid lines produce viable seeds allowing recombination of characters and selection of new lines. Diploid lines also have narrow leaf blades, which gives them a much finer and more aesthetically pleasing texture. As of yet, it is not known by which mechanism resistance to southern chinch bug operates in these resistant lines.

Understanding the mechanisms of resistance in St. Augustinegrass to southern chinch bug is crucial. This knowledge may allow turf breeders to design appropriate screening assays, which would expedite their development of insect resistant varieties. The objective of this research was to characterize the mechanisms of southern chinch bug resistance in the polyploid ('FX-10') and diploid (NUF-76) St. Augustinegrass lines. To meet this objective we conducted choice and no-choice tests to examine adult chinch bug preference for and survival on resistant

and susceptible St. Augustinegrass lines. We also quantified reproduction of adult chinch bugs and studied development and survival of immature chinch bugs on susceptible and resistance lines.

Materials and Methods

Plants and Insects

St. Augustinegrass lines selected for the experiments included NUF-76 (resistant diploid), 'FX-10' (resistant polyploid), 'Floritam' (once resistant polyploid), 'Bitter Blue' (susceptible polyploid) and 'Palmetto' (susceptible diploid). Plant material was supplied by R. Nagata (Everglades Research and Education Center, Belle Glade, FL). Rooted sprigs from single node cuttings were grown in plastic pots (12.5 cm diameter \times 12.5 cm depth) containing a potting medium of 1:1 (v/v) sand and Fafard mix #2 (Conrad Fafard, Agawam, MA). Test plants were 6 to 8 weeks old. Plants were fertilized with Osmocote Plus 15-9-12 (Scotts-Sierra Horticultural Products Company, Marysville, OH) at the rate of 5g/kg of growing medium. Stolons approximately 30 to 40 cm in length with a minimum of three nodes were used for testing.

Nagata and Cherry (2003) reported that most populations of southern chinch bugs in Florida had overcome 'Floritam' resistance. Thus for all experiments, southern chinch bugs were collected from Royal Palm Beach, Palm Beach Co., FL. Preliminary trials were conducted to determine their ability to develop on 'Floritam'. The insects were collected by vacuuming St. Augustinegrass lawns as described by Cherry (2001) and then sorting through debris in the laboratory for fifth instars and adults (depending on the needs of the experiment). After collection, insects were provided with fresh 'Floritam' stolons as needed and maintained at 18°C in 20-liter plastic buckets.

Choice Tests

Choice tests were conducted to study the behavior of southern chinch bugs on resistant and susceptible lines of St. Augustinegrass. Arenas were constructed from circular polypropylene food storage containers measuring 33.6 (top diameter) × 33.0 (bottom diameter) × 10.6 cm (height) (Rubbermaid 3907, Wooster, OH) that were modified in the following manner. Five holes of 6-mm diameter, equally spaced around the perimeter of the arena and 3 cm below the open edge were drilled. A channel was then cut from the open edge of the container to the hole drilled in the side. The majority of the lid surface area was removed, leaving approximately 3 cm around the sealing edge. Anti-virus insect screen (hole size of 0.0105 × 0.0322 inches, Green-Tek, Edgeton, WI) covered the opening in the lid to facilitate aeration.

A moist paper towel was placed on the bottom of the arena to prevent desiccation of insects. Plant stolons, still attached to potted plants, were passed through the channels to rest in the holes on the side of the arena. The portion of the stolons in contact with the plastic arena was wrapped with parafilm strips (American National Can, Greenwich, CT) so that the plant would not be damaged. After positioning the stolons, the channels were sealed with clear cellophane tape to prevent southern chinch bugs from escaping. Plants were watered as needed. NUF-76, 'FX-10', 'Floritam', 'Bitter Blue' and 'Palmetto' were evaluated in this choice test.

Twenty chinch bug adults (10 males and 10 females) of undetermined age were released at the center of the arena and the lid was replaced. The location of the insects was recorded as 'on' one of the five lines or 'off' all plants at 24 h, 48 h, 72 h and 120 h after release. The experimental design was a completely randomized block design. The experiment was conducted in a laboratory room that was maintained at 30 ± 1°C and 14:10 L:D h photoperiod and was replicated 15 times.

No-Choice Tests

No-choice tests were conducted to investigate the possibility of antibiosis in resistant St. Augustinegrass lines. One stolon of each line (NUF-76, 'FX-10', 'Floritam', 'Bitter Blue' and 'Palmetto'), still attached to the plant, was introduced into separate rectangular arenas. The arena was made of a food storage container (Rectangle 1.8 qt, Rubbermaid #7A23, Wooster, OH) with dimensions of 27 cm × 15 cm × 5.7 cm. One hole was drilled on the 15-cm side and a channel was cut as described in the choice-test section so that a stolon could be passed unharmed to the interior of the box. The majority of the lid surface area was removed leaving approximately 3 cm around the sealing edge. Anti-virus insect screen was glued over the opening to facilitate aeration. The insects were starved for 12 h before the start of the experiment

A moist paper towel was placed on the bottom of the arena to prevent desiccation of insects. A sheet of heavy-duty aluminum foil (Reynolds Kitchens, Richmond, VA) was cut to the size of the arena and placed on the moist paper towel. Twenty chinch bug adults (10 males and 10 females) were released inside the arena. The exact locations of feeding of the adults such as leaf sheaths, internodes, or nodal regions, were observed 24 h, 48 h, 72 h and 120 h after release. The aluminum foil sheet was replaced after each observation and the number of excretory spots was recorded with the naked eye. Use of aluminum foil sheets to collect the southern chinch bug excreta was originally reported by Busey and Zaenker (1992). The experimental design was a randomized complete block design with 15 replicates. The experiment was conducted in a laboratory room that was maintained at 30 ±1°C and 14:10 L:D h photoperiod. Plants were watered as needed.

Ovipositional Studies

Studies were conducted to investigate the effect of St. Augustinegrass lines on fecundity of southern chinch bugs. Rectangular arenas used in no-choice tests were used for this experiment and St. Augustinegrass lines used included 'FX-10', NUF-76 and 'Floritam'. In order to obtain adults of similar age, fifth instar nymphs were selected from the field collection and then provided with fresh 'Floritam' runners. Insects that molted to the adult stage within a 5-d period were selected for the experiment. Five pairs of adults were released into the arena. A week after release, surviving chinch bugs were transferred gently using a mouth aspirator from one arena to a new arena with an undamaged stolon from the same individual plant. Adult mortality was recorded and the stolons were dissected to count the number of eggs. This was continued for a total of 4 weeks. This experiment was conducted at the Envirotron, IFAS, University of Florida that was maintained at $30 \pm 1^\circ\text{C}$. Plants were watered as needed. The experimental design was a randomized complete block design with 13 replications.

Developmental Studies

This experiment was conducted to study the influence of St. Augustinegrass lines on growth and development of southern chinch bug nymphs. The experimental set up was as explained in the no-choice test except the arena size was $28 \times 16 \times 11$ cm (l \times w \times h) (Rubbermaid #3901, Wooster, OH). Five pairs of adults (0-5 d old) were released into the arena. After 32 d boxes were opened and stolons were dissected to count the number of nymphs and adult chinch bugs surviving. This experiment was conducted at the Envirotron, IFAS, University of Florida that was maintained at $30 \pm 1^\circ\text{C}$. Plants were watered as needed. The experimental design was completely randomized block design with 11 replications.

Statistical Analysis

The distribution of chinch bugs in choice tests among the five lines taken over a period of 5 d was analyzed as a repeated measures design using mixed model analyses (PROC MIXED, SAS Institute 1999) after data were transformed by arcsine \sqrt{x} . In no-choice tests, the numbers of excretory spots counted over a period of 5 d were analyzed as a repeated measures design using mixed model analyses (PROC MIXED). In ovipositional studies, the number of eggs laid per female was transformed by $\sqrt{(x + 0.375)}$ to correct for nonhomogeneity of variances and data were analyzed using a repeated measures analysis with a mixed model (PROC MIXED). For the developmental studies, the number of nymphs produced and adults surviving after 32 d was analyzed using PROC GLM (SAS Institute 1999). When significant F-tests were obtained, means were compared using Tukey's test with a significance level of $P < 0.05$.

Results

Choice Tests.

More chinch bugs were observed on the three susceptible lines than the two resistant lines at three out of four observation times (Table 1). Southern chinch bug adults showed significant preference for susceptible lines at 24 h, 72 h and 120 h after release ($F = 10.43$; $df = 4, 70$; $P < 0.0001$). Forty-eight hours after release, the proportion of the chinch bugs on plants found on 'FX-10' was not significantly lower than the proportion counted on susceptible plants. NUF-76 had a significantly lower proportion of chinch bugs on it than on all susceptible lines except 'Bitter Blue'. The preferences of chinch bugs for different lines did not differ among time periods ($F = 0.43$; $df = 3, 207$; $P = 0.7300$). Between 69 and 72 % of the released adults were found either feeding or resting on a stolon; the remaining adults were either off the plant or dead. The proportions of chinch bugs not found on the plants were 0.30, 0.27, 0.28, and 0.31 at 24 h,

48 h, 72 h, and 120 h after release, respectively. A significant interaction was detected between St. Augustinegrass lines and time after release ($F = 2.1$; $df = 12, 207$; $P = 0.0157$). This significant interaction indicates that, in general most lines had a similar proportion infestation over time. However ‘Palmetto’ was relatively more infested early in the experiment whereas ‘Floritam’ was relatively more infested later in the experiment. Mortality of adults in the arenas was not analyzed statistically but ranged between 5 and 10% (one or two insects out of 20). Adult chinch bugs were observed feeding on all plants, including the resistant ‘FX-10’ and NUF-76, and fecal spots were noticed underneath all plants.

No-Choice Tests

On most of the observation periods, 40 to 45 % of the adult chinch bugs were found inside the leaf sheaths, a few were observed on nodal and internodal regions, and the remaining 55 to 60% of the insects were off the plant. The number of excretory spots counted differed significantly among St. Augustinegrass line ($F = 27.16$; $df = 4, 56$; $P < 0.0001$) (Table 2). The number of excretory spots found under ‘FX-10’ was consistently significantly less than under the susceptible lines at all the time periods. At 48 h and 72 h, the mean number of excretory spots produced on NUF-76 was approximately half the number found on the susceptible lines, however the differences were not statistically significant. At 120 h the mean number of excretory spots produced on NUF-76 was significantly lower than found on the susceptible lines. There were significant differences among time periods ($F = 6.19$; $df = 3, 135$; $P < 0.0006$). In general production of excretory spots was greatest at 24 h, most likely because insects were starved for 12 h before the start of the experiment, and declined afterwards. There was no significant interaction of time with St. Augustinegrass line ($F = 1.23$; $df = 12, 135$; $P = 0.27$).

Ovipositional Studies

Southern chinch bug adults confined on 'Floritam' produced 11 and five times more eggs than those on 'FX-10' and NUF-76, respectively ($F = 57.6$; $df = 2, 36$; $P < 0.0001$) (Table 3). There were significant effects due to time and the interaction between St. Augustinegrass lines and time (time: $F = 9.79$; $df = 3, 108$; interactions: $F = 5.24$; $df = 6, 108$; $P < 0.0001$). This interaction was due to the higher mean number of eggs laid during the second and third weeks of the experiment compared to the first and fourth weeks on 'Floritam' and 'FX-10' but higher oviposition on NUF-76 on the first and third weeks than the second and fourth weeks. During the third and fourth weeks of the experiment, fewer females were alive on 'FX-10' and NUF-76 than on 'Floritam' ($F = 32.77$; $df = 2, 108$; $P < 0.0001$) (Table 3). However, during the first week, numbers of female alive on different lines did not differ significantly whereas during the second week fewer females were alive on 'FX-10' than on NUF-76 and 'Floritam'. The eggs were often found inside the folded emerging leaf or partially opened leaf sheaths and occasionally on adventitious roots.

Developmental Studies

The five pairs of adults released on 'FX-10', NUF-76 and 'Floritam' produced an average of 9.5 ± 3.6 (mean \pm SEM), 20.3 ± 6.7 , and 180.5 ± 20.1 nymphs, respectively, after 32 d. Nymphal production on 'FX-10' and NUF-76 was significantly less than that on 'Floritam' ($F = 284.7$; $df = 2, 53$; $P < 0.0001$). The number of adults surviving on 'FX-10' (0.8 ± 0.3) and NUF-76 (1.9 ± 0.6) was significantly less than that on 'Floritam' (8.0 ± 0.4) ($F = 71.90$; $df = 2, 20$; $P < 0.0001$). Most of the nymphs were first and second instars; a very few third instar nymphs were found on 'Floritam'. Thus we assume that the adults observed after 32d were the originally released adults rather than offspring.

Discussion

Choice tests, no-choice tests, ovipositional and developmental studies were helpful to determine the presence or absence of antixenosis and antibiosis in NUF-76 and 'FX-10', chinch bug-resistant St. Augustinegrass lines. In choice tests, southern chinch bugs significantly preferred the susceptible lines to both 'FX-10' and NUF-76, suggesting the presence of antixenosis in the resistant lines. The finding of significantly fewer excretory spots under 'FX-10' in no-choice tests confirms the presence of antixenosis. However, the mean number of excretory spots found under NUF-76 was not consistently different from the susceptible lines. At 24 h, NUF-76 was not significantly different from any of the susceptible lines however at 48 h and 72 h, the mean number of spots found under NUF-76 was significantly different from 'Palmetto', a susceptible diploid, and at 120 h the mean number of spots were significantly different from all the susceptible lines. Our data indicate a trend that feeding of southern chinch bug on NUF-76 is reduced over time. While reduced feeding (antixenosis) on 'FX-10' and NUF-76 could impact chinch bug survival it is unlikely to have been the sole reason for the low survivorship of chinch bugs on these lines documented previously (Nagata and Cherry 2004).

Adults released on 'FX-10' and NUF-76 laid many fewer eggs than on 'Floritam'. The reduction in fecundity could be due to reduced feeding on 'FX-10' and NUF-76 shown in the no-choice tests but was so extreme that it is more likely that reduced fecundity is due to a nutritional inadequacy for egg development or some toxin that influenced reproduction (i.e., antibiosis).

Studies on the mechanisms of resistance in St. Augustinegrass to southern chinch bug have been confined to those done on 'Floritam'. Reinert and Dudeck (1974) and Reinert (1978) reported that 'Floritam' and 10 other St. Augustinegrass accessions exhibited antibiosis to southern chinch bug. Based on adult and nymphal mortalities, the mechanism of resistance in

‘Floritam’ was categorized as antibiosis. Reinert et al. (1980) and Crocker et al. (1989) used the term ‘antibiosis’ to describe the resistance to southern chinch bug in St. Augustinegrass lines. Unfortunately, none of the above-mentioned reports explained the exact cause of the nymphal and adult mortality.

Resistance to other pest chinch bugs has been studied. Heng-Moss et al. (2003) investigated the mechanisms of resistance to western chinch bug (*Blissus occiduus* Barber) in buffalograss (*Buchloe dactyloides* (Nuttall) Engelman). They found an array of modalities of resistance among germplasm tested (antixenosis, antibiosis and tolerance) but reported no morphological or biochemical differences to account for these different susceptibilities.

Our studies confirm that both ‘FX-10’ and NUF-76 have a high level of southern chinch bug resistance. However, because of the course texture and other negative characteristics such as slow ground coverage, proneness to weeds, ‘FX-10’ was never extensively grown in Florida (Nagata and Cherry 2003). Another problem with ‘FX-10’ was difficulty in transferring its resistance to southern chinch bug into other desirable lines because of non-viable seeds. Being a diploid and therefore much easier to cross, NUF-76 is a very important source of chinch bug resistance for turfgrass breeding programs. NUF-76 allows very little population growth and should need many fewer applications of insecticides. Studies are underway to understand how ‘FX-10’ and NUF-76 are morphologically or biochemically different from ‘Floritam’ and other chinch bug-susceptible lines and how the polyploid damaging population of southern chinch bug overcame resistance in ‘Floritam’.

Table 2-1. Distribution (mean proportion \pm SEM) of adult southern chinch bugs among five St. Augustinegrass lines in a choice situation at varying time intervals after release *

Lines	Proportion of <i>B. insularis</i> found on St. Augustinegrass lines*			
	Time after chinch bug release			
	24 h	48 h	72 h	120 h
'FX-10'	0.15 \pm 0.03b	0.18 \pm 0.03ab	0.14 \pm 0.02b	0.10 \pm 0.02b
NUF-76	0.09 \pm 0.03b	0.10 \pm 0.03b	0.08 \pm 0.02b	0.11 \pm 0.04b
'Floritam'	0.23 \pm 0.03a	0.25 \pm 0.03a	0.29 \pm 0.03a	0.29 \pm 0.05a
'Bitter Blue'	0.21 \pm 0.04a	0.21 \pm 0.04ab	0.23 \pm 0.04a	0.23 \pm 0.04a
'Palmetto'	0.33 \pm 0.02a	0.27 \pm 0.02a	0.25 \pm 0.02a	0.27 \pm 0.02a

* Proportions calculated as no. of chinch bugs on one St. Augustinegrass line divided by the number of chinch bugs counted on all lines. Those chinch bugs off the plants at a particular observation time were not included in the totals. Means within the same column with a letter in common are not significantly different ($\alpha = 0.05$) by Tukey's test.

Table 2-2. Mean number (\pm SEM) of excretory spots deposited per living chinch bug on St. Augustinegrass lines in a no-choice situation at varying time intervals after release

Lines	Number of excretory spots per living insect*			
	Time after chinch bug release			
	24 h	48 h	72 h	120 h
'FX-10'	1.7 \pm 0.3b	1.5 \pm 0.3c	1.3 \pm 0.3c	1.7 \pm 0.4b
NUF-76	3.6 \pm 0.5a	2.0 \pm 0.2bc	2.6 \pm 0.3bc	2.6 \pm 0.5b
'Floritam'	5.2 \pm 0.5a	3.9 \pm 0.4a	3.5 \pm 0.3ab	5.1 \pm 0.5a
'Bitter Blue'	3.9 \pm 0.5a	3.6 \pm 0.3ab	4.0 \pm 0.5ab	4.7 \pm 0.5a
'Palmetto'	4.0 \pm 0.4a	3.9 \pm 0.3a	4.4 \pm 0.3a	5.4 \pm 0.6a

* Number of excretory spots produced during a certain time period was calculated as the number of spots divided by number of adults alive that time period. Means in a column with the same letter in common are not significantly different ($\alpha = 0.05$) by Tukey's test.

Table 2-3. Mean number (\pm SEM) of eggs produced per female alive on St. Augustinegrass lines at weekly intervals after release

Lines	Weeks after chinch bug release			
	Number of eggs per live female			
	1	2	3	4
'FX-10'	1.4 \pm 0.6c	2.2 \pm 1.1b	2.5 \pm 1.7b	0.7 \pm 0.5b
NUF-76	6.5 \pm 1.4b	2.5 \pm 0.8b	5.4 \pm 3.4b	0.8 \pm 0.6b
'Floritam'	13.9 \pm 1.6a	25.3 \pm 2.4a	28.3 \pm 6.3a	9.2 \pm 2.6a
	Number of live females			
'FX-10'	4.15 \pm 0.25a	1.30 \pm 0.5b	0.69 \pm 0.4b	0.46 \pm 0.3b
NUF-76	4.07 \pm 0.32a	3.15 \pm 0.4a	1.46 \pm 0.5b	0.30 \pm 0.2b
'Floritam'	4.61 \pm 0.1a	4.15 \pm 0.2a	4.0 \pm 0.2a	3.58 \pm 0.4a

* Means in a column with the same letter in common are not significantly different ($\alpha = 0.05$) by Tukey's test.

CHAPTER 3
OXIDATIVE RESPONSES OF ST. AUGUSTINEGRASSES CHALLENGED BY
SOUTHERN CHINCH BUG, *BLISSUS INSULARIS* BARBER (HEMIPTERA: BLISSIDAE)

Introduction

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.), is the most widely used turfgrass in tropical and subtropical climatic regions (Sauer 1972). It is a commonly grown residential turfgrass species in Florida and is estimated to cover more than one million acres. St. Augustinegrass accounts for 85% of total sod production in Florida and is worth more than \$262 million annually. The southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae), is the most serious insect pest of St. Augustinegrass (Crocker 1993). The annual cost of southern chinch bug management and losses in Florida alone was estimated at \$5 million in 1983 (Hamer 1985) and is undoubtedly much more today. The economic importance of this insect is further increased by its development of resistance to several key insecticides (Reinert and Portier 1983; Cherry and Nagata 2005, Cherry and Nagata 2007).

Historically, host plant resistance has been one of the most successful pest management tools for this insect. Floratam, a polyploid variety of St. Augustinegrass with resistance to southern chinch bug, has been available in Florida and Gulf Coast areas since its release in 1973 (Horn et al. 1973). However, in 1985 a population of chinch bug was reported to have overcome Floratam's resistance in Florida (Busey and Center 1987). Nagata and Cherry (2003) collected southern chinch bug populations from nine widely-dispersed cities or their suburbs in Florida and found all populations capable of overcoming the resistance in Floratam.

As potential alternatives to Floratam, two southern chinch bug resistant St. Augustine lines were developed: a polyploid line, 'FX-10' (Busey 1993) and a diploid line, NUF-76 (Nagata and Cherry 2003). However, the mechanisms of resistance in these lines are unknown. Deciphering these mechanisms will greatly facilitate our efforts to breed cultivars with durable chinch bug

resistance in St. Augustinegrass. Although the mechanisms of plant resistance against phloem-feeding aphids have been studied extensively (Thompson and Goggin 2006), aphids feed on their host plants by probing intracellularly, while southern chinch bugs probe intercellularly, likely resulting in more and different kinds of cellular damage and plant response.

In our recent investigations, we showed that both 'FX-10' and NUF-76 deterred feeding of southern chinch bugs (antixenosis) (Rangasamy et al. 2006). NUF-76 also had adverse effects on the biology of the insect (antibiosis) (Rangasamy et al. 2006), suggesting a metabolic basis for insect resistance. One of the most prominent plant responses to insect herbivore attack is the induction of oxidative enzymes such as polyphenol oxidase (PPO), peroxidase (POX), lipoxygenase (LOX) and catalase (CAT) (Green and Ryan 1972; Hildebrand et al. 1986; Felton 1989; Felton et al. 1994a b; Stout et al. 1999; Constabel et al. 2000; Chaman et al. 2001; Ni et al. 2001; Heng-Moss et al. 2004). These enzymes, because of their potential roles in the synthesis of defense compounds and/or in oxidative stress tolerance, have often been implicated in plant resistance to insect herbivores.

The objective of this investigation was to compare the enzymatic responses of resistant and susceptible lines of diploid and polyploid St. Augustinegrass to feeding by a population of southern chinch bugs that has overcome the resistance of Floratam. Our study focused on the induction of oxidative enzymes POX, PPO, CAT and LOX in four cultivars of St. Augustinegrass following the introduction of southern chinch bugs.

Materials and Methods

Plants, Insects, Sample Collection

St. Augustinegrass lines selected for comparison included NUF-76 (resistant diploid), FX-10 (resistant polyploid), Floratam (susceptible polyploid), and 'Palmetto' (susceptible diploid). Both polyploid and diploid lines were included to determine whether the ploidy level has any

role in resistance to southern chinch bugs. Rooted sprigs from single node cuttings were grown in plastic pots (12.5 cm diam. × 12.5 cm depth) containing a 1:1 (v/v) mixture of sand and Fafard mix #2 (Conrad Fafard, Agawam, MA). Plants were fertilized with Osmocote Plus 15-9-12 (Scotts-Sierra Horticultural Products Company, Marysville, OH) at the rate of 5 g kg⁻¹ of growing medium. Stolons used in experiments were approximately 30 to 40 cm in length, with a minimum of three nodes, located on plants that were 6 to 8 weeks old.

Southern chinch bugs were collected from Royal Palm Beach (Palm Beach County, FL) for all experiments. The insects were collected by vacuuming southern chinch bug-infested St. Augustinegrass lawns as described by Cherry (2001). In the laboratory, fifth instars and adults were separated from the debris and insects were provided with fresh Floratam runners and maintained at 18°C in 20-l plastic buckets. Twenty adult chinch bugs (1:1 sex ratio) were confined in organandy sleeve cages on the youngest three nodes of one runner of a St. Augustinegrass plant. The chinch bugs were removed from the runner before collecting the sample for protein analysis. A 2-g amount of sample including leaf sheaths and blades, excised from each runner, was collected 1, 3, 5, and 8 d after infestation and frozen immediately in liquid nitrogen. The samples were stored at -80°C until analyses. Five replicate plants were infested for each line at each time period along with an equal number of control plants whose runners were confined in cages without chinch bugs.

Sample Preparation

Frozen St. Augustinegrass samples were ground into powder using a mortar chilled with liquid nitrogen. Soluble proteins were extracted using 5 ml 20 mM HEPES (4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid) buffer (pH 7.2) containing protease inhibitors [0.3 ml/g plant tissue, a mixture of 1 μM bestatin, 0.2 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 1 μM pepstatin A, 1 μM E-64, 10 μM leupeptin and 1 mM 1, 10-phenanthroline

(Sigma, St. Louis, MO) and 1% (wt/v) polyvinylpyrrolidone]. The homogenate was filtered through a 4X muslin cloth and centrifuged at $10,000 \times g$ for 10 min at 4°C . The supernatant was collected, desalted using a PD-10 desalting column (Sephadex G-25M, Amersham Biosciences Inc., Piscataway, NJ), and stored at -80°C until protein and enzyme analyses.

Estimation of Total Protein

Total protein content and enzyme activities were determined using a Beckman Model DU 640 Ultraviolet-Visible spectrophotometer. The total protein content was determined by following the modified method of Lowry et al. (1951) using the Folin-Ciocalteu phenol reagent (Pierce Chemical, Rockford, IL) and bovine serum albumin as a standard.

Enzyme Activities

The soluble POX specific activity was measured with guaiacol (Sigma, St. Louis, MO) as a substrate. Increase in absorbance at 470 nm was monitored for 2 min using a protocol modified from Hildebrand et al. (1986) and Hori et al. (1997). The reaction was started by adding $10\mu\text{l}$ of 30% (v/v) H_2O_2 to a quartz cuvette containing $100\mu\text{l}$ 250 mM HEPES-KOH, pH 7.0, $5\mu\text{l}$ protein extract, $300\mu\text{l}$ 18 mM guaiacol, and $585\mu\text{l}$ distilled water. The POX specific activity ($\mu\text{M mg protein}^{-1} \text{ min}^{-1}$) was calculated using the molar absorptivity of guaiacol at 470 nm ($\epsilon = 26.6 \text{ M}^{-1} \text{ cm}^{-1}$).

Catalase-specific activity was determined following a modified protocol from Hildebrand et al. (1986). The reaction was started by adding $200\mu\text{l}$ protein extract to a quartz cuvette containing $100\mu\text{l}$ 250 mM HEPES-KOH, pH 8.0, $100\mu\text{l}$ 75 mM H_2O_2 (Sigma, St. Louis, MO) and $600\mu\text{l}$ distilled water. The reaction was monitored at 240 nm for 2 min and the CAT specific activity was determined using the molar absorptivity of H_2O_2 at 240 nm ($\epsilon = 43.6 \text{ M}^{-1} \text{ cm}^{-1}$) and expressed as $\mu\text{moles mg protein}^{-1} \text{ min}^{-1}$.

PPO activity was measured using a protocol modified from Hori et al. (1997). The reaction was started by adding 25 μ l of protein extract to a cuvette containing 100 μ l 250 mM HEPES-KOH, pH 6.0, 500 μ l 1.6% catechol (Sigma, St. Louis, MO) and 375 μ l distilled water. PPO activity was monitored at 470 nm for 2 min and calculated as the change in A_{470} $\text{mg protein}^{-1} \text{ min}^{-1}$.

LOX activity was determined using a protocol modified from Skorzynska-Polit and Krupa (2002). Linoleic acid (Fluka, St. Gallen, Switzerland) substrate was prepared by following a protocol described in Koch et al. (1992). A 10 mM stock solution of sodium linoleate was prepared by mixing 14 μ l linoleic acid, 14 μ l Tween-20 and 2 ml oxygen-free distilled water by drawing back and forth with a Pasteur pipette which produced a milky emulsion. The emulsion was cleared on addition of 0.11 ml 0.5 M NaOH. The volume was then made up to 5 ml with oxygen-free distilled water and dispensed to 2-ml screw-capped vials which were flushed with N_2 and stored at -20°C . The reaction was started by adding 10 μ l 10 mM linoleic acid to a quartz cuvette containing 50 μ l protein extract, 100 μ l 250 mM HEPES-KOH (pH 7.0) and 860 μ l distilled water. The change in absorbance at 234 nm was recorded for 5 min. The LOX activity was expressed as the change in A_{234} $\text{mg protein}^{-1} \text{ min}^{-1}$.

Isozyme Profile Studies

Isozyme expression of POX, PPO and LOX was studied using native gel electrophoresis (Mini-Protean III, Bio-Rad Laboratories, Inc., Hercules, CA). Equal amounts of protein (30 μ g/well) were loaded into 10-well 4.5 % (wt/v) and 7.5% (wt/v) polyacrylamide discontinuous gels for POX and PPO and 5.0% (wt/v) and 12.5% (wt/v) gels for LOX. Tris-glycine (pH 8.3) continuous buffer system was used to run the gel for 120 min at 30 mA at 4°C . POX and PPO isozymes were stained using a protocol modified from Vallejos (1983) and LOX isozymes were stained according to a protocol by Heinisch et al. (1996).

Gels to be stained for POX activity were incubated in 50 mM sodium acetate buffer (pH 5.0) at $25 \pm 2^\circ\text{C}$ on a platform shaker set at 50 rpm. After 10 min of incubation, 20 μl 30% H_2O_2 and 10 mg 4-chloronaphthol (dissolved in 0.5 ml methanol) were added to the buffer.

Peroxidase activity was visualized as dark blue bands after approximately 20 min. Gels to be stained for PPO activity were soaked in 20 mM HEPES (pH 7.2) buffer containing 5 mM DL- β -(3,4-dihydroxyphenylalanine) (DOPA, Sigma, St. Louis, MO) for 30 min at $25 \pm 2^\circ\text{C}$ on a platform shaker set at 50 rpm. PPO activity was visualized as dark brown bands on a clear background after 30 min.

Gels to be stained for LOX activity were briefly rinsed in distilled water and incubated for 30 min in linoleic acid substrate solution at 4°C on a platform shaker set at 50 rpm. The gel was rinsed again in distilled water, and then 20 ml of the staining solution was added. It was incubated on a shaking platform set at 50 rpm at $25 \pm 2^\circ\text{C}$ for 10 min. LOX activity was observed as pink bands. The substrate solution was prepared by mixing 50 μl linoleic acid and 50 μl 95% ethanol using a magnetic stirrer in a 2-ml screw cap vial at 4°C by exclusion of light. The solution was transferred into a 50-ml flask and the volume was made up to 50 ml with 0.2 M sodium borate buffer (pH 9.0). The staining solution was prepared by mixing 0.5g *N, N*-dimethyl-*p*-phenylenediamine, 4.5 ml methanol and 0.5 ml acetic acid (1:1, acid : water) and the volume was made up to 50 ml with distilled water.

Statistical Analysis

The study was set up using a completely randomized design with five replications. The means of enzyme activities in polyploid lines (FX-10 and Floratam) and diploid lines (NUF-76 and Palmetto) were analyzed separately using PROC GLM (SAS 1999) and Tukey's test was used for mean separations. In the two-way ANOVA model, time after treatment and grass lines were considered fixed effects, while replicate plants were random.

Results

Total Protein Content

There were no significant differences in total extractable protein content between chinch bug-infested and control plants for any of the St. Augustinegrass lines at any time periods after infestation ($P > 0.05$, $n=5$) (Figure 3-1).

Peroxidase Activities

FX-10 and NUF-76 plants infested with southern chinch bug had nearly two-fold higher POX-specific activities 5 and 8 d after infestation compared to their respective controls ($F = 52.68$, $df = 3, 64$, $P < 0.0001$, $n = 5$) (Figure 3-2). Two-way ANOVA found no significant interaction between St. Augustinegrass lines and time ($F = 1.8$, $df = 9, 64$, $P < 0.07$). POX activities in infested FX-10 plants on 5 and 8 d were significantly higher than levels in the susceptible polyploid, Floratam, whether infested or uninfested. The southern chinch bug-susceptible Floratam and Palmetto did not have any significant difference in POX specific activity between infested and control plants at any of the time periods studied ($F = 1.69$, $df = 3, 64$, $P = 0.08$) (Figure 3-2).

Polyphenol Oxidase Activities

The southern chinch bug-infested NUF-76 plants had significantly higher PPO activity 5 and 8 d after infestation compared to their respective control plants ($F = 21.39$, $df = 3, 64$, $P < 0.05$, $n = 5$) (Figure 3-3). There were no significant differences in PPO specific activity between the southern chinch bug-infested and control plants of FX-10, Floratam ($F = 1.98$, $df = 3, 64$, $P = 0.1255$) and Palmetto ($F = 1.61$, $df = 3, 64$, $P = 0.10$) at any of the time periods after infestation. With the exception of NUF-76 plants 5 and 8 d after infestation, there were no significant differences in PPO activities among infested and control NUF-76 and Palmetto plants.

Lipoxygenase Activities

FX-10 plants infested with southern chinch bug had significantly higher LOX specific activity 3, 5 and 8 d after infestation compared to their respective control plants ($F = 312.96$; $df = 3, 64$; $P < 0.0001$, $n = 5$) (Figure 3-4). There was a significant interaction between St. Augustinegrass line and time ($F = 25.46$, $df = 9, 64$; $P < 0.0001$). LOX specific activity in southern chinch bug-infested FX-10 plants peaked 3 d after infestation when it was 2-fold higher than 1 d after infestation, and then declined 5 and 8 d after infestation. The LOX activities in southern chinch bug-infested and control Floratam plants were not significantly different. However chinch bug-infested FX-10 plants had significantly higher LOX activity than uninfested FX-10 controls, Floratam controls and FX-10 and Floratam infested plants on 1 and 3 d (Figure 3-4). Two-way ANOVA found no difference in LOX activities between FX-10 controls, Floratam control and infested plants at any of the time periods studied. There were no significant differences in LOX specific activities between southern chinch bug-infested and control NUF-76 and Palmetto plants ($F = 1.69$, $df = 3, 64$, $P = 0.18$).

Catalase Activities

There were no significant differences observed in CAT specific activity between southern chinch bug-infested and control plants at any time period after infestation in any of the St. Augustinegrass lines studied (Figure 3-5).

Isozyme Profile Studies

Native gels stained for POX-specific activity showed five isozymes in FX-10; two of them were induced 5 and 8 d after infestation compared to their respective controls (Figure 3-6). There were three POX isozymes in NUF-76 with no apparent difference between infested and control plants. There were three POX isozymes in Palmetto and five in Floratam with no difference between infested and control plants at any of the times after infestation (Figure 3-6).

Native gels stained for PPO identified two isozymes in NUF-76, Palmetto and Floratam and three in FX-10 with no apparent difference between southern chinch bug-infested and controls plants at any of the time periods studied (Figure 3-7). There were two LOX isozymes seen in NUF-76, four in Palmetto, three in FX-10, and one in Floratam (Figure 3-8). There were no apparent differences in LOX isozyme intensity or number between southern chinch bug-infested and controls plants.

Discussion

There was no significant difference in extractable total protein content of southern chinch bug-infested and control St. Augustinegrass plants at any time after infestation, although southern chinch bug-infested FX-10 plants showed an overall increase in protein content over time. Our results agree with those of Heng-Moss et al. (2004) who found no increase in total protein content after infestation by the western chinch bug, *Blissus occiduus*, of resistant Buffalograss varieties ‘Cody’ and ‘Tatanka’. However Ni et al. (2001) reported a significant increase in total protein content in wheat varieties infested with the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), and the corn leaf aphid, *Rhopalosiphum padi* (L.), in contrast to an earlier report of a two-fold decrease in total protein 2 d after infestation by *D. noxia* (Van der Westhuizen and Pretorius, 1995).

We found both chinch bug-resistant lines, FX-10 and NUF-76 had a nearly two-fold increase in POX specific activity levels 5 and 8 d after infestation compared to uninfested control plants. Our results agreed with many previous reports that POX specific activity levels increase after herbivore infestation (Hildebrand et al. 1986; Felton et al. 1994a, b; Van der Westhuizen et al. 1998; Ni et al. 2001; Chaman et al. 2001; Heng-Moss et al. 2004; Khattab, 2007). Lupin varieties resistant to green peach aphid, *Myzus persicae* (Sulzer), had significantly different

levels of POX and PPO activity, protease inhibitors and soluble phenolics. However, the levels of PPO and POX were not increased after the aphid infestation (Cardoza et al. 2005)

Herbivore damage is known to induce production of H_2O_2 in plants (Dowd and Lagrimini, 1997). The level of H_2O_2 in plants is regulated by oxidative enzymes such as POX and CAT, and it has been demonstrated *in vitro* that POX activity is dependent on the supply of H_2O_2 (Duffey and Felton, 1991). An increase in POX activity in insect-resistant plants may detoxify the peroxides, thus reducing plant tissue damage compared to susceptible plants (Hildebrand et al. 1986). Peroxidases are involved in plant cell wall-building processes, by mediating the oxidation of hydroxycinnamyl alcohols into free radical intermediates, cross-linking of polysaccharides and extensin monomers, lignification and suberization (Chittoor et al. 1999). Brisson et al. (1994) suggested that the oxidative cross-linking of the plant cell wall could enhance its ability to slow pathogen ingress. Such a reinforcement of the cell wall in response to insect feeding may serve as a mechanical barrier and prevent the insect's stylets from reaching their feeding sites, phloem sieve elements in the case of southern chinch bug. An enhancement of the POX isozyme activity in maize (*Zea mays* L.) increased resistance to herbivores and pathogens (Dowd and Lagrimini, 1997). Increased POX activities in FX-10 and NUF-76 in our study suggest that these two lines may have increased cell wall lignification.

Plant catalases scavenge H_2O_2 that occurs in mitochondria and peroxisomes and reduce them into H_2O and O_2 . Zhu-Salzman (2004) found that the CAT genes were down-regulated in resistant sorghum plants infested with greenbug and speculated that the reduced CAT activity could help the plant to maintain high H_2O_2 levels thus causing damage to the insect midgut. Park et al. (2006) also found that CAT genes were down regulated in greenbug resistant sorghum lines. However, Heng-Moss et al. (2004) reported a loss in CAT activity in response to feeding

by *B. occiduus* on susceptible buffalograss varieties. Our study found no association between CAT-specific activity levels and southern chinch bug resistance in St. Augustinegrasses, and supports previous reports using other plant-insect combinations indicating no relationship between CAT activity and resistance (Faccioloi, 1979; Matkovics et al. 1981; Hildebrand et al. 1986).

The association of PPO activity with host plant resistance to insects has been reported in many plants including tomato, potato, coffee and poplar (Duffey and Felton, 1991; Constabel et al. 1996; Wang and Constabel, 2004; Chaman et al. 2001; Thipyapong et al. 2006). In our studies, only NUF-76 had significantly higher levels of PPO specific activity as a result of southern chinch bug infestation, whereas FX-10 showed no such induction. Heng-Moss et al. (2004) found no association between PPO activity in buffalograsses and their resistance to *B. occiduus*.

Our studies showed that FX-10 had a two-fold higher LOX-specific activity 3 d after southern chinch bug infestation, and a reduced but still significantly higher activity 5 and 8 d after infestation compared to the control plants (Figure 3-4). Increased activity of LOX has been reported in soybean in response to two-spotted spider mite infestation (Hildebrand et al. 1986) and attack by larvae of *Helicoverpa zea* (Boddie) (Felton et al. 1994b). The increased levels of LOX activity caused oxidative damage in midgut epithelial cells of *H. zea* larvae and reduced their growth (Felton et al. 1994b). Bi et al. (1997) showed significant increase in LOX in *H. zea* damaged cotton squares along with altered phenolic contents. Gene expression studies showed that LOX transcripts were strongly elicited in tomato plants in response to attack by the aphids, *Macrosiphium euphorbiae* (Thomas) and *M. persicae* (Fidantsef et al. 1999) and were locally upregulated in *Nicotiana attenuata* after *Macrosiphium nicotianae* Blackman infestation

(Voelckel et al. 2004). LOX-deficient *N. attenuata* plants, when planted into native habitats, became more vulnerable to adapted herbivores such as *Manduca sexta* (L.) and attracted new opportunistic herbivores, such as *Empoasca* spp. (Kessler et al. 2004).

Lipoxygenases are ubiquitous enzymes that catalyze the hydroperoxidation of polyunsaturated fatty acids having *cis*, *cis*-pentadiene moieties (Hildebrand et al. 1986). Linoleic acid and linolenic acid are the major LOX substrates in plants (Hildebrand et al. 1986). Lipid peroxidation by LOX results in formation of fatty acid hydroperoxides which may enzymatically and/or chemically be degraded to highly reactive aldehydes, γ -ketols, epoxides (Gardner, 1991), hydroxyl radicals, singlet oxygen, superoxide ion and peroxy, acyl and carbon-centered radicals (Kanofsky and Axelrod, 1986). Gardner (1979) mentioned that the interactions between lipid hydroperoxides and proteins results in protein-protein cross linking, and amino acid damage. These chemical changes in proteins may affect the assimilation of amino acids of insects. These lipid peroxidation end products could be repellent to insect feeding and operate via antixenosis or be toxic and function via antibiosis (Felton et al. 1994b). Both modalities of resistance have been reported previously in St. Augustinegrass against the southern chinch bug (Rangasamy et al 2006). Shukle and Murdock (1983) demonstrated that LOX could directly act as a defense in soybean against insect attack. Lipoxygenases are also involved in inducible herbivore resistance; the LOX oxidation of linolenic acid is the first step in jasmonic acid signaling pathway, which has been implicated in coordination of direct plant defense such as production of oxidative enzymes and protease inhibitors (Staswick and Lehman, 1999, Mao et al. 2007) and indirect plant defense such as production of volatiles (Dicke et al. 1999; Thaler et al. 1999). Rayapuram and Baldwin (2006) investigated the performance of *M. sexta* larvae on *N. attenuata* plants silenced in LOX3 expression and jasmonic acid signaling and found that LOX3-mediated

defense in wild plants reduced larval growth, consumption and frass production compared to the mutant. Mao et al. (2007) transformed maize plants with the wheat oxalate oxidase gene and reported that the up-regulated LOX transcripts and 14-fold higher free phenolics in transgenic plants were positively associated with their resistance to the European corn borer (*Ostrinia nubilalis* H.) compared to the control plants.

Based on the results from our research, we associate the increased levels of POX and LOX in FX-10 and POX and PPO in NUF-76 with southern chinch bug resistance in these lines. The increased POX and LOX activities could be a cause of antixenosis in FX-10 and NUF-76 and antibiosis in NUF-76 found in our previous studies (Rangasamy et al. 2006, Chapter 2). Increased POX activity seems to be due to the induction of specific isozymes. Together, our results support the hypothesis that oxidative enzymes play a vital role in herbivore resistance in plants. Our data suggest that identification of genes encoding inducible PPO, POX, and LOX enzymes could be the first step in understanding southern chinch bug resistance in St. Augustinegrass and will be valuable markers for breeding southern chinch bug resistant lines. The presence of different kinds of southern chinch bug resistance in FX-10 and NUF-76 is in agreement with our previous studies (Rangasamy et al. 2006, Chapter 2). Our research supports the hypothesis that chinch bug resistance in St. Augustinegrass has a metabolic basis and highlights the possible involvement of lignification and oxidative enzymes in secondary product synthesis in chinch bug resistance in St. Augustinegrass.

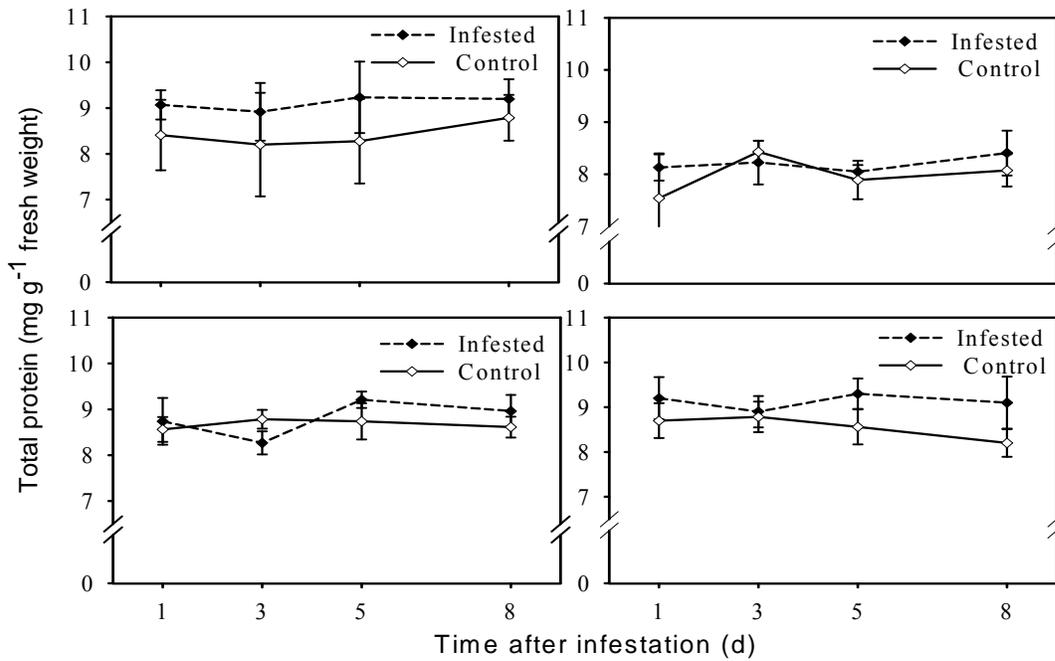


Figure 3-1. Total protein content in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding. A. FX-10, SCB resistant polyploid. B. Floritam, SCB susceptible polyploid. C. NUF-76, SCB resistant diploid. D. Palmetto, SCB susceptible diploid. The values shown are mean \pm SE of five replicates.

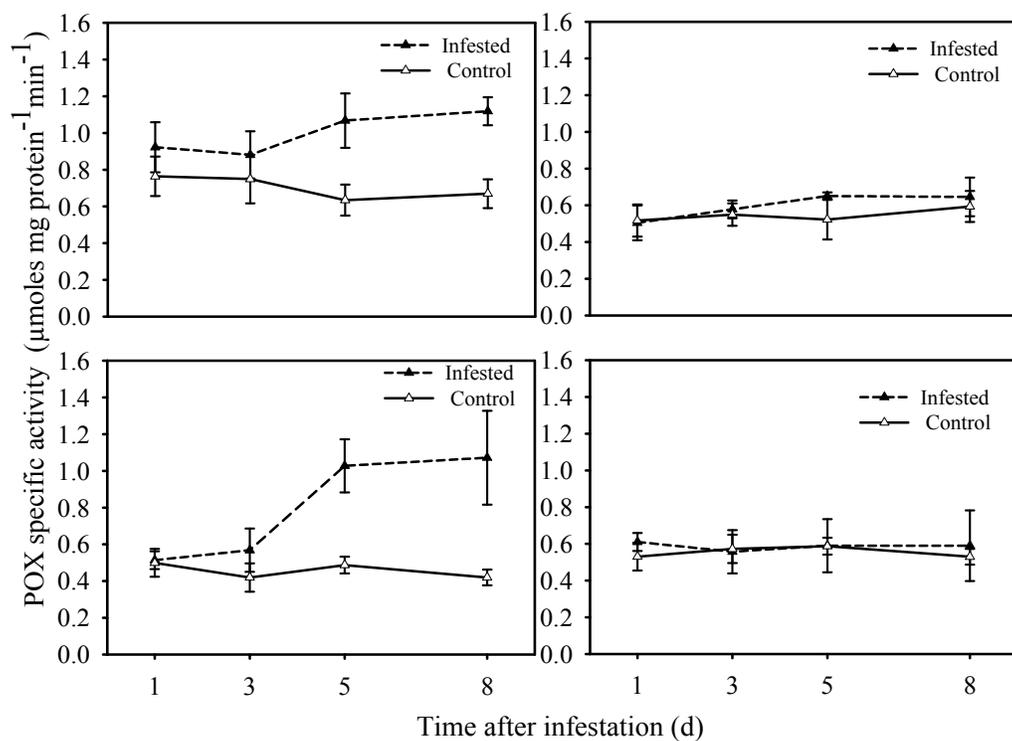


Figure 3-2. Levels of peroxidase (POX) specific activity levels in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding. The values shown are mean \pm SE of five replicates. Means marked with * differ significantly between control and infested treatments ($P < 0.05$, Tukey's test).

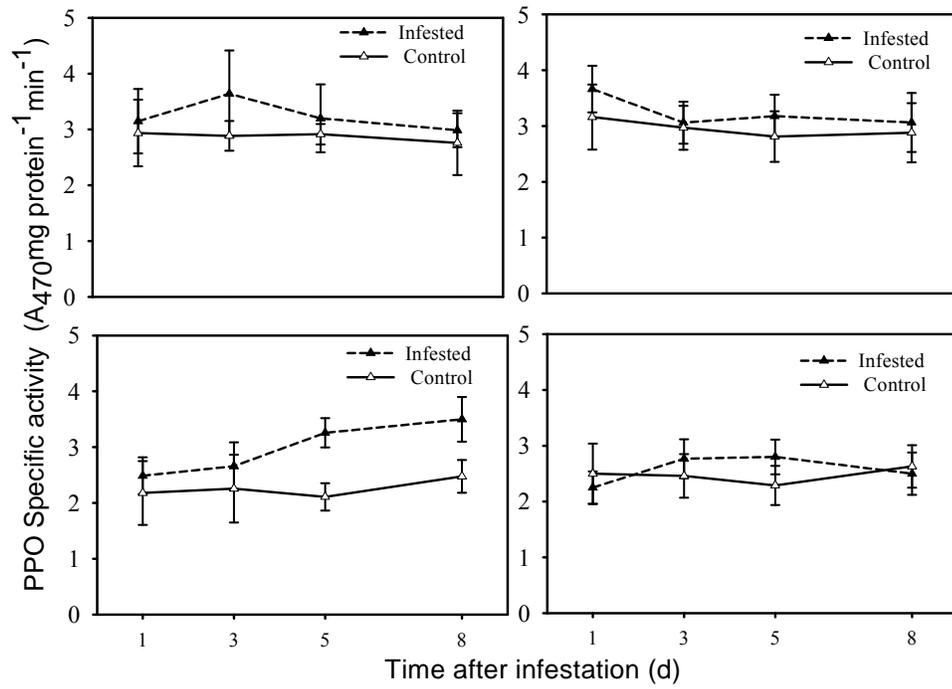


Figure 3-3. Levels of polyphenoloxidase (PPO) specific activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding. The values shown are mean \pm SE of five replicates. Means marked with * differ significantly between control and infested treatments ($P < 0.05$, Tukey's test).

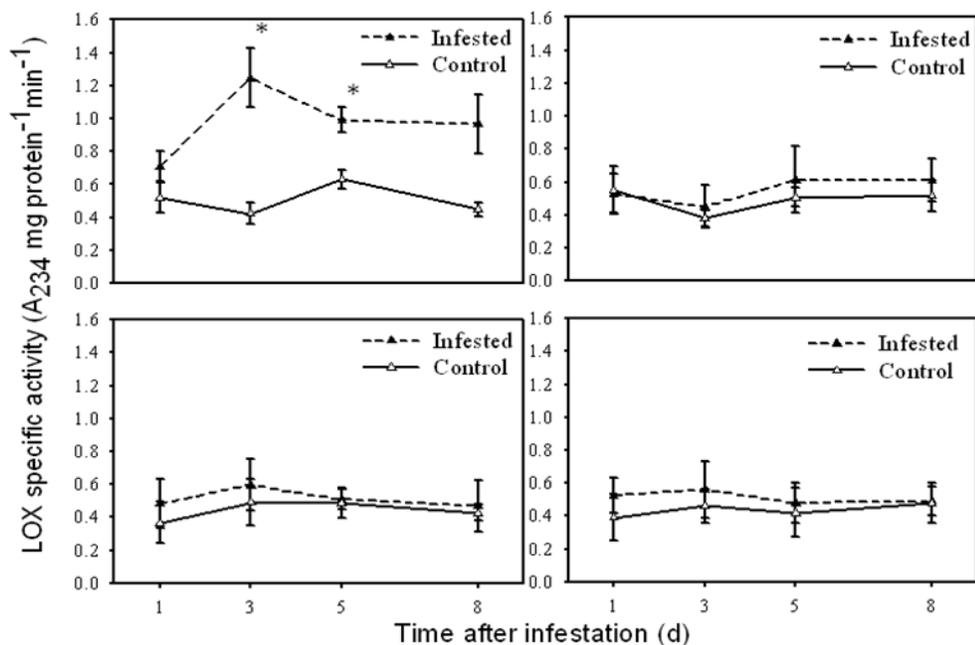


Figure 3-4. Levels of lipoxygenase (LOX) specific activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding. The values shown are mean \pm SE of five replicates. Means marked with * differ significantly between control and infested treatments ($P < 0.05$, Tukey's test).

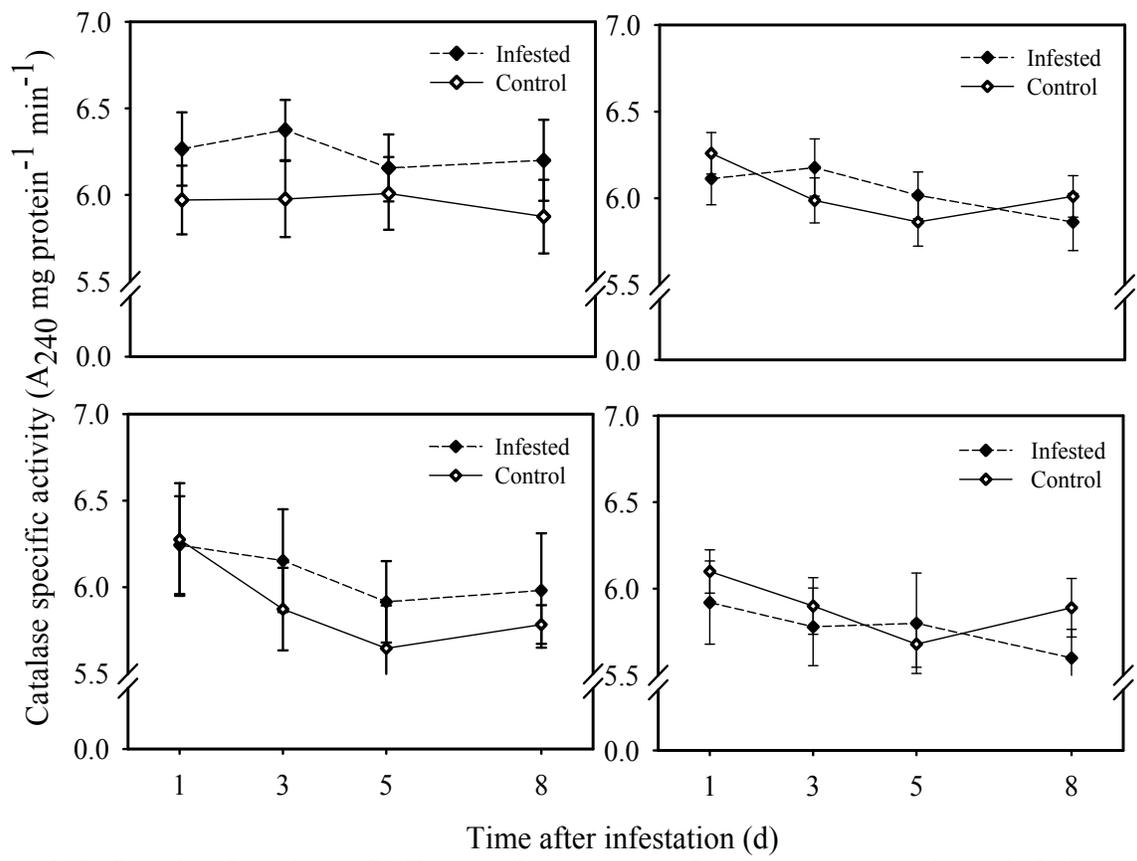


Figure 3-5. Levels of catalase (CAT) specific activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding. The values shown are mean \pm SE of five replicates. Means marked with * differ significantly between control and infested treatments ($P < 0.05$, Tukey's test).

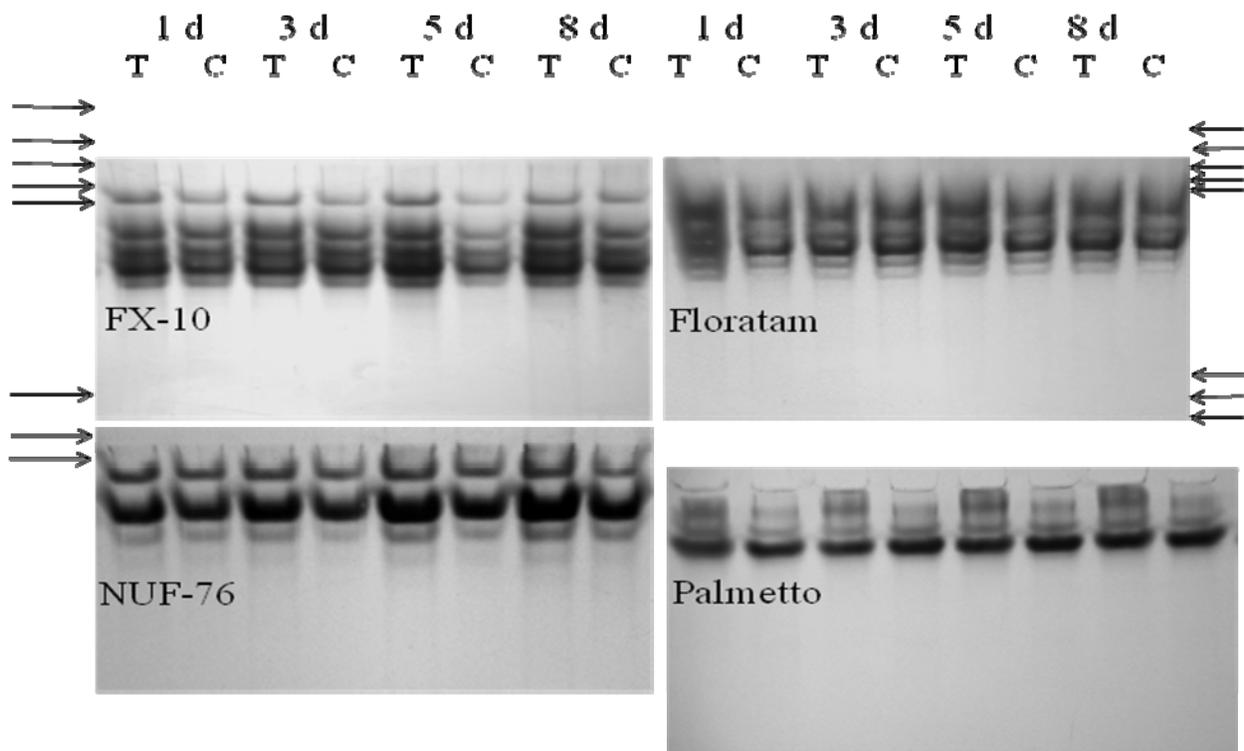


Figure 3-6. Native gels stained for POX activity in St. Augustinegrass lines 1, 3, 5, and 8 d after initiation of southern chinch bug feeding. T and C refer to the infested (i.e., treated) and control samples, respectively. Arrows indicate the number of POX isoforms.

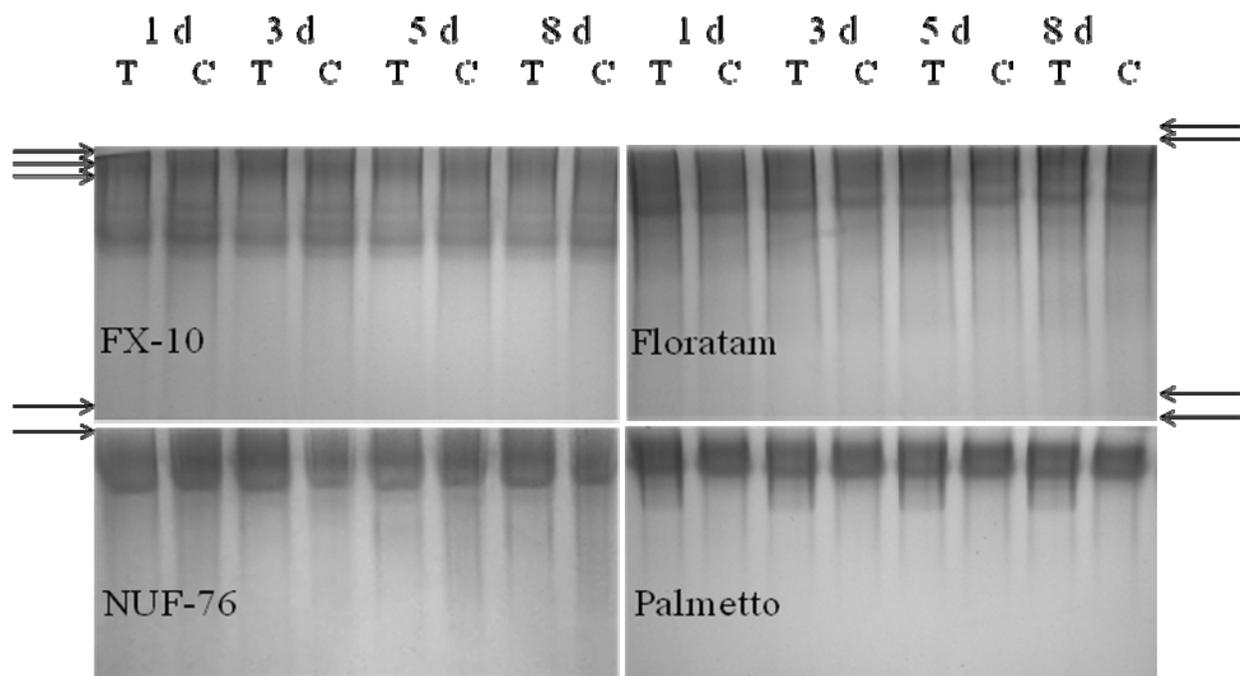


Figure 3-7. Native gels stained for PPO activity in St. Augustinegrass lines 1, 3, 5, and 8 d after challenge by southern chinch bug. T and C refer to the infested (i.e., treated) and control samples, respectively. Arrows indicate the number of PPO isoforms.

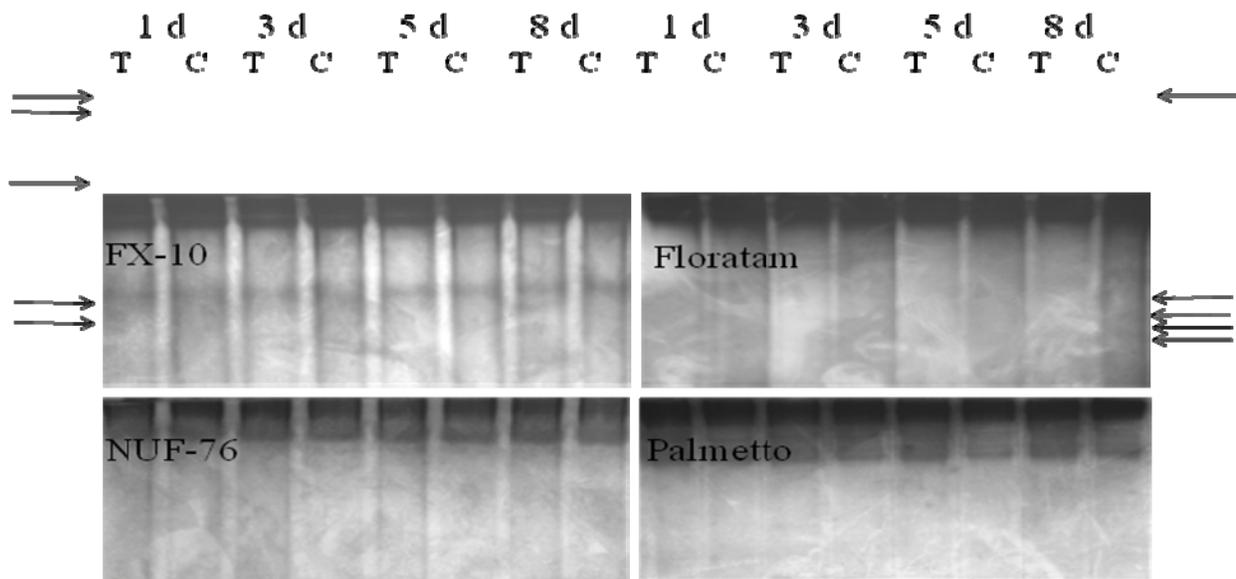


Figure 3-8. Native gels stained for LOX activity in St. Augustinegrass lines 1, 3, 5, and 8 d after challenge by southern chinch bug. T and C refer to the infested and control samples, respectively. Arrows indicate the number of LOX isoforms.

CHAPTER 4
QUANTITATIVE ANALYSIS OF FEEDING BEHAVIOR OF SOUTHERN CHINCH BUG,
BLISSUS INSULARIS BARBER (HEMIPTERA: BLISSIDAE), ON RESISTANT AND
SUSCEPTIBLE ST. AUGUSTINEGRASSES

Introduction

St. Augustinegrass is the most widely planted turfgrass in Florida and other Gulf coast states. St. Augustinegrass is estimated to cover more than one and a half million acres in Florida (Hodges et al. 1994) and accounts for 85% of the sod industry in Florida with a value of \$262 million annually (Haydu et al. 2005). The southern chinch bug, *Blissus insularis* Barber, is the most serious insect pest of St. Augustinegrass (Crocker 1993). The annual cost of southern chinch bug management and losses in Florida alone was estimated at \$5 million in 1983 (Hamer 1985) and is undoubtedly much more today. The economic importance of this insect is further augmented by its development of resistance to several key insecticides (Reinert and Portier 1983, Cherry and Nagata 2005, 2007).

Historically, host plant resistance has been one of the most successful pest management methods for this insect. ‘Floritam’, a polyploid variety of St. Augustinegrass with resistance to southern chinch bug, has been available in Florida and Gulf Coast areas since its release in 1973 (Horn et al. 1973). However, by 1985 a population of chinch bug had overcome Floritam’s resistance in Florida (Busey and Center 1987). Although significant progress has been made in identifying new sources of southern chinch bug resistance in St. Augustinegrass lines, such as the polyploid FX-10 (Busey 1993) and the diploid NUF-76 (Nagata and Cherry 2003), the mechanisms of resistance in these lines are unknown. Rangasamy et al. (2006) investigated categories of resistance to southern chinch bug in FX-10 and NUF-76 and reported high levels of antixenosis in both lines and possible antibiosis in NUF-76 (Chapter 2). Higher mortality of southern chinch bugs has been reported on resistant FX-10 and NUF-76 than on susceptible

Floritam, Palmetto and Bitter Blue (Nagata and Cherry 2003); however, the cause of mortality is still unknown. The mortality may be caused by toxic secondary metabolites present in phloem sap, lack of adequate nutrition after sustained feeding or starvation due to reduced feeding on resistant varieties. Gaining a better understanding of the feeding behavior of southern chinch bugs on these resistant and susceptible St. Augustinegrasses is essential to elucidate further the mechanisms of resistance in FX-10 and NUF-76.

Studying the feeding behavior of insects with piercing and sucking mouth parts is more difficult compared with insects with chewing mouthparts and requires specialized techniques. McLean and Kinsey (1964) developed a unique technique, the electronic feeding monitor which was later referred to as the electrical penetration graph (EPG) (Tjallingii 1985) to study the feeding behavior of hemipterans. The EPG monitor has gone through many modifications and improvements (Brown and Holbrook 1976, Kawabe and McLean 1980, Kimsey and McLean 1987, Backus and Bennett 1992, Backus and Bennett, unpublished) and today is the most rigorous technique for studying feeding behavior and plant interactions of hemipterans. The basic principles of EPG have been well described by Walker (2000). In brief, the EPG monitor consists of a voltage source and an amplifier which are connected to each other. Both the insect and plant are connected to the EPG monitor thus becoming a part of the circuit. The insect is connected to the amplifier using a very thin gold wire and silver conducting paint and the plant is connected to the voltage source in the EPG monitor using a conductive probe (an uninsulated stiff copper wire). Whenever the insect attempts to probe or inserts its stylet into the plant, the circuit becomes complete and the current flows from the voltage source through the plant, the insect, the amplifier (via its input resistor) and then back to the voltage source (Walker 2000). The plant and the insect act as the biological component of the circuit and resist current flow.

The changes in resistance in the insect-plant interface, as well as biopotentials generated, are measured as electrical signals and converted into waveforms which are unique to the insect's activities inside the plant.

In the research reported here, we used the EPG technique to investigate the stylet probing of southern chinch bugs on St. Augustinegrass. The St. Augustinegrasses selected for our studies included FX-10 (resistant polyploid), NUF-76 (resistant diploid), Floratam (susceptible polyploid), and 'Palmetto' (susceptible diploid).

Materials and Methods

Plants

Plants were grown as described in Rangasamy et al. (2006) (Chapter 2). Stolons used in experiments were approximately 30 to 40 cm in length with a minimum of three nodes and were cut from plants that were 6 to 8 weeks old.

Insects

Southern chinch bugs were collected from Royal Palm Beach (Palm Beach County, FL) by vacuuming infested St. Augustinegrass lawns as described by Cherry (2001a). Adults and nymphs were collected by suction into a modified WeedEater[®] Barracuda blower/vacuum (Poulan/Weedeater, Shreveport, LA). In the laboratory, fifth instars were separated from the debris and provided with fresh Bitter Blue runners and maintained at 26°C in 20-l plastic buckets with a moist paper towel at the bottom to avoid desiccation of insects. Only newly emerged 3-5 d old adult females were used for the experiment.

Wiring and Acclimating of the Insects

Insects were removed from the colony and starved for 3 h before wiring. A 12.7- μm -diameter (0.0005 in.) gold wire (Sigmund Cohn Corp., Mt. Vernon, NY) was glued to the

pronotum of the insect using silver conductive paint (Ladd Industries, Burlington, VT). The insect was held immobile during application of the wire tether by placing its abdomen in a Pasteur pipette tip under low vacuum suction pressure. After wiring, the insects were allowed to rest on a moist paper towel and acclimate to the wire for a 1-h period before recording.

EPG Equipment and Data Acquisition

A four-channel AC-DC electrical penetration graph (EPG) monitor (Backus and Bennett, unpublished data) was used for recording the feeding behavior of southern chinch bug on *St. Augustinegrass*. Plants, insects and the head-stage amplifiers were all placed in a Faraday cage, constructed of 1.27-cm mesh galvanized hardware metal cloth (LG Sourcing Inc., N. Wilkesboro, NC), to avoid any external electrical noise. An input impedance level of $10^7 \Omega$ was used for all four channels and all the test plants were supplied with a 10 mV, 1000 Hz AC substrate voltage. The pots containing the plants were placed on wooden blocks to electrically isolate them from the Faraday cage. A selected runner from each *St. Augustinegrass* plant was laid down along a Plexiglas stage measuring 20 cm length \times 5 cm width and fixed in place using strips of Parafilm (Pechiney Plastic Packaging, Menasha, WI). The Plexiglas stage was held horizontally by an alligator clip connected to a “helping hand” holder (van Sickle Electronics, St. Louis, MO). The use of the horizontal stage allowed insects that had left the plant the opportunity to return by walking to the plant (unless they fell off the stage and hung suspended from their wire tether). The changes in electrical resistance as well as biopotentials during stylet probing were amplified, rectified and digitized at a rate of 100 samples per second using a DI-720 analog-to-digital board as described by Almedia and Backus (2004) and recorded using a Dell Pentium notebook with WinDaq Pro software (Dataq Instruments, Akron, OH). All recording started at 1600 h (in an isolated room at $26 \pm 2^\circ\text{C}$ with photoperiod of L18:D6) and

continued for 24 h simultaneously on four St. Augustinegrass lines; probing was artificially terminated for insects with mouthparts still inserted in the plant at 24 h. There were 35 recordings per treatment; each consisting of all four St. Augustinegrasses arranged randomly inside the Faraday cage and connected individually to each of the four channels in the monitor. However, recordings where one or more of the insects within the replicate were not active or dead were discarded, leaving 22 replicates that were analyzed statistically. Waveform

Characterization and Measurements

The waveforms produced by southern chinch bug feeding on St. Augustinegrass were categorized based on stereotypical patterns (Backus et al., ms. in prep.). In brief, there are four waveform families (Z, G, H, and I), and six waveform types (Z1, Z2, G1, G2, I1 and I2) within those families (Figure 1). For the purpose of comparing the waveforms produced on southern chinch bug resistant and susceptible St. Augustinegrasses, only the major waveform categories of Z1, Z2, P (pathway, G1 + G2 + H) and I (ingestion, I1 + I2) were analyzed in this research. The duration measurements for each waveform were made with WinDaq /Pro +Waveform Browser software at a compression of 10 (2 sec/div.) using 2 sec as the threshold duration for a particular waveform event. The files were copied into a Microsoft Excel file developed for EPG analysis by van Giessen and Jackson (1998) and modified for *B. insularis*.

Waveform Categories

The following waveform categories used in the analysis are depicted in Figure 4-1.

- **Z1:** Insects off the plant,
- **Z2:** Non-probing baseline,
- **G1:** variable-frequency and high-amplitude waveforms; always occur at the beginning of a probe after Z2 and followed by G2, presumably associated with stylet pathway activities,
- **G2:** hump-like, high-amplitude putative pathway waveform,

- **H:** low-amplitude putative pathway waveform, always preceded by G2,
- **I:** Putative ingestion, very uniform, repetitive (7-8 Hz) waveform with peaks and waves (Figure 4-1).

E. A. Backus (personal communication) suggests that I1 and I2 may be associated with either active and passive ingestion or salivation and ingestion, respectively. No biological correlation for either stylet activities or location has been established to differentiate between I1 and I2 waveform types at this time; however it is likely that at least part of this ingestion is from phloem.

Definition of Non-Sequential Parameters

Non-sequential parameters used in this study were defined by Backus et al. (2007). A probe is defined as all behaviors occurring from the beginning of stylet penetration probing into plant tissue until stylet withdrawal. A waveform event is defined as a continuous, uninterrupted occurrence of one waveform type (Almeida and Backus 2004, Backus et al. 2007). An accumulator parameter for an insect is designated “by insect” and a parameter averaged across the N insects assayed is termed “per insect”.

Statistical Analysis

Statistics of waveforms from the experiment were analyzed using statistical analysis software (SAS 1999) and the procedure described by Almeida and Backus (2004) and Backus et al. (2007). The descriptive statistics for feeding behaviors were compiled at the heuristic levels of waveform event, probe, insect and cohort as described in Backus et al. (2007). A cohort-level waveform analysis will show the total behavioral repertoire of the southern chinch bug population tested. Non-sequential parameters for EPG data were the same as those named and described in Backus et al. (2007). In brief, the basic parameters of EPG data at the event level are the number of waveform events per probe (NWEP) and waveform duration per event (WDE).

The values in these parameters are combined to achieve parameters at the probe level, such as the number of probes per insect (NPI), and probing duration per probe (PDP). Probe level values were combined for parameters at the insect level, such as probing duration per insect (PDI), waveform duration per insect (WDI), and number of waveform events per insect (NWEI). When more than one waveform event occurs during a probe, the probe-level data provide sequence information, transition information or both (Backus et al. 2007). The insect-level data provide behavioral information on individual insects as well as of an average insect in the cohort.

Restricted maximum likelihood estimation (REML-ANOVA) (PROC MIXED, SAS 1999) was used to find out whether the frequencies or durations of each waveform type or overall probing were significantly different on the four St. Augustinegrass lines, per event, per probe, per insect or per cohort (Backus et al. 2007). When required, means were then separated using the LSMEANS statement (SAS 1999). Frequency and duration data were square root- and log-transformed, respectively, before ANOVA to improve homogeneity of variances. Differences were considered significant at $\alpha = 0.05$.

Results

Cohort Level

Southern chinch bugs spent only 28% and 46% of their total 24-h plant access time in stylet probing (total probing duration, TPD) on FX-10 and NUF-76, respectively (combination of P and I, Table 4-1; Figure 4-2). However, chinch bugs confined on Floratam and Palmetto spent 56% and 66%, respectively, of their access times in probing. More probing events (TNPE) were recorded from chinch bugs confined on FX-10 and NUF-76 than on Floratam and Palmetto (Table 4-1). Overall, ingestion duration was highest on Palmetto, followed by Floratam, then NUF-76 and lowest on FX-10 (Figure 4-3). It is also valuable to note that only a third of stylet

penetrations in which pathway waveform P began successfully transitioned to the ingestion phase (I1) on FX-10 and NUF-76, whereas nearly half of the probes on Floratam and Palmetto resulted in ingestion waveforms (Table 4-2). To statistically analyze the data, cohort level data were subdivided to event level and rebuilt through probe and insect levels.

Event Level

Probing duration per event (PDE) is the averaged duration of all events, regardless of waveform type. In general, the longest events were made on FX-10, intermediate on Floratam and Palmetto, and shortest events were made on NUF-76 ($F = 46.22$; $df = 3, 2500$; $P < 0.0001$) (Table 4-1). A waveform event is defined as the smallest, continuous duration of a particular waveform type. The duration of each Z1 waveform (i.e., time southern chinch bugs were off the plant) was the longest on FX-10, intermediate on Palmetto and NUF-76 and shortest on Floratam ($F = 3.38$; $df = 3, 111$; $P = 0.02$) (Table 4-3). The duration of Z2 waveform (non-probing, walking or resting on the plant) per event was not significantly different among FX-10, Floratam and Palmetto; however, the duration of Z2 was shorter on NUF-76 compared to the others ($F = 45.62$; $df = 3, 7640$; $P = 0.0001$). The longest duration of the pathway waveform type (P) occurred on FX-10 whereas the shortest pathway waveform event occurred on Floratam; intermediate durations were recorded on NUF-76 and Palmetto, which were not significantly different from one another ($F = 29.51$; $df = 3, 11000$; $P = 0.0001$). The durations of ingestion waveform event (I1, I2 waveforms) on Floratam and Palmetto were four and two times longer than on FX-10 and NUF-76, respectively (I1: $F = 96.44$; $df = 3, 2772$; $P = 0.0001$, I2: $F = 29.45$; $df = 3, 672$; $P = 0.0001$) (Table 4-3).

Probe Level

Southern chinch bugs feeding on resistant FX-10 and NUF-76 produced significantly more probes per insect (NPI) than bugs feeding on susceptible Floratam and Palmetto ($F = 17.64$; $df =$

3, 172; $P = 0.0001$) (Table 4-1). In addition, the mean duration of probes (PDP) on FX-10 and NUF-76 were only one-third to one-half as long as those on Floratam and Palmetto; the duration of probes on the latter two were not significantly different ($F = 46.22$; $df = 3, 25000$; $P = 0.0001$) (Table 4-1). Considering waveforms on a per probe basis, the insects spent the longest time in pathway activities (P waveforms) on FX-10 and the shortest time on Floratam however, no difference was observed between NUF-76 and Palmetto ($F = 7.56$; $df = 3, 697$; $P = 0.0001$) (Table 4-4). The mean durations of ingestion waveforms (both I1, I2) during a probe on FX-10 were only one third that of the duration on Floratam. For I1, duration per probe on FX-10 was not significantly different from that on NUF-76 but both FX-10 and NUF-76 were significantly different from the intermediate duration on Floratam and the longest probe duration on Palmetto ($F = 18.77$; $df = 3, 450$; $P = 0.0001$). The longest durations of I2 on Floratam and Palmetto were not significantly different, but both were greater than the intermediate duration of I2 on NUF-76 and the shortest duration on FX-10 ($F = 15.03$; $df = 3, 257$; $P = 0.0001$) (Table 4-4).

Insect Level

There were no significant differences in the number of times that insects walked off the plant (Z1), on a per insect basis, among the four St. Augustinegrass lines ($F = 0.71$; $df = 3, 45$; $P = 0.51$) (Table 4-5). However, the duration of the times off the plant was longer for FX-10, NUF-76 and Palmetto than for Floratam (Figure 4-3). There was no significant difference observed among FX-10, NUF-76 and Palmetto (Z1, $F = 8.92$; $df = 3, 45$; $P = 0.0001$). The numbers of nonprobing events on the plant (Z2, $F = 8.62$; $df = 3, 84$; $P = 0.0001$) were higher on FX-10 and NUF-76 than on Floratam and Palmetto (Table 4-5). The duration of nonprobing waveforms (Z2) was longest on FX-10, significantly different from the intermediate durations observed on Floratam and NUF-76 and the shortest duration on Palmetto (Z2, $F = 8.92$; $df = 3,$

84; $P = 0.0001$) (Figure 4-3). Chinch bugs confined on FX-10 and NUF-76 produced significantly more pathway waveform events per insect ($F = 5.75$; $df = 3, 84$; $P = 0.0013$) (Table 4-5) that were of longer duration ($F = 8.23$; $df = 3, 84$; $P < 0.0001$) (Figure 4-3) than those produced by insects confined on Floratam and Palmetto. Fewest pathway waveform events per insect were produced on Palmetto whereas an intermediate number of pathway waveform events per insect was observed on Floratam (Table 4-5). Although the number of ingestion (I1 and I2) waveform events per insect did not differ among the four St. Augustinegrass varieties ($F = 0.60$; $df = 3, 84$; $P = 0.6168$) (Table 4-5), the mean durations of ingestion waveform I1 were three and two times longer on Floratam and Palmetto than on FX-10 and NUF-76, respectively ($F = 11.63$; $df = 3, 84$; $P < 0.0001$) (Table 4-3). The mean durations of ingestion waveform I2 were four and two times longer on Floratam and Palmetto than on FX-10 and NUF-76, respectively ($F = 9.82$; $df = 3, 84$; $P < 0.0001$) (Figure 4-3).

Discussion

For the first time, the EPG technique has been used for studying the feeding behavior of southern chinch bug on St. Augustinegrasses. EPG results revealed that the southern chinch bugs were off the plant (Z1 waveforms) on FX-10 and NUF-76 for a longer time than on Floratam and Palmetto. These results agree with our previous studies which showed that fewer southern chinch bugs were found on resistant FX-10 and NUF-76 than on Floratam, Palmetto and Bitter Blue in a choice experiment, suggesting the presence of strong antixenosis (Chapter 2; Rangasamy et al. 2006). When the insects were on the resistant FX-10 and NUF-76 plants, they spent more time in non-probing activities, such as resting or walking, than in ingestion than when on susceptible Floratam and Palmetto. The short durations of sustained ingestion on FX-10 and NUF-76 and long duration of sustained ingestion on Floratam and Palmetto also

corroborate our previous results (Rangasamy et al. 2006; Chapter 2), which showed that significantly fewer excretory spots were produced by chinch bugs fed on FX-10 and NUF-76 than on the susceptible varieties.

Successful insect colonization on a host plant depends on host plant acceptability. For phloem-feeding, piercing and sucking insects such as aphids, host acceptability depends partly on the ability of the insect's stylets to reach the phloem and partly on the quantitative and qualitative properties of phloem sap (Klingauf 1987). Tarn and Adams (1982) and Dreyer and Campbell (1987) discussed that aphid stylet penetration on resistant plants is often characterized by more frequent, but shorter probes, and longer waveforms associated with pathway activities. We found that southern chinch bugs confined on resistant FX-10 and NUF-76 probed more frequently and that their pathway waveforms were longer than on the susceptible Floratam and Palmetto. The durations of ingestion, on per event, per insect and per probe levels, were all significantly reduced in chinch bugs feeding on the resistant St. Augustinegrass varieties compared to the susceptible varieties. Thus, the present study clearly demonstrates that southern chinch bugs spent longer on FX-10 and NUF-76 in activities that produced the pathway waveforms (P) and less time in activities that resulted in ingestion waveforms (I1, I2). These results indicate the presence of a physical barrier around the vascular bundle or chemical deterrents from the phloem of the resistant cultivars. Similar to our results, the aphids, *Myzus persicae* (Sulz.) and *Nasonovia ribisnigri* (Mosley), produced many probes, longer waveforms associated with extracellular stylet pathway activities and shorter waveforms associated with phloem ingestion on resistant lettuce plants, *Lactuca sativa* L., suggesting that aphid resistance might involve both mesophyll and phloem factors (Montllor and Tjallingii 1989).

Our results suggest that southern chinch bug can perceive significant differences among the four St. Augustinegrasses before their stylets reach the phloem sieve elements; thus, the resistance factors in FX-10 and NUF-76 may reside in tissue encountered by the chinch bug stylets prior to reaching the vascular bundle. Dreyer and Campbell (1984) and Campbell and Dreyer (1985) found that a chemical modification of pectin in the middle lamella of some sorghum varieties, *Sorghum bicolor* L. (Moench) made it difficult for the intercellular stylet penetration by certain biotypes of greenbug, *Schizaphis graminum* (Rondani). It is possible that a similar resistant mechanism occurs in the resistant St. Augustinegrasses. Transmission electron microscopic studies on St. Augustinegrass leaf sheath anatomy revealed that the sclerenchyma cells in NUF-76 and FX-10 have thicker cell walls (Chapter 5) which may serve as a physical barrier and prevent or make it difficult for southern chinch bug stylets to reach the phloem sieve elements.

Resistance factors residing in sieve elements are often indicated by short durations of ingestion waveforms (Campbell et al. 1982, Ryan et al. 1987, van Helden and Tjallingii 1993, Cole 1994, Caillaud et al. 1995, Paul et al. 1996, Klingler et al. 1998, Sauge et al. 1998, Kaloshian et al. 2000, Tjallingii 2006). Jiang and Walker (2007) found that the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, terminated its ingestion waveforms more often and spent reduced amount of time in ingestion on whitefly-resistant alfalfa clones than on susceptible clones, and suggested that this ingestion behavior could be due to the chemical constituency of the phloem sap, such as the presence of toxins or repellents or lack of stimulants. The shorter duration of ingestion waveforms could also be caused by a physical blockage of phloem sap by callose deposition causing a long term plugging (Eschrich 1975) or by phloem-specific proteins as a temporary defense measure or by other sieve tube sealing mechanisms (van

Bel 2003). As evidence for the blockage of sap flow, Jiang and Walker (2007) reported that on one of the whitefly-resistant alfalfa clones, phloem sap ingested per unit time (correlated with honeydew production) was reduced and a longer lag time was observed between the last drop of honeydew produced and the termination of ingestion waveform. They speculated that the whitefly might have tried to continue the ingestion even after a possible phloem blockage and the last observed drop of honeydew, but eventually gave up because of difficulty in ingestion.

In our research, the phloem sap in FX-10 and NUF-76 did not stimulate sustained feeding, as shown by reduced duration of ingestion waveforms compared to those on Palmetto and Floratam. The shorter duration of ingestion waveforms could be due to the presence of resistance factors in phloem sap or blockage of sieve elements and possibly could be an important component of southern chinch bug resistance in FX-10 and NUF-76. Although we have no direct evidence that the resistant St. Augustinegrasses have phloem-sealing mechanisms or feeding deterrents in phloem sap, stylectomy technique has been used to explore these possibilities in aphids and it could also be tried in southern chinch bugs. Caillaud et al. (1995) found that the resistance factors for the aphid, *Sitobion avenae* (Fabr.), were present in the sieve elements of some lines of *Triticum monococcum* L. and inhibited phloem sap ingestion by the aphid. Caillaud and Niemeyer (1996) investigated the reasons for the rejection of some lines of *T. monococcum* by *S. avenae*. They found no significant difference in the concentration of hydroxamic acids, a family of aphid resistance factors in cereals, between the favorable *T. monococcum* line Tm47 and the resistant lines, Tm46 and Tm44, suggesting that the rejection of aphid resistant lines was not due to these chemical factors examined. They collected phloem sap from young seedlings of aphid resistant and susceptible *T. monococcum* and found that the success in stylectomy, the proportion of stylets resulting in longer exudations (>1 min), the

average duration of exudation and the final volume of phloem sap exuded, were significantly higher in the aphid-susceptible genotypes than the resistant ones. This suggests that phloem-sealing caused the aphid to reject feeding in the aphid-resistant *T. monococcum* lines.

Our previous investigations showed that southern chinch bugs did not prefer resistant lines and their oviposition and development were affected on them (Rangasamy et al. 2006, Chapter 2). The current study, which employed EPG technique to elucidate the feeding behavior of chinch bugs, showed that the southern chinch bugs made more probes, took longer time to reach the phloem sieve elements and spent less time on ingestion in resistant lines, suggesting that these resistant lines may have some physical or biochemical factor(s) impeding the stylet's uptake from the phloem. While not previously reported on homopterans, chemical analysis of St. Augustinegrass phloem sap, collected using the stylectomy technique, could be used to investigate the presence of feeding deterrents or role of sieve tube sealing mechanisms in southern chinch bug resistance in FX-10 and NUF-76. Considering the economic importance of St. Augustinegrass and the continuing race to find chinch bug resistant varieties, our current research provides exact details on feeding behavior of southern chinch bugs and will eventually further elucidate the mechanisms of resistance.

Table 4-1. Durations and frequencies of probing parameters of EPG data recorded on four southern chinch bug resistant and susceptible St. Augustinegrass lines. Values within rows followed by the same letter are not significantly different at $\alpha = 0.05$ separated by LSD means test. *Mean \pm SE.

Variables	St. Augustinegrasses				P values
	FX-10	Floritam	NUF-76	Palmetto	
Total probing duration in min (TPD)	8922	17819	14909	21082	
Total no. of probing events (TNPE)	2291	1545	2571	1352	
Probing duration per event (PDE) (min)*	9.94 \pm 0.59a	8.98 \pm 0.61b	6.53 \pm 0.49c	7.85 \pm 0.57b	<0.0001
No. of probes per insect (NPI)*	101.37 \pm 8.08a	68.96 \pm 6.22b	114.37 \pm 13.20a	59.69 \pm 6.83b	<0.0001
Probing duration per probe (PDP) (min)*	4.01 \pm 0.23c	11.75 \pm 1.39a	5.93 \pm 0.71b	16.06 \pm 2.15a	<0.0001
Probing duration per insect (PDI) (min)*	1034.45 \pm 44.32	630.19 \pm 58.35	762.33 \pm 71.18	482.03 \pm 66.18	0.7833

Table 4-2. Total number of major waveform events (TNWE) on each of four southern chinch bug-resistant or -susceptible St. Augustinegrass varieties (n = 22).

Major waveforms		No. of waveform events (TNWE)			
		FX-10	Floritam	NUF-76	Palmetto
Z1	insect off the plant	33	21	36	25
Z2	non-probing, insect walking	2258	1524	2535	1327
P	pathway	2253	1534	2532	1314
I1	ingestion	744	630	806	596
I2		134	170	185	187

Table 4-3. Mean waveform durations per event (WDE) for major waveforms recorded for southern chinch bug feeding on resistant and susceptible St. Augustinegrasses. Values within the rows with the same letter are not significantly different at $\alpha = 0.05$ separated by LSD means test.

Major waveforms		Waveform duration per event (WDE) (min) (mean \pm SE)				P values
		FX-10	Floratam	NUF-76	Palmetto	
Z1	insect off the plant	60.41 \pm 18.37a	18.62 \pm 9.95c	39.43 \pm 17.75b	44.14 \pm 12.12a	0.0200
Z2	non-probing, insect walking	9.20 \pm 0.52a	8.85 \pm 0.6a	6.06 \pm 0.42b	7.16 \pm 0.52a	<0.0001
P	pathway	1.50 \pm 0.30a	1.25 \pm 0.04c	1.29 \pm 0.05b	1.28 \pm 0.05b	<0.0001
I1	ingestion	4.28 \pm 0.31c	17.72 \pm 1.40a	10.24 \pm 0.80b	21.99 \pm 1.92a	<0.0001
I2		6.69 \pm 0.58b	20.34 \pm 1.93a	10.29 \pm 1.37b	27.46 \pm 4.18a	<0.0001

Table 4-4. Mean duration of major waveforms per probe (WDP) on each of four southern chinch bug-resistant or -susceptible St. Augustinegrasses. Values within rows followed by the same letter are not significantly different at $\alpha = 0.05$ separated by LSD means test

Major waveforms		Waveform duration per probe (WDP) (min) (mean \pm SE)				P values
		FX-10	Floratam	NUF-76	Palmetto	
P	pathway	1.51 \pm 0.06a	1.17 \pm 0.04c	1.33 \pm 0.06b	1.44 \pm 0.09b	<0.0001
I1	ingestion	5.19 \pm 0.49c	15.18 \pm 1.59b	7.32 \pm 0.88c	20.45 \pm 2.84a	<0.0001
I2		7.46 \pm 0.73c	19.18 \pm 2.33a	12.45 \pm 1.77b	21.66 \pm 3.16a	<0.0001

Table 4-5. Mean number of major waveform events per insect (NWEI) on each of four southern chinch bug-resistant or -susceptible St. Augustinegrass varieties. Values within rows followed by the same letter are not significantly different at $\alpha = 0.05$ separated by LSD means test.

Major waveforms		No. of waveform events per insect (NWEI) (mean \pm SE)				P values
		FX-10	Floratam	NUF-76	Palmetto	
Z1	insect off the plant	2.20 \pm 0.46	2.10 \pm 0.51	3.00 \pm 0.60	2.09 \pm 0.40	0.5111
Z2	non-probing, insect walking	102.64 \pm 8.10a	69.28 \pm 6.21b	115.23 \pm 13.29a	60.32 \pm 6.84b	<0.0001
P	pathway	140.41 \pm 12.64a	108.05 \pm 9.30b	154.36 \pm 17.05a	89.63 \pm 9.07c	<0.0013
I1	ingestion	33.82 \pm 5.13	28.64 \pm 2.52	36.64 \pm 4.07	27.10 \pm 3.21	0.4018
I2		6.10 \pm 0.83	7.73 \pm 1.15	8.41 \pm 1.80	8.50 \pm 1.37	0.6168

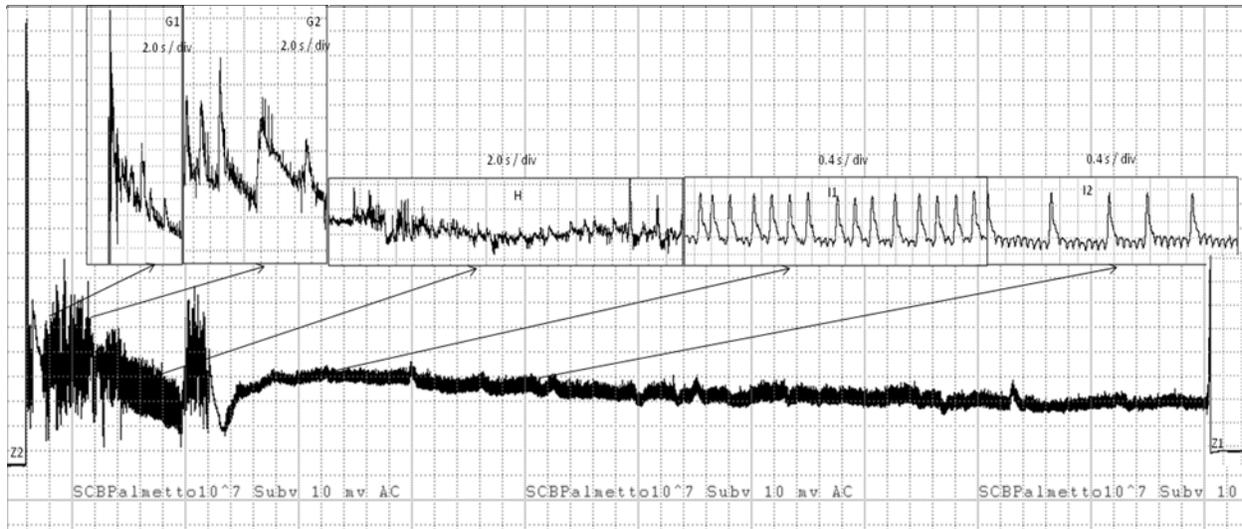


Figure 4-1. EPG waveforms produced by southern chinch bug feeding on St. Augustinegrass, recorded using an AC/DC EPG monitor. Major waveform categories: Z2 – baseline, G1 and G2 - high-amplitude pathway waveforms, H - low-amplitude pathway waveform, I1 and I2 – ingestion waveforms.

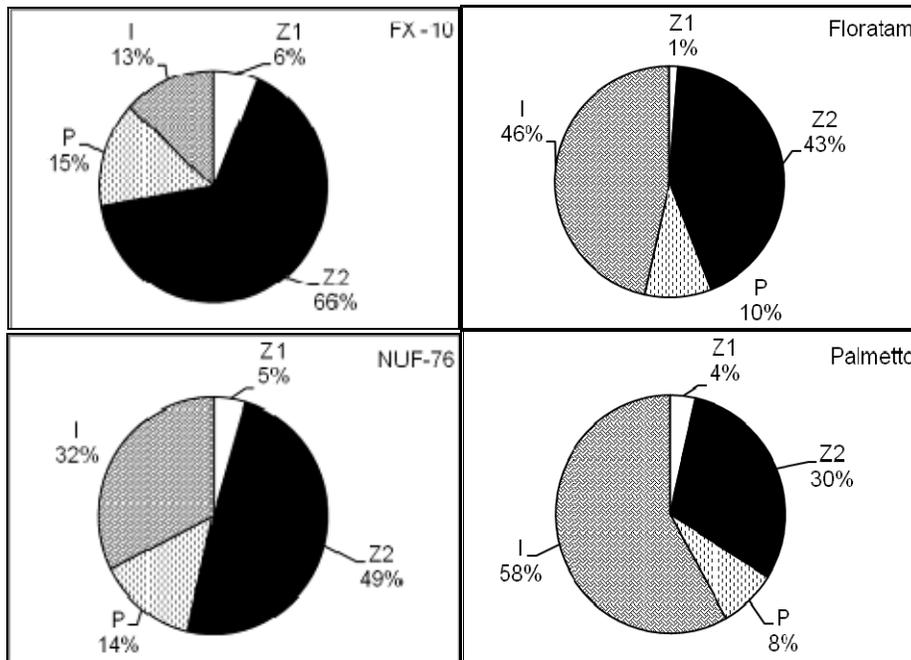


Figure 4-2. Total percentage of time spent on total wave form duration (TWD) by southern chinch bugs on resistant and susceptible St. Augustinegrasses in different waveform categories. Z1 – Insects off the plant, Z2- insects on the plant, resting, walking; P – combined pathway waveforms (G1+G2+H), I – combined ingestion waveforms (I1+I2).

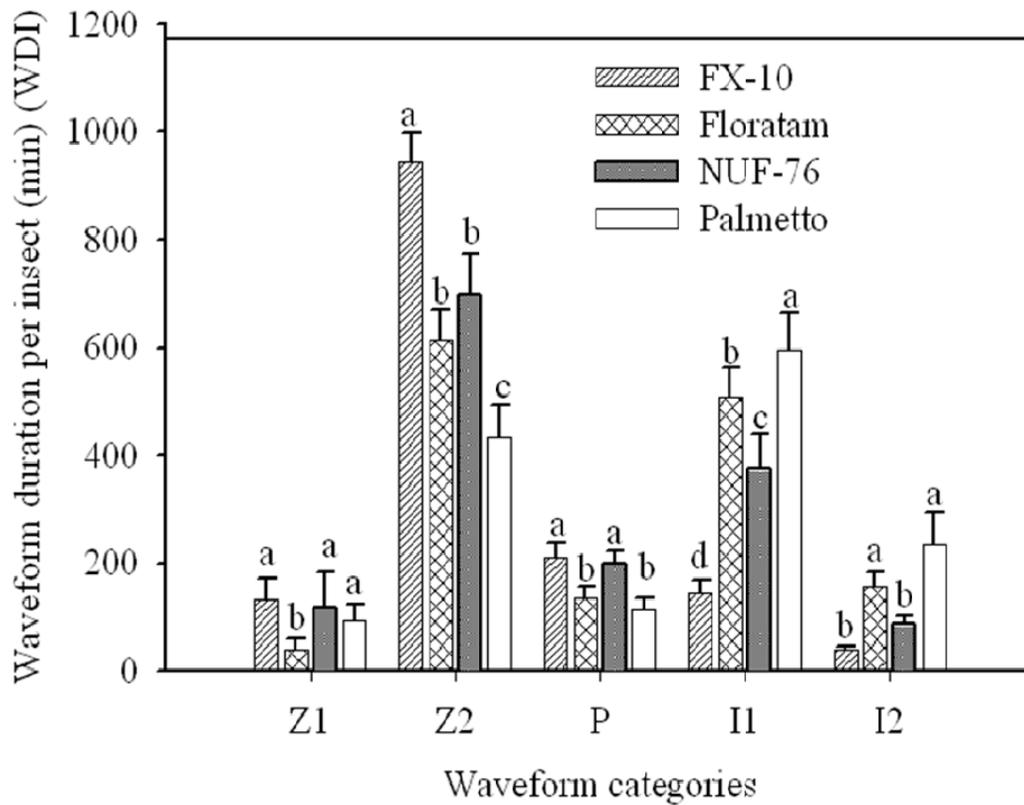


Figure 4-3. Waveform duration per insect (WDI) for major waveforms on resistant and susceptible *St. Augustinegrasses*. Z1 – insects off the plant, Z2 - insects on the plant, resting, walking; P – combined pathway waveforms (G1+G2+H), I – combined ingestion waveforms (I1+I2). Values (mean \pm SE, n = 22) with the same letter in a waveform category are not significantly different at $\alpha = 0.05$ separated by LSD means test.

CHAPTER 5
ROLE OF LEAF SHEATH LIGNIFICATION AND ANATOMY IN RESISTANCE AGAINST
SOUTHERN CHINCH BUG, *BLISSUS INSULARIS* BARBER (HEMIPTERA: BLISSIDAE) IN
ST. AUGUSTINEGRASS LINES

Introduction

St. Augustinegrass, *Stenotaphrum secundatum*, is the most widely used turfgrass in tropical and subtropical climatic regions (Sauer 1972). It is a commonly grown residential turfgrass species in the southern United States, covering more than one million acres and accounting for 85% of the sod industry in Florida alone, with a value estimated at \$262 million (Haydu et al. 2005). The southern chinch bug is the most serious insect pest of St. Augustinegrass (Crocker 1993). The annual cost of southern chinch bug management and losses in Florida alone was estimated at \$5 million in 1983 (Hamer 1985). Management of this insect has further been challenged by its development of resistance to several key insecticides (Reinert and Portier 1983, Cherry and Nagata 2005, Cherry and Nagata 2007).

Historically, host plant resistance has been one of the most successful management tools for this insect. ‘Floratom’, a polyploid variety of St. Augustinegrass with resistance to southern chinch bug, has been available in Florida and other Gulf Coast states since its release in 1973 (Horn et al. 1973). Within a decade after its release, in 1985, a population of chinch bug was reported to have overcome Floratom’s resistance in Florida (Busey and Center 1987). Significant progress has been made in identifying new sources of southern chinch bug resistance in St. Augustinegrass lines. However the registered polyploid FX-10 with a high level of resistance to southern chinch bug (Busey 1993), is coarse and tough and never became popular among growers (Nagata and Cherry 2003). The discovery of southern chinch bug resistance in diploid NUF-76 was considered important because of its fine texture and slow growth (Nagata and Cherry 2003). However, the mechanisms of resistance in these lines are unknown.

Rangasamy et al. (2006) investigated categories of resistance to southern chinch bug in FX-10 and NUF-76 and found high levels of antixenosis in both lines and possible antibiosis in NUF-76 (Chapter 2). Electrical penetration graph (EPG) studies revealed that southern chinch bugs took longer to reach the phloem sieve elements presumably and spent less time on ingestion on FX-10 and NUF-76 compared to their susceptible controls, Floratam and Palmetto, respectively (Chapter 4). Relatively more stylet probings per insect on FX-10 and NUF-76 than on Floratam and Palmetto also suggested the presence of stylet penetration impediments around the vascular bundle in resistant varieties. In addition, the short duration of ingestion on FX-10 and NUF-76 suggested the presence of feeding deterrents within phloem sap and/or sieve tube plugging mechanisms.

Lignin content of plant tissues has often been correlated with resistance to insects and plant pathogens. Blum (1968) identified lignin as a major resistance factor in sorghum against shoot fly, *Atherigona variocata*. Shoot fly resistant sorghum plants had highly lignified and thickened cells around the vascular bundles in the young leaves of the central whorl preventing penetration by larvae. Toughness of plant tissues has often been considered as a major factor in their unsuitability to insect feeding (Feeny 1970, Coley 1983). Leaf beetles (*Plagioderma versicolora* (Laicharting)) feeding on mature *Salix* sp. leaves succumbed to increased mandible wear compared to those feeding on young leaves which resulted in reduced leaf consumption and beetle fecundity (Raupp 1985). Bark beetles, *Dendroctonus micans* Hugel feeding on spruce trees with higher bark lignin content had reduced larval weight, survival and development rate (Wainhouse 1998). *Solanum integrifolium* plants transformed with horseradish peroxidase gene had 30% higher lignin content compared to wild-type plants and a high level of resistance to corn earworm (*Heliothis armigera* (Hübner), Lepidoptera: Noctuidae) (Tsuduki et al. 2006). Our

previous investigation into the activities of oxidative enzymes such as peroxidase, lipoxygenase, catalase and polyphenol oxidase in response to the feeding of southern chinch bug on resistant and susceptible St Augustinegrass lines revealed that the chinch bug-resistant FX-10 had increased peroxidase activity 5 and 8 d after southern chinch bug infestation (Chapter 3). Because plant peroxidases play diverse roles in plants including lignin biosynthesis in the cell wall (Lagrimini et al. 1987) and oxidation of toxic compounds (Gasper et al. 1982) and because lignification of cells could be an impediment to insect pests (Coors 1987, Buendgen et al. 1990, Wainhouse et al. 1990, Wainhouse et al. 1998, Tsuduki et al. 2006), we hypothesized that increased lignification could be a possible component of southern chinch bug resistance in these St. Augustinegrass lines.

The objectives of this research were to investigate the possibility that increased lignification and differences in leaf sheath anatomy could account for resistance in diploid NUF-76 and polyploid FX-10 St. Augustinegrasses. Specifically, we compared stylet probing frequencies, leaf sheath lignin content and vascular bundle anatomy among two southern chinch bug-resistant and two susceptible St. Augustinegrass varieties.

Materials and Methods

Plants

St. Augustinegrass varieties used in the following experiments included FX-10 (resistant polyploid), Floratam (susceptible polyploid), NUF-76 (resistant diploid) and Palmetto (susceptible diploid). The plants were grown as described by Rangasamy et al. (2006). Runners used for the stylet probing study and lignin staining were 30 to 40 cm in length with a minimum of four nodes; runners used for the lignin quantification had a minimum of 15 nodes.

Insects

Southern chinch bugs were collected from Royal Palm Beach (Palm Beach County, FL). The insects were collected by vacuuming St. Augustinegrass lawns as described by Cherry (2001a) and then sorting through debris in the laboratory for fifth instars and adults. After collection, insects were provided with fresh Floratam stolons and maintained at 18°C in 20-l plastic buckets with a moistened paper towel at the bottom to increase humidity within the bucket.

Staining for Salivary Sheaths

Visual observations suggested that chinch bugs were residing in and feeding on the axillary shoot. Hence, we focused our studies on the axillary shoot which consisted of an outermost leaf sheath and several layers of inner leaf sheaths with leaf blades and meristematic tissues (Figure 5-1). Ten chinch bug adults (5 males and 5 females) were confined for 48 h in organandy sleeve cages to the third youngest node of one runner of each St. Augustinegrass plant. The chinch bugs were removed from the plant before collecting the sample for staining for salivary sheaths. Southern chinch bug-infested axillary shoots were excised from the plants and placed in McBride's staining solution. The staining solution was prepared using 0.2% (wt/v) acid fuchsin in a 1:1 mixture of 95% (v/v) ethanol and glacial acetic acid (Sigma-Aldrich, St. Louis, MO) according to the methods of Backus et al. (1988). The plant tissues were stained for 24 h at 24 ± 2°C, then placed in the clearing solution and autoclaved for 15 min at 120°C and 15 psi. The clearing solution was prepared using 99% (v/v) glycerol, 85% (v/v) lactic acid and distilled water (1:1:1, v/v/v, Sigma-Aldrich, St. Louis, MO) (Backus et al. 19988). The stained salivary sheaths in each leaf sheath within an axillary shoot were counted under a dissection microscope at 40X (Figure 5-2)

Lignin Quantification

Total lignin content of uninfested axillary shoots was quantified using the Klason method (Kirk and Obst 1988). Fresh axillary shoot were air dried and ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 20-mesh screen. A 200-mg sample was weighed into a small beaker and 1 ml of 72% (v:v) H₂SO₄ per 100 mg sample was added. The sample-acid mixture was digested by placing in a water bath at 30°C for 1 h and was stirred frequently to get a complete solution. After exactly 1 h, the solution was diluted using 28 ml of distilled water for each 1 ml of acid and transferred to an Erlenmeyer flask. The diluted solution was then hydrolyzed in an autoclave at 120°C for 1 h and filtered through a 50-ml Pyrex Gooch crucible with fritted disc of 4 – 5.5 µm porosity (Sigma-Aldrich, St. Louis, MO). The filtrate was saved for estimating the acid-soluble lignin. The Klason lignin residue was washed with hot water to remove the acid. The Klason lignin was then dried to constant weight at 105°C and expressed as a percent dry weight. The acid-soluble lignin present in the filtrate was determined spectrophotometrically at 205 nm using an absorptivity value of 110L/g-cm (extinction coefficient) to calculate the total lignin content (Ehrman 1996).

Staining for Lignin

Fresh sections of 10-15 µm of the outermost leaf sheath of an axillary shoot were prepared using a cryostat (MICROM HM 505E, Microm Lobergerate GMBH, Strasse, Germany) and stained with 2% (wt/v) phloroglucinol (Sigma-Aldrich, St. Louis, MO) solution in ethanol/water (95/5 v/v) for 2 min followed by treatment with 50% (v/v) HCl for 1 min for the Weisner reaction (Rastogi and Dwivedi 2006). The stained sections were photographed at 400X under an epifluorescence light microscope (Olympus BH2-RFCA, Olympus America, Inc., Lake Success, NY, USA).

Transmission Electron Microscopy

The axillary shoot from the fourth youngest node of each of the four St. Augustinegrasses was collected and cut into small segments of 1-2 mm in length and fixed in Trump's EM fixative (1% wt/v glutaraldehyde - 4% wt/v paraformaldehyde in phosphate buffer saline (PBS), pH 7.3), incubated in 2% (wt/v) osmium tetroxide and then dehydrated in a graded ethanol-acetone series (25%, 50%, 75%, 95%, 100% (v/v) ethanol, 100% (v/v) acetone). After dehydration, the tissues were embedded with Spurr's epoxy resin (Spurr 1969) at concentrations of 30%, 50%, 75%, 100% and placed in an oven at 60°C for 48 h. Ultra-thin sections of 1 μ m were made using an ultramicrotome (EM-UC 6, Leico Microsystems, Bannockburn, IL) equipped with a glass knife (Type 7801B, LKB Knife Maker, Rockville, MD). The ultra-thin sections were post-stained with 2% (wt/v) uranyl acetate and viewed in a Hitachi H7000 electron microscope (Hitachi, Tokyo, Japan) operated at an accelerating voltage of 75 kV. To measure the thickness of the epidermal layer of the leaf sheath, photomicrographs of leaf sheath cross-sections were taken using a light microscope at 400X and the epidermal layer thickness was measured using Adobe Photoshop CS2 (Adobe Systems Inc., San Jose, CA). The proportion of the sclerenchyma cell wall area relative to the total sclerenchyma cell area was calculated from the TEM micrographs (3500X) of the leaf sheath vascular bundle using the SPOT image analysis program (Version 3.5, Diagnostic Instruments, Inc. Sterling Heights, MI). Twenty five sclerenchyma cells were measured for each of the three replicate plants in each of the four St. Augustinegrass lines.

Experimental Design and Statistical Analysis

The experiment to investigate the salivary sheath distribution was a completely randomized design with four treatments (FX-10, Floratam, NUF-76, and Palmetto) and 20 replications. The samples for lignin staining and electron microscopic studies were collected

from three replicate plants of each of the four St. Augustinegrasses. Parameters such as number of salivary sheaths in each leaf sheath layer relative to the outermost leaf sheath, total lignin content, leaf sheath epidermal layer thickness and the proportion of sclerenchyma cell wall area relative to total cell area were analyzed using PROC GLM (SAS 1999). When appropriate, means were separated using Tukey's test at a significance level of $\alpha = 0.05$.

Results

Most of the 20 axillary shoots examined from each of the four St. Augustinegrass lines contained five leaf sheaths, arranged in concentric whorls. Significantly more salivary sheaths were counted on the outermost leaf sheath layer in FX-10 and NUF-76 than on Floratam and Palmetto ($F = 16.78$; $df = 3, 76$; $P < 0.0001$) (Figure 5-3) and these salivary sheaths were branched more often in FX-10, Floratam and NUF-76 compared to Palmetto ($F = 2.58$; $df = 3, 39$; $P < 0.0080$) (Figure 5-4). A significantly smaller proportion of salivary sheaths reached the innermost layer of FX-10 and NUF-76 axillary shoots compared to Floratam and Palmetto ($F = 63.16$; $df = 3, 76$; $P < 0.0001$) (Figure 5-3).

There was no significant difference in total axillary shoot lignin content between FX-10 and Floratam or between NUF-76 and Palmetto, although polyploid lines had significantly more lignin than the diploid lines ($F = 23.22$; $df = 3, 15$; $P = 0.0002$) (Figure 5-5). Staining for lignin did not show any distinct difference between southern chinch bug-resistant and -susceptible St. Augustinegrass lines (Figure 5-6). In addition, there was no significant difference in the thickness of the leaf sheath epidermal layer among the St. Augustinegrass lines studied ($F = 1.17$; $df = 3, 6$; $P = 0.28$) (Figs. 5-7, 5-8). However, TEM studies on the leaf sheath vascular bundle indicated significantly thickened sclerenchyma cell walls in NUF-76 and FX-10 compared to Floratam and Palmetto (Figure 5-9). Quantification of cell wall thickness in

sclerenchyma cells confirmed these observations (Figure 5-10). The cell walls accounted for a much larger proportion of total sclerenchyma area in the resistant St. Augustinegrasses than in the susceptible grasses ($F = 4.65$; $df = 3, 6$; $P = 0.0181$) (Figs. 5-9, 5-10).

Discussion

Chinch bugs, among other hemipterans, lay down a salivary sheath produced by gelling saliva during the intracellular passage of their stylets through plant tissue. Painter (1928) noted the presence of a 'stylet-sheath' during feeding of the common chinch bug (*B. leucopterosus leucopterosus*), a pest of sorghum, *Sorghum bicolor* (L.) Moench. The production of salivary sheaths has often been used as a quantitative measure of homopteran feeding (Bowling 1979, Marion-Poll et al. 1987, Bing et al. 1991). The research reported herein investigated the possible presence of impediments to stylet penetration and access to phloem in southern chinch bug-resistant St. Augustinegrass lines.

In this research, I found a significantly higher number of salivary sheaths observed on the outermost leaf sheath layers in FX-10 and NUF-76 than on susceptible Floratam and Palmetto, corroborating our EPG studies which indicated that chinch bugs probed significantly more often on FX-10 and NUF-76 than on the susceptible varieties (Chapter 4). Oxidative enzymes such as peroxidase, lipoxygenase and polyphenol oxidase had increased activities in FX-10 and NUF-76 plants infested by southern chinch bugs compared to infested Palmetto and Floratam plants (Chapter 3); the induction of these enzymes could be due to the mechanical damage resulting from the higher number of probings on the leaf sheath. EPG studies (Chapter 4) showed that southern chinch bugs spent more time on pathway waveforms in FX-10 and NUF-76 which is corroborated by the presence of more branched salivary sheaths on FX-10 and NUF-76. The large proportion of salivary sheaths reaching the innermost leaf sheath on Floratam and Palmetto

demonstrates a strong preference by the southern chinch bugs for the intercalary meristematic tissues, which are continuing to differentiate and are covered by the mature outermost leaf sheaths. In other insect systems, Ni and Quisenberry (1997) found no significant difference in the number of salivary sheaths of Russian wheat aphid *Diuraphis noxia* (Mordvilko) between resistant ('Halt') and susceptible ('Arapahoe') varieties of wheat, *Triticum aestivum* L. However, more salivary sheaths were initiated through leaf stomata on the aphid-resistant 'Halt' than on the susceptible 'Arapahoe', suggesting the possible presence of antixenotic epidermal characters such as leaf toughness and epicuticular waxes on 'Halt' leaves. Bing et al (1991) similarly observed that 57% of the time the stylets of the bird cherry-oat aphid, *Rhopalosiphum maidis* (L.) entered through stomata in maize, *Zea mays* L, plants in the late whorl stage however only 8% of the stylets penetrated through stomata in the seedling stage. This suggested that the higher rate of stomatal entry of stylets was due to easier penetration. Anderson et al. (2006) found that *Blissus occiduus* Barber probed more frequently on buffalograss, a preferred host, than on less-preferred sorghum and St. Augustinegrass.

We found that a smaller proportion of the initiated salivary sheaths reached the innermost layer of the axillary shoot in resistant FX-10 and NUF-76 than they did in the susceptible varieties. Based on the results from the EPG studies (Chapter 4) and stylet probing distribution, these probes were probably terminated during pathway phase prior to ingestion (presumably from phloem). We postulated that lignified tissue may be impeding stylet progress towards or access to the tissue in which the southern chinch bug prefers to feed. Painter (1928) noted that the phloem tissue was the target of the stylets of the common chinch bug. Because of a strong preference by the common chinch bug to feed within the leaf sheath (Painter 1928) and our own observations of feeding by southern chinch bug (Chapter 2), we quantified the total lignin

content of the leaf sheath and stained the leaf sheaths to look for quantitative and qualitative differences, if any.

In *St. Augustinegrass*, the vascular bundle is surrounded by the highly lignified sclerenchyma cells (Figure 5-3). Sclerenchyma cells have thickened secondary cell walls which contain cellulose, hemicelluloses and lignin. In general, the lignin content of sclerenchyma cell wall ranges from 18 – 35% (wt/wt) (Mauseth 1988) and the presence of lignin provides a stable protection to the vascular bundle. Our TEM studies found a larger proportion of leaf sheath sclerenchyma cell wall area in FX-10 and NUF-76 relative to the total cellular area consistent with our hypothesis that the sclerenchyma cells may serve as a physical barrier to stylet penetration.

Our previous studies suggested that chinch bug resistance mechanisms of the polyploid line FX-10 may be different in certain aspects than that found in NUF-76 since NUF-76 showed antibiosis but both lines had antixenosis (Rangasamy et al. 2006). It is interesting to note here that both resistant lines had thicker sclerenchyma cell walls compared to the susceptible lines, suggesting that NUF-76 may have additional features for antibiosis. A greater proportion of sclerenchyma cell wall area was observed on NUF-76, a diploid line with total lignin content less than the polyploids FX-10 and Floratam. It may be that deposition of lignin in a few sclerenchyma cells with thick cell walls rather than total lignin is important as a barrier to the southern chinch bug stylets.

In summary, our results showed that stylet penetration to innermost leaf sheaths occurred more frequently in susceptible lines than in resistant lines, suggesting an impediment to stylet penetration. However, this impediment is not due to differences in their epidermal layer or total lignin content. Based on qualitative and quantitative analyses of TEM of vascular bundles, the

resistant lines FX-10 and NUF-76 had a greater degree of lignification in sclerenchyma cells around the vascular bundles. We propose that thicker sclerenchyma cells around the vascular bundles is one of perhaps several features of the resistance in St. Augustinegrass lines to southern chinch bug and may be useful as a marker in breeding for southern chinch bug resistance.

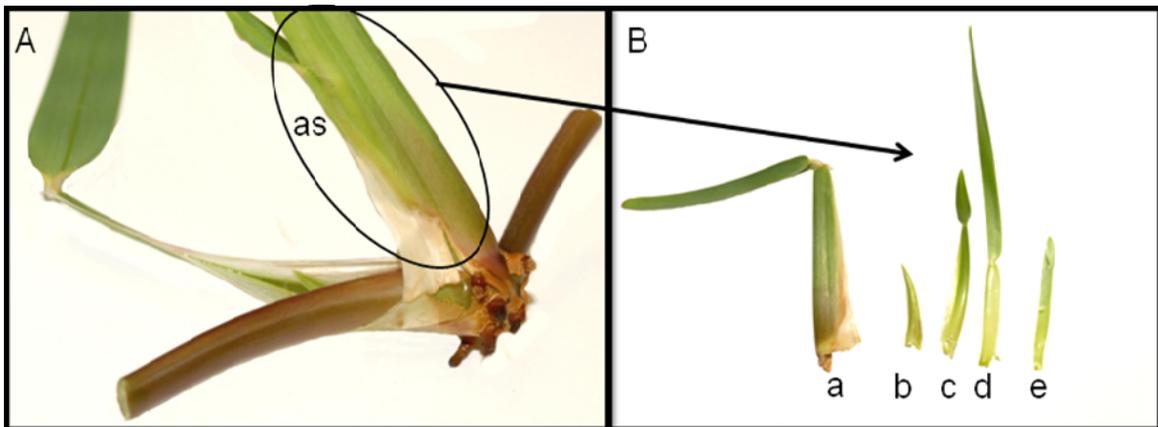


Figure 5-1. St. Augustinegrass stolon A. with the axillary shoot (as); B. Parts of axillary shoot, a - outermost leaf sheath with leaf blade, b - prophyll, c - second leaf sheath layer with leaf blade, d - third leaf sheath layer with leaf blade, e - axillary meristem.

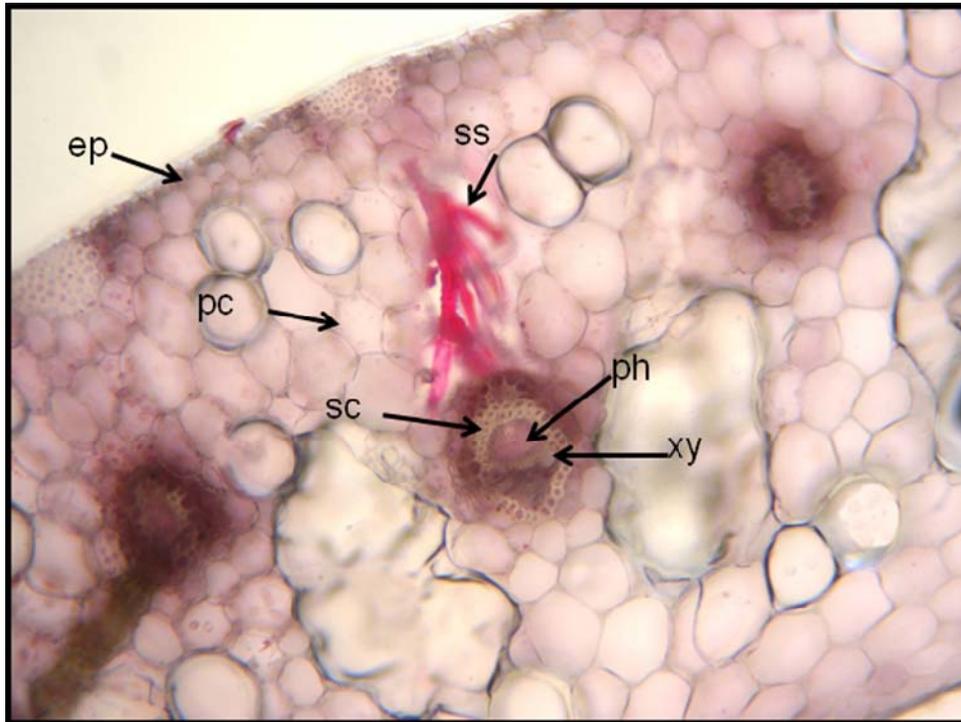


Figure 5-2. Transverse section of outermost St. Augustinegrass leaf sheath showing southern chinch bug salivary sheath stained with McBride's stain (400X). ep – epidermal layer, pc – parenchyma cells, ss – salivary sheath, sc – sclerenchyma cells, ph – phloem, xy – xylem.

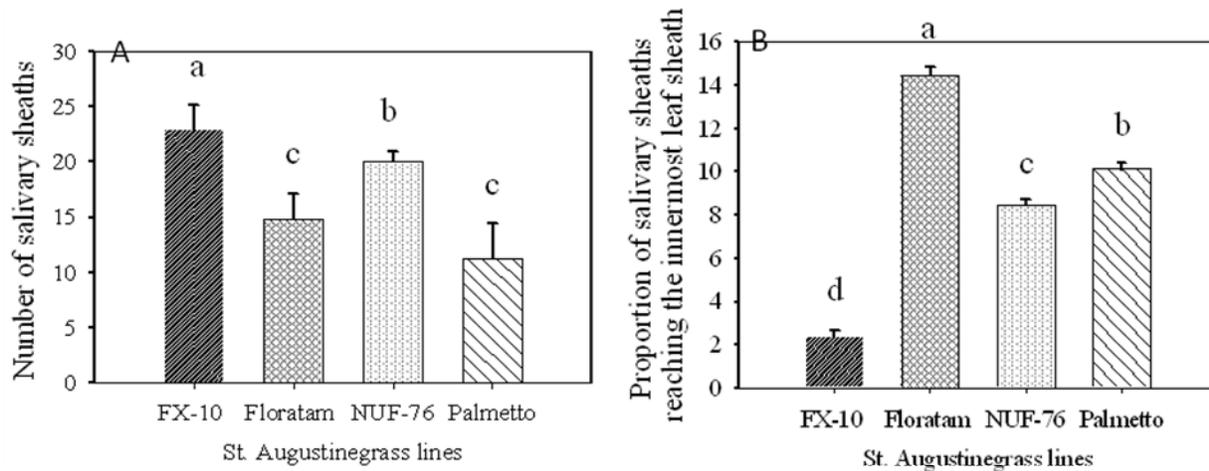


Figure 5-3. Number of southern chinch bug salivary sheaths found on the outermost leaf sheath of axillary shoots (A) and the proportion of salivary sheaths reaching the innermost sheath (B) of southern chinch bug-resistant and -susceptible St. Augustinegrasses. The values shown are mean \pm SE. Means followed by the same letter are not significantly different at $\alpha = 0.05$ level separated by Tukey's test ($n = 20$).

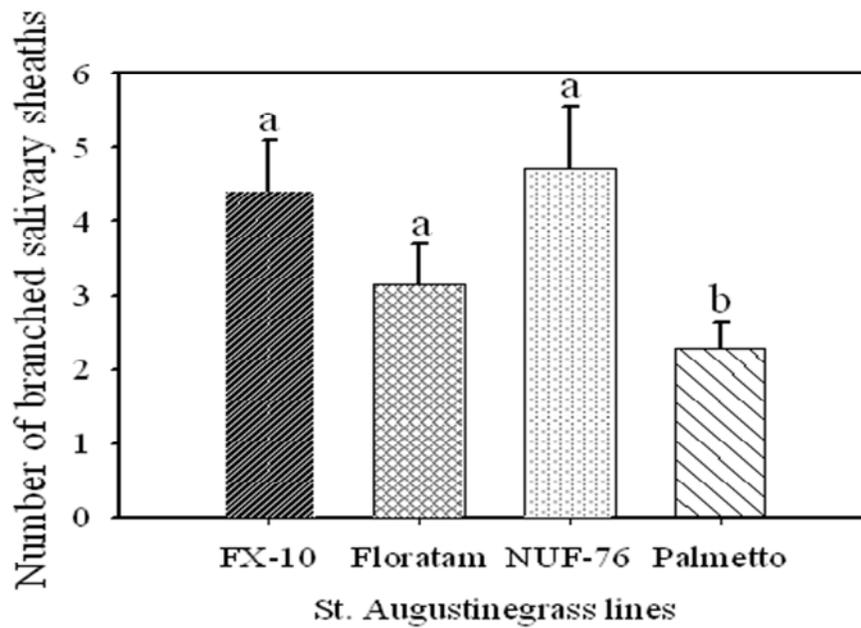


Figure 5-4. Number of southern chinch bug branched salivary sheaths on the outermost leaf sheath of axillary shoots from southern chinch bug-resistant and -susceptible St. Augustinegrasses. The values shown are mean \pm SE. Means followed by the same letter are not significantly different at $\alpha = 0.05$ level separated by Tukey's test ($n = 14$).

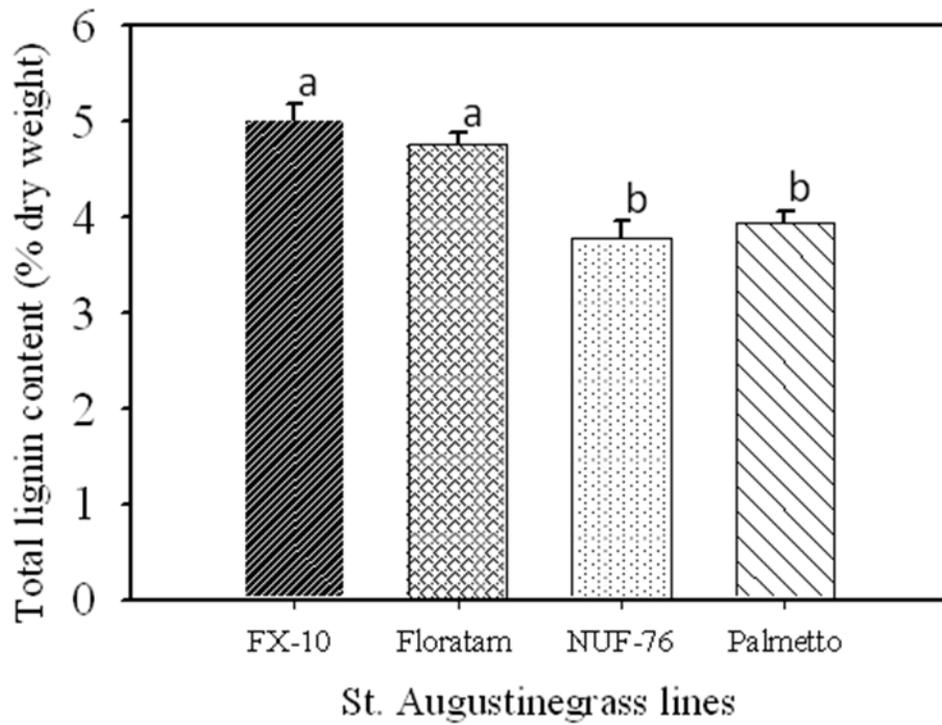


Figure 5-5. Total lignin content of axillary shoot in southern chinch bug-resistant and -susceptible St. Augustinegrasses. The values shown are mean \pm SE of six replicates. Means followed by the same letter are not significantly different at $\alpha = 0.05$ level separated by Tukey's test ($n = 6$).

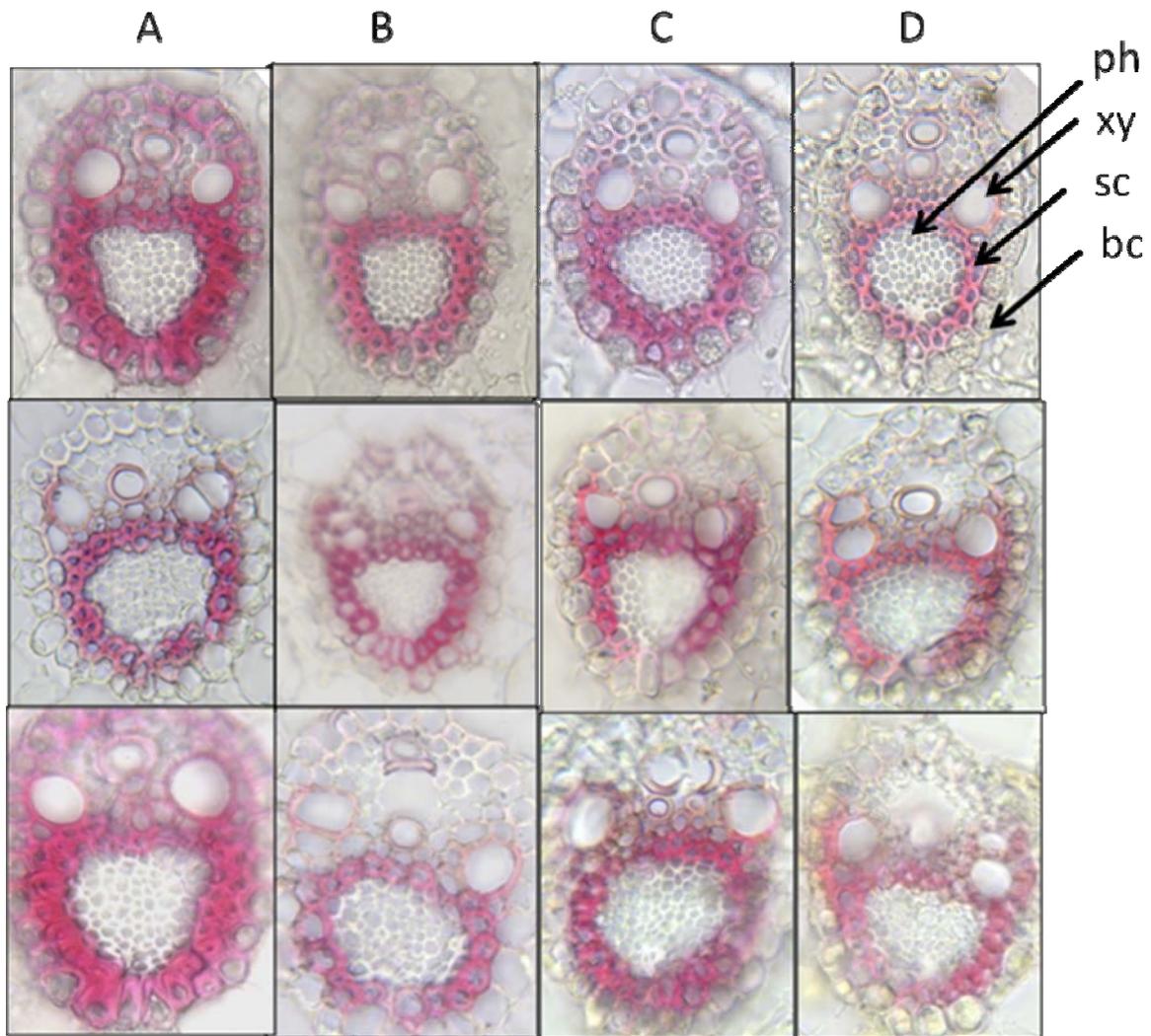


Figure 5-6. Transverse sections of outermost leaf sheath vascular bundles stained for lignin in southern chinch bug-resistant and -susceptible St. Augustinegrasses (400X) (n = 3). A. FX-10, B. Floritam, C. NUF-76, D. Palmetto. ph - phloem, xy - xylem cells, sc - sclerenchyma cells, bc - bundle sheath cells.

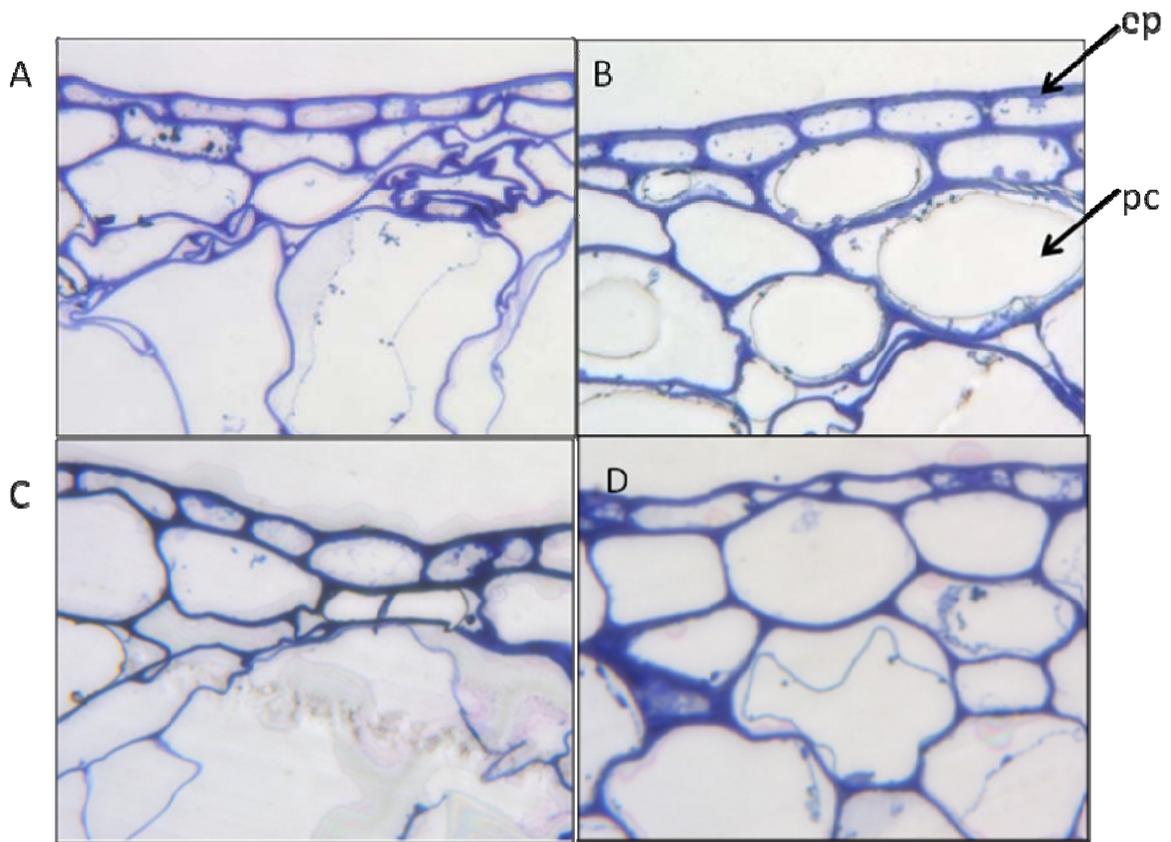


Figure 5-7. Transverse sections of the epidermal layer of the outermost leaf sheath in southern chinch bug-resistant and -susceptible St. Augustinegrasses (400X) (n = 3). A. FX-10, B. Floritam, C. NUF-76, D. Palmetto. ep – epidermal layer, pc – parenchyma cells.

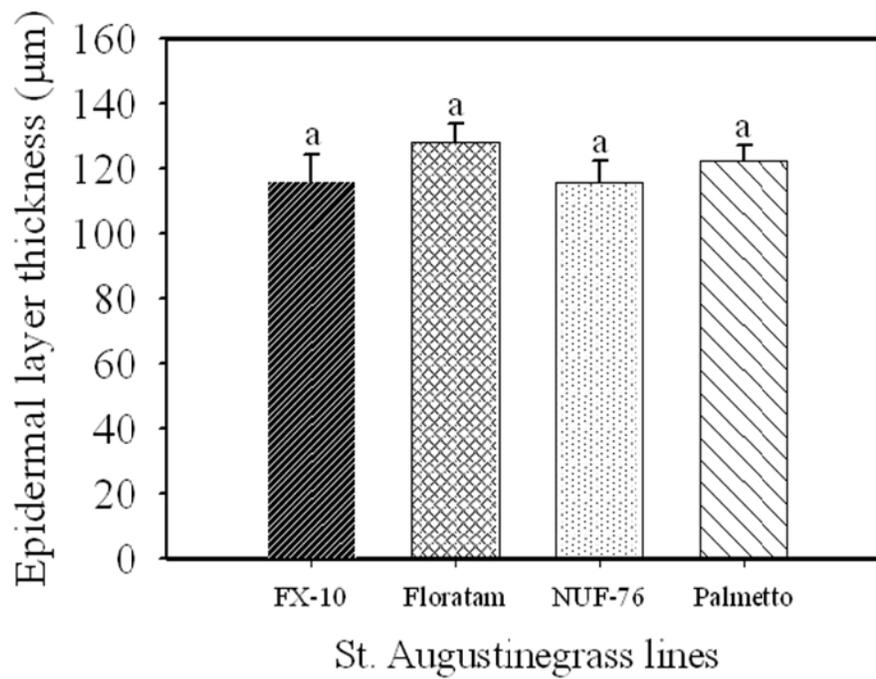


Figure 5-8. Thickness of epidermal layer of outermost leaf sheath in southern chinch bug-resistant and -susceptible St. Augustinegrasses. Means (\pm SE) followed by the same letter are not significantly different at $\alpha = 0.05$ level separated by Tukey's test ($n = 3$).

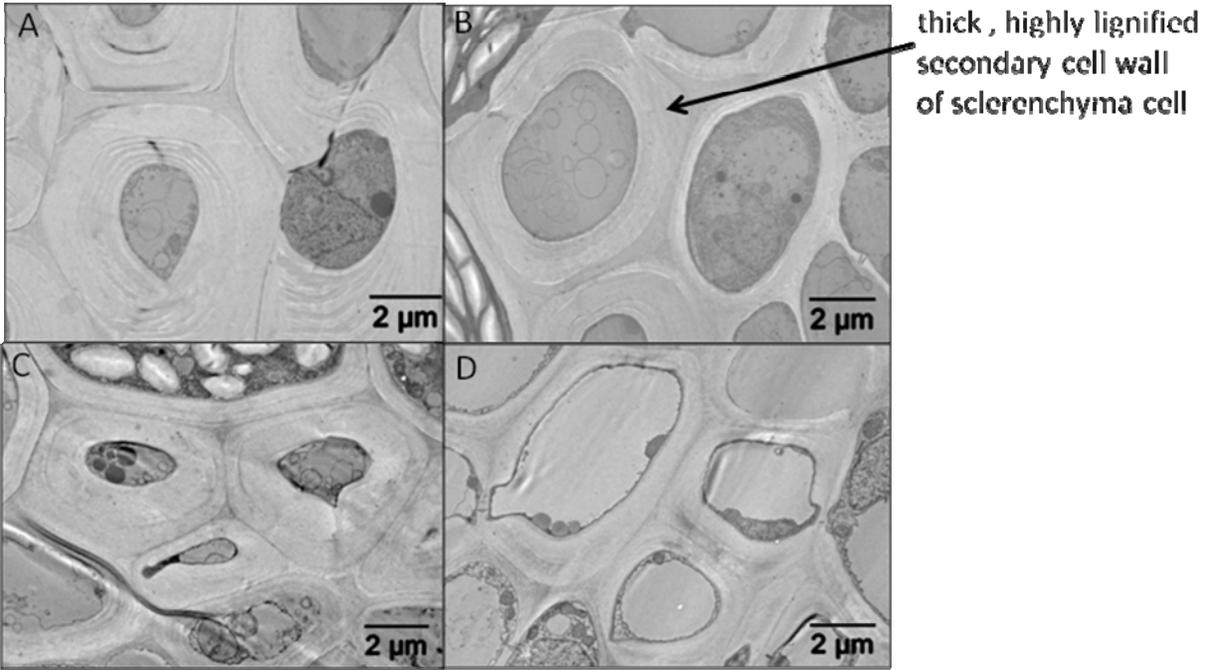


Figure 5-9. Transmission electron micrographs (7000X) of the sclerenchyma cells of the outermost leaf sheath of St. Augustinegrasses resistant and susceptible to southern chinch bug (n = 3). A. FX-10, B. Floratam, C. NUF-76, D. Palmetto.

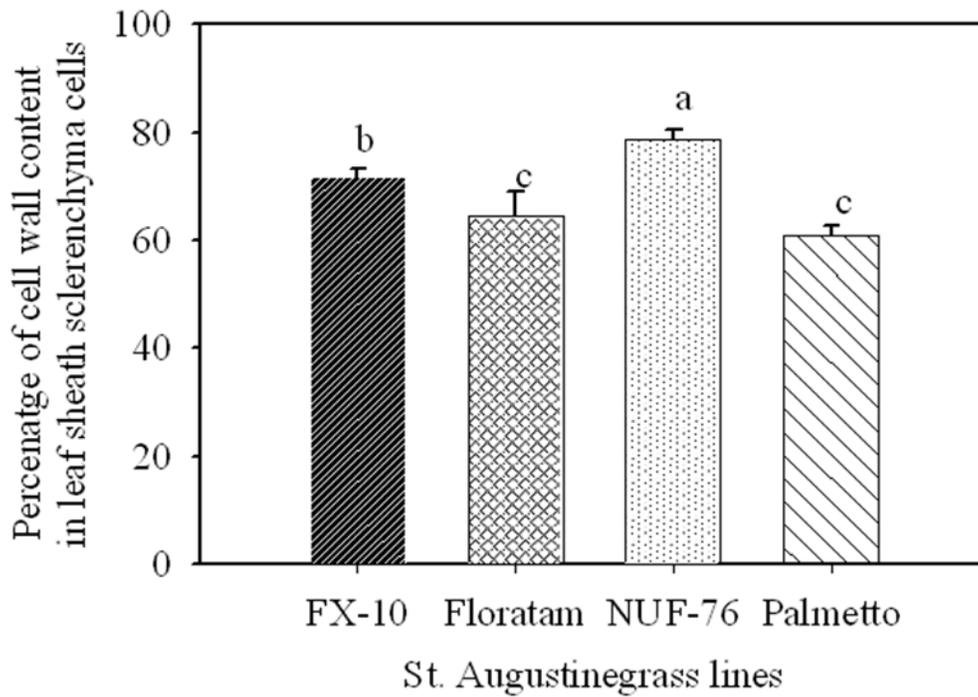


Figure 5-10. Percentage of cell wall area relative to total cell area of sclerenchyma of the outermost leaf sheath of southern chinch bug-resistant and -susceptible St. Augustinegrasses. Means (\pm SE) followed by the same letter are not significantly different at $\alpha = 0.05$ level separated by Tukey's test ($n = 3$).

CHAPTER 6 SUMMARY

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is the most commonly grown lawn grass in Florida and other Gulf coast states.. It is estimated to cover more than one and a half million acres in Florida and accounts for 85% of the sod industry in Florida, worth \$262 million annually. The southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae), is the most serious insect pest of St. Augustinegrass. The nymphs and adult chinch bugs feed on the inside of the leaf sheath; the infested plants turn yellow and eventually die. Repeated use of insecticides led to development of insecticide resistance in southern chinch bug to chlorinated hydrocarbons, organophosphates, carbamates, synthetic pyrethroids and insecticides with novel modes of action, such as imidacloprid and lambda-cyhalothrin. Host plant resistance has long been one of the successful management tools for control of the southern chinch bug.

‘Floritam’, a polyploid St. Augustinegrass variety with initially high level of resistance to southern chinch bug was released in 1974. However, 12 years after its release, a population of southern chinch bug from Florida overcame Floritam’s resistance. FX-10, another southern chinch bug resistant polyploid variety was registered in 1993 with a high level of resistance to southern chinch bug. However, it never became popular among growers because of its coarse texture, slow ground coverage and susceptibility to weeds. In 2003, NUF-76, a diploid St. Augustinegrass line was reported to have a high level of southern chinch bug resistance. Its discovery was considered as a breakthrough in breeding for southern chinch bug resistance because of its diploid genome, making the transfer of southern chinch bug resistance easier. Understanding the mechanisms of southern chinch bug resistance in resistant St. Augustinegrass is essential for maintaining host plant resistance and the development of future cultivars with

better resistance. The main objective of this research was to investigate mechanisms of southern chinch bug resistance in FX-10 and NUF-76.

Choice and no-choice tests and ovipositional and developmental studies were conducted to determine the categories of resistance in FX-10 and NUF-76 to the southern chinch bug. When adult chinch bugs had a choice among attached stolons of three susceptible lines (Floritam, and 'Bitter Blue', a susceptible polyploid, and 'Palmetto', a susceptible diploid) and the two resistant lines, chinch bugs were found significantly more often on the susceptible lines over a 5-d exposure period. This result indicated the presence of antixenosis factors in the resistant lines FX-10 and NUF-76. In a no-choice study, chinch bugs produced fewer excretory spots (suggesting less ingestion) on FX-10 and NUF-76 than on the susceptible lines. The no-choice study confirmed a high level of antixenosis in FX-10, a moderate level of antixenosis in NUF-76, and possible antibiosis in NUF-76. Ovipositional and developmental studies conducted using Floritam, FX-10 and NUF-76 demonstrated that the adults released on Floritam produced more eggs and offspring than adults confined on FX-10 and NUF-76.

Higher mortality of southern chinch bugs has been reported on resistant FX-10 and NUF-76 than on susceptible Floritam, Palmetto and Bitter Blue (Nagata and Cherry 2003); however, the cause of mortality is still unknown. Understanding the mechanisms of chinch bug resistance in these two cultivars is essential for future turfgrass breeding programs. Plant defenses against herbivores are usually characterized as constitutive or induced. Constitutive defense is always present in plants whereas induced defense occurs only in response to herbivore attack which further reduces or deters the herbivory. Induced plant defense could be direct when they have a direct negative effect on the herbivore or indirect when the natural enemies of the herbivores are attracted. Increased activities of oxidative enzymes such as polyphenol oxidase, peroxidase, and

lipoxygenase are often correlated with induced plant defense to insects. FX-10 and NUF-76 had significantly higher peroxidase activity 5 and 8 d after infestation and NUF-76 had significantly higher polyphenol oxidase activity 5 and 8 d after infestation compared to control plants. FX-10 had significantly higher lipoxygenase activity 3, 5 and 8 d after infestation compared to control plants. Catalase activities did not differ significantly between infested and control plants in any of the lines tested. Native gels stained for peroxidase indicated that some isozymes in FX-10 and NUF-76 were induced 5 and 8 d after infestation. Isozyme profiles of polyphenol oxidase and lipoxygenase did not differ between control and infested FX-10, NUF-76 and Floratam. The increased levels of peroxidase and lipoxygenase in FX-10 and peroxidase and polyphenol oxidase in NUF-76 are associated with southern chinch bug resistance in these lines.

Southern chinch bugs are intracellular feeders. Induction of these oxidative enzymes could involve a general plant response to mechanical damage caused by the chinch bug stylets and also include hypersensitive response of cells around the site of stylet penetration and reactions specific salivary proteins. Increased oxidative enzyme activities could cause a combination of induced plant defense mechanisms such as enhanced plant toughness through lignification, poor nutritional quality and toxicity. The increased toughness could potentially be caused by increased peroxidase activities. Peroxidases and polyphenol oxidases are known to produce protein binding quinones thus reducing the nutritional quality of the plants. Subsequent to the generation of activated products due to the action of these oxidative enzymes, reactions may occur that could potentially result in cross-linking or polymerization of materials toughening the plant tissues, and nutrient complexes which are relatively unavailable and in cell death. The reduced oviposition, development and survival of southern chinch bugs on FX-10 and NUF-76 could be due the poor nutritional quality caused by induced activity of these oxidative enzymes.

Lines of experimental evidence shows that plant responses to attack by hemipterans are similar to that of plant pathogens (Kaloshian and Walling 2005). Fungal-rust resistant wheat plants showed induction of lignin biosynthetic pathway in cells exhibiting hypersensitive reactions in response to pathogen infection thus preventing the further hyphal development (Menden et al. 2007). St. Augustinegrasses resistant to the southern chinch bugs may also respond in the same way and prevent further stylet penetration.

Understanding the feeding behavior of southern chinch bugs on the resistant FX-10 and NUF-76 is important to elucidate the mechanisms of resistance. For the first time, the electrical penetration graph (EPG) technique was used to quantify southern chinch bug feeding behavior on resistant and susceptible St. Augustinegrass lines. Southern chinch bugs made more frequent probes, produced longer-duration waveform events for pathway-related behaviors (searching for an ingestion site) and spent less time in ingestion-related waveforms on FX-10 and NUF-76, compared to the susceptible Floratam and Palmetto. Relatively more stylet probes per insect on FX-10 and NUF-76 than on Floratam and Palmetto suggest the presence of stylet penetration impediments around the vascular bundle in resistant varieties. In addition, the short duration of presumed phloem sap ingestion on FX-10 and NUF-76 suggests the possible presence of resistance factors in phloem sap or blockage of sieve elements. A significantly higher number of salivary sheaths were observed in the outermost leaf sheath in FX-10 and NUF-76, which further supports the possible presence of a physical barrier or hypersensitive reactions. Also, the significantly greater number of branched salivary sheaths in FX-10 and NUF-76 corroborates our EPG studies that the southern chinch bugs spent more time on pathway related activities (during which the salivary sheath is constructed) on these two resistant lines.

Plant peroxidases play diverse roles in plants such as lignin biosynthesis, scavenging hydrogen peroxides, and oxidation of reduced toxic compounds and 3-indoleacetic acid (IAA). EPG studies showed that southern chinch bugs probe more frequently on FX-10 and NUF-76 but their stylets took a longer time to reach the phloem sieve elements. Based on the above results, I hypothesized that lignin content and/or leaf sheath anatomy may play a role in southern chinch bug resistance in FX-10 and NUF-76. However, there was no significant difference between FX-10 and Floratam or between NUF-76 and Palmetto in total lignin in the axillary shoot. Therefore, total lignin content does not appear to influence southern chinch bug resistance; however, subtle differences in lignin structure may be important. In general, lignins are made of polymerized phenolic monolignols such as *p*-coumaryl-, coniferyl- and sinapyl-alcohols which are generated through the phenyl propanoid pathway (Barber and Mitchell 1997). The lignins in graminaceous plants are characterized by *p*-hydroxy-phenyl, guaiacyl, and syringyl units (Iiyama et al. 1990). In rust-resistant wheat plants, total lignin content was increased in response to pathogen infection and the increase was exclusively due to increase in syringyl units thus changing the syringyl to guaiacyl ratio from 0.55 to 1.43 (Menden et al. 2007). Previous studies showed that the increase in syringyl-rich lignin or pure syringyl lignin content occurs in plants exhibiting hypersensitive-resistance responses to plant pathogens (Mitchell et al. 1999). The total lignin content of the axillary shoot in southern chinch bug resistant and susceptible St. Augustinegrasses did not differ significantly. However, a change in the ratio of these lignin monomers could have occurred in cells around the stylet pathway, thus preventing further stylet penetration and leading to an increased number of stylet probes on southern chinch bug resistant plants, as shown by both the EPG and salivary sheath distribution studies. There was no significant difference in the thickness of the epidermal layer among any of the St.

Augustinegrasses studied. Transmission electron microscopic studies on axillary shoot anatomy revealed that the cell walls of the highly lignified sclerenchyma cells in the leaf sheath contributed a greater percentage of total cell area in NUF-76 and FX-10 than in Floratam and Palmetto, suggesting that the sclerenchyma cells around the vascular bundle in FX-10 and NUF-76 could serve as an impediment to the southern chinch bug stylet and prevent them from reaching the phloem sieve elements. Thus, these results show for the first time that at least part of southern chinch bug resistance mechanism is a physical impediment to stylet penetration into the vascular bundles.

The presence of thicker sclerenchyma cells in FX-10 and NUF-76 appears to be the constitutive plant defense mechanism against the southern chinch bug and the reason why the southern chinch bugs probe more and change direction more frequently, producing branched salivary sheaths, on these resistant lines. More probing could result in more cellular damage thus leading to a release of plant defense signaling molecules such as salicylic and jasmonic acid. The induction of oxidative enzymes in FX-10 and NUF-76 could be an induced plant defense mechanism and could cause an adverse effect on southern chinch bug survival and development on these resistant lines either by reduced phloem sap ingestion or reduced food absorption. Apart from the excessive cellular damage, more probes also causes deposition of more salivary proteins into the damaged plant tissues. The role of salivary proteins in southern chinch bug-St. Augustinegrass interactions is not known. However, considering the diverse role of insect salivary proteins in plants, it is possible that an excessive deposition of salivary proteins on the resistant cultivars may activate the induced plant defense mechanism in FX-10 and NUF-76. In susceptible plants, these salivary proteins could act as suppressors of plant defense.

This research suggests that the resistant mechanisms formerly found in Floratam were different from any of the mechanisms described above in FX-10 and NUF-76. When Floratam was resistant, the cellular damage and the presence of salivary proteins may have been detected by plant receptors and resulted in the activation of induced plant defense. However, over time, the southern chinch bugs could have evolved to make a change in the nature or content of salivary protein which is not recognized by the receptors in the plant leading to susceptibility. The susceptibility in Palmetto, a diploid, and Bitter Blue, a polyploid, may be due the absence of receptors to perceive the southern chinch bug attack. It is possible that the resistant mechanisms present in each St. Augustinegrass are unique because of the genetic dissimilarities between the lines.

From this research, I conclude that FX-10 and NUF-76 have strong antixenosis to southern chinch bug, while NUF-76 has antibiosis. The southern chinch bug resistance in FX-10 and NUF-76 could be due to a combination of induction of oxidative enzymes, thicker sclerenchyma cells around the vascular bundle, and possible presence of feeding deterrents or toxins in phloem sap or sieve tube blocking mechanisms. Studies outlined here will serve as a solid foundation to further test: (a) phloem sap properties for potential antifeedants or altered nutritional properties, (b) plant metabolic responses including insect-induced polyphenoloxidase, peroxidase and lipoxygenase, and (c) sieve tube blocking mechanisms in FX-10 and NUF-76. The results have important implications for breeding St. Augustinegrass lines with improved resistance to southern chinch bugs.

LIST OF REFERENCES

- Almeida, R. P. P., and E. A. Backus. 2004.** Stylet penetration behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae): EPG waveform characterization and quantification. *Annal. Entomol. Soc. Am.* 97: 838-851.
- Anderson, W. G., T. M. Heng-moss, F. P. Baxendale, L. M. Baird, G. Sarath, and L. Higley. 2006.** Chinch bug (Hemiptera: Blissidae) mouthpart morphology, probing frequencies, and locations on resistant and susceptible germplasm. *J. Econ. Entomol.* 99: 212-221.
- Annan, I. B., G. A. Schaefers, W. M. Tingey and W. F. Tjallingii. 1997.** Stylet activity of cowpea aphid (Homoptera: Aphididae) on leaf extracts of resistant and susceptible cowpea cultivars. *J. Insect Behav.* 10: 603-618.
- Backus, E. A. 1994.** History, development and applications of the AC electronic insect feeding monitor, pp. 1-51. *In* M. E. Ellsbury, D. E. Ullman and E. A. Backus (eds.), History, development and application of the AC electronic insect feeding monitor. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.
- Backus, E. A., and W. H. Bennett. 1992.** New AC electronic insect feeding monitor for Pn-structure analysis of waveforms. *Ann. Entomol. Soc. Am.* 85: 437-444.
- Backus, E. A., W. B. Hunter, and C. N. Arne. 1988.** Technique for staining leafhopper (Homoptera: Cicadellidae) salivary sheath and eggs within unsectioned plant tissue. *J. Econ. Entomol.* 81: 1819-1823.
- Backus, E. A., J. Habibi, F. Yan, and M. Ellersieck. 2005.** Stylet penetration by adult *Homalodisca coagulata* on grape: electrical penetration graph waveform characterization, tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. *Annal. Entomol. Soc. Am.* 98: 787-813.
- Backus, E. A., A. R. Cline, M. R. Ellerseick, and M. S. Serrano. 2007.** *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of nonsequential electrical penetration graph data. *Annal. Entomol. Soc. Am.* 100: 296-310.
- Barber, H. G. 1918.** A new species of *Leptoglossus*: A new *Blissus* and varieties. *Brooklyn Ent. Soc. Bull* 13: 35-39.
- Barber, M. S., and H. J. Mitchell. 1997.** Regulation of phenylpropanoid metabolism in relation to lignin biosynthesis in plants, pp. 243-293. *In* K. J. Jeon (ed.), International review of cytology - a survey of cell biology. Academic Press Inc., San Diego.
- Baxendale, F. P., T. M. Heng-Moss, and T. P. Riordan. 1999.** *Blisus occiduus* Barber (Hemiptera: Lygaeidae): a new chinch bug pest of buffalograss. *J. Econ. Entomol.* 92: 1172-1176.
- Beard, J. B. 1973.** Turfgrass: science and culture. Prentice Hall, Englewood Cliffs, NJ.

- Beyer, A. H. 1924.** Chinch bug control on St. Augustinegrass. Fla. Sta. Hort. Soc. Proc. 37: 216-219.
- Bi, J. L., and G. W. Felton. 1995.** Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. J. Chem. Ecol. 21: 1511-1530.
- Bi, J. L., J. B. Murphy, and G. W. Felton. 1997.** Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. J. Chem. Ecol. 23: 97-117.
- Bing, J. W., M. G. Novak, J. J. Obrycki, and W. D. Guthrie. 1991.** Stylet penetration and feeding sites of *Rhopalosiphum maidis* (Homoptera : Aphididae) on two growth stages of maize. Ann. Entomol. Soc. Am. 84: 549-554.
- Bird, R. D., and A. V. Mitchner. 1950.** Insects of the season 1949 in Manitoba. Can. Insect Pest Rev. 67: 169-172.
- Blum, A. 1968.** Anatomical phenomena in seedlings of sorghum varieties resistant to the sorghum shoot fly (*Atherigona variasocata*). Crop Sci. 8: 388-390.
- Bowling, C. C. 1979.** The stylet sheath as an indicator of feeding activity of the rice stink bug. J. Econ. Entomol. 72: 259-260.
- Brisson, L. F., R. Tenhaken, and C. J. Lamb. 1994.** Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell. 6: 1703-1712.
- Bronner, R., E. Westphal, and F. Dreger. 1991.** Enhanced peroxidase activity associated with the hypersensitive response of *Solanum dulcamara* to the gall mite *Aceria cladophthirus* (Acari: Eriophyoidea). Can. J. Bot. 69: 2192-2196.
- Brown, C. M., and F. R. Holbrook. 1976.** An improved electric system for monitoring feeding of aphids. Am. Potato J.: 457-462.
- Bruton, B. D., R. W. Toler, and J. A. Reinert. 1979.** Combined resistance in St. Augustinegrass to the southern chinch bug and the St. Augustine decline strain of Panicum mosaic virus. Plant Dis. 67: 171-172.
- Buendgen, M. R., J. G. Coors, A. W. Grombacher, and W. A. Russell. 1990.** European corn borer resistance and cell wall composition of three maize populations. Crop Sci 30: 505-510.
- Busey, P. 1979.** What is Floratam? Proc. Fla. State Hort. Soc. 92.
- Busey, P., T. K. Broschat, and B. J. Center. 1982.** Classification of St. Augustinegrass. Crop Sci. 22: 469-473.

- Busey, P. 1986.** Morphological identification of St. Augustinegrass cultivars. *Crop Sci.* 26: 28-32.
- Busey, P., and B. J. Center. 1987.** Southern chinch bug (Hemiptera: Heteroptera: Lygaeidae) overcomes resistance in St. Augustinegrass. *J. Econ. Entomol.* 80: 608-611.
- Busey, P., and B. L. Coy. 1988.** Vulnerability of St. Augustinegrass to the southern chinch bug. *Proc. Fla. State Hort. Soc.* 101: 132-135.
- Busey, P. 1990a.** Inheritance of host adaptation in the southern chinch bug (Heteroptera: Lygaeidae). *Ann. Entomol. Soc. Am.* 83: 563-567.
- Busey, P. 1990b.** Polyploid *Stenotaphrum* germplasm: resistance to the polyploid damaging population southern chinch bug. *Crop Sci* 30: 588-93.
- Busey, P., and E. I. Zaenker. 1992.** Resistance bioassay from southern chinch bug (Heteroptera: Lygaeidae) *J. Econ. Entomol.* 85: 2032-2038.
- Busey, P. 1993.** Registration of FX-10 St. Augustinegrass. *Crop Sci.* 33: 214-215.
- Busey, P. 2003.** St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, pp. 309 - 330. *In* M. D. Casler and R. R. Duncan (eds.), *Biology, breeding, and genetics of turfgrasses.* John Wiley and Sons, Inc., Hoboken, NJ.
- Caillaud, C., J. Pierre, B. Chaubet, and J. Di Pietro. 1995.** Analysis of wheat resistance to the cereal aphid, *Sitobion avenae*, using electrical penetration graphs and flow charts combined with correspondence analysis. *Entomol. Exp. Appl.* 75: 9-18.
- Caillaud, C. M., and H. M. Niemeyer. 1996.** Possible involvement of the phloem sealing system in the acceptance of a plant as host by an aphid. *Cell. Mol. Life Sci.* 52: 927-931.
- Campbell, B. C., and D. L. Dreyer. 1985.** Host-plant resistance of sorghum: differential hydrolysis of sorghum pectic substances by polysaccharases of greenbug biotypes (*Schizaphis graminum*, Homoptera: Aphididae). *Arch. Insect Biochem. Physiol.* 2: 203-215.
- Campbell, B. C., D. L. McLean, M. G. Kinsey, K. C. Jones, and D. L. Dreyer. 1982.** Probing behavior of the greenbug (*Schizaphis-graminum*, biotype-c) on resistant and susceptible varieties of sorghum. *Entomol. Exp. Appl.* 31: 140-146.
- Cardoza, Y. J., J. Reidy-Crofts, and O. R. Edwards. 2005.** Differential inter- and intra-specific defense induction in *Lupinus* by *Myzus persicae* feeding. *Entomol. Exp. Appl.* 117: 155-163.
- Carter, R. P., and R. L. Duble. 1976.** Variety evaluations in St. Augustinegrass for resistance to the southern lawn chinch bug, pp. 39. *Tex. Agric. Exp. Stn. Prog. Rep.* PR-3374C.

- Chaman, M. E., L. J. Corcuera, G. E. Zuniga, L. Cardemil, and V. H. Argandona. 2001.** Induction of soluble and cell wall peroxidases by aphid Infestation in Barley. *J. Agri. Food Sci* 49: 2249-2253.
- Chen, Z., H. Silva, and D. F. Klessig. 1993.** Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* 262: 1883-1886.
- Chen, H., C. G. Wilkerson, J. A. Kuchar, B. S. Phinney, and G. A. Howe. 2005.** Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proc Natl. Acad. Sci. USA* 102: 19237-19242.
- Cherry, R. H., and R. T. Nagata. 1997.** Ovipositional preference and survival of southern chinch bugs (*Blissus insularis* Barber) on different grasses. *Int. Turfgrass Soc. Res. J.* 8: 981-986.
- Cherry, R. H. 2001a.** Seasonal wing polymorphism in southern chinch bugs (Hemiptera: Lygaeidae). *Fla. Entomol.* 84: 737-39.
- Cherry, R. H. 2001b.** Spatial distribution of southern chinch bugs (Hemiptera: Lygaeidae) in St. Augustinegrass. *Fla. Entomol.* 84: 151-153.
- Cherry, R. H., and A. Wilson. 2003.** Morphology and fertility of wing polymorphic adults of southern chinch bugs (Hemiptera: Lygaeidae). *J. Entomol. Sci.* 38: 688-691.
- Cherry, R. H., and R. T. Nagata. 2005.** Development of resistance in southern chinch bug (Hemiptera: Lygaeidae) to the insecticide bifenthrin. *Fla. Entomol.* 88: 219-221.
- Cherry, R., P. Stansly, R. Nagata, and S. Halbert. 2006.** *Liburnia pseudoseminigra* (Delphacidae: Homoptera), a new and unusual pest of St. Augustinegrass. *Fla. Entomol.* 89: 459-461.
- Cherry, R., and R. Nagata. 2007.** Resistance to two classes of insecticides in southern chinch bugs (Hemiptera: Lygaeidae). *Fla. Entomol* 90: 431-434.
- Chittoor, J. M., J. E. Leach, and F. F. White. 1999.** Induction of peroxidase during defense against pathogens, pp. 171- 193. *In* S. K. Datta and S. Muthukrishnan (eds.), *Pathogenesis-related proteins in plants*. CRC, Boca Raton, FL.
- Cipollini, D., S. Enright, M. B. Traw, and J. Bergelson. 2004.** Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. *Mol. Ecol.* 13: 1643-1653.
- Cline, A. R., and E. A. Backus. 2002.** Correlations among AC electronic monitoring waveforms, body postures, and stylet penetration behaviors of *Lygus hesperus* (Hemiptera: Miridae). *Environ. Entomol* 31: 538-549.
- Cole, R. 1994.** Locating a resistance mechanism to the cabbage aphid in two wild Brassicas. *Entomol. Exp. Appl.* 71: 23-31.

- Coley, P. D. 1983.** Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.* 53: 209-233.
- Congdon, C., and E. A. Buss. 2002.** Southern chinch bug management on St. Augustinegrass. *Fla. Coop. Ext. Serv. Eny-325.*
- Constabel, C. P., D. R. Bergey, and C. A. Ryan. 1996.** Polyphenol oxidase as a component of the inducible defense response in tomato against herbivores, pp. 231-252. *In* J. T. Romeo, J. A. Saunders and P. Barbosa (eds.), *Phytochemical diversity and redundancy in ecological interactions.* Plenum, New York, USA.
- Constabel, C. P., Y. Peter, P. Lynn, J. Joseph, and M. E. Christopher. 2000.** Polyphenol oxidase from hybrid poplar: cloning and expression in response to wounding and herbivory. *Plant Physiol.* 124: 285-296.
- Coors, J. G. 1987.** Resistance to the European corn borer, *Ostrinia nubilalis* (Hu' bner), in maize, *Zea mays* L., as affected by soil silica, plant silica, structural carbohydrates, and lignin , pp 445-456. *In:* H. W. Gabelman and B. C. Loughman (eds) *Genetics aspects of plant mineral nutrition.* Dordrecht:, Martinus, Nijhoff.
- Crocker, R. L., R. W. Toler, and J. B. Beard. 1989.** St. Augustinegrass antibiosis to southern chinch bug (Hemiptera: Lygaeidae) and to St. Augustine decline strain of Panicum mosaic virus. *J. Econ. Entomol.* 82: 1729-1732.
- Crocker, R. L. 1993.** Chemical control of southern chinch bug in St. Augustinegrass. *Int. Turfgrass Soc. Res. J.* 7: 358-365.
- Dahms, R. G. 1948.** Effect of different varieties and ages of sorghum on the biology of the chinch bug. *J. Agric. Res.* 76: 271-288.
- Denno, R. F., and M. A. Peterson. 2000.** Caught between the devil and the deep blue sea, mobile plant-hoppers elude natural enemies and deteriorating host plants. *Amer. Entomol.* 46: 95-109.
- Dicke, M., R. Gols, D. Ludeking, and M. A. Posthumus. 1999.** Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *J. Chem. Ecol.* 25: 1907-1922.
- Dowd, P. F., and L. M. Lagrimini. 1997.** The role of peroxidase in host insect defenses, pp. 195-223. *In* Carozzi J. N and M. Koziel ([eds.), *Advances in insect control: the role of transgenic plants* Taylor and Francis, London, UK.
- Dowd, P. F., L. M. Lagrimini, and D. A. Herms. 1998.** Differential leaf resistance to insects of transgenic sweetgum (*Liquidambar styraciflua*) expressing tobacco anionic peroxidase. *Cell. Mol. Life Sci.* 54: 712-720.

- Dowd, P. F., R. A. Holmes, T. S. Pinkerton, E. T. Johnson, L. M. Lagrimini, and R. S. Boston. 2006.** Relative activity of a tobacco hybrid expressing high levels of a tobacco anionic peroxidase and maize ribosome-inactivating protein against *Helicoverpa zea* and *Lasioderma serricornis*. *J. Agri. Food Sci.* 54: 2629-2634.
- Dreyer, D. L., and B. C. Campbell. 1984.** Association of the degree of methylation of intercellular pectin with plant resistance to aphids and with induction of aphid biotypes. *Experientia* 40: 224-226
- Dreyer, D. L., and B. C. Campbell. 1987.** Chemical basis of host-plant resistance to aphids. *Plant Cell Environ.* 10: 353-361.
- Dudeck, A. E., J. A. Reinert, and P. Busey. 1986.** Registration of 'Floralawn' St. Augustinegrass. *Crop Sci.* 26: 1033.
- Duffey, S. S., and M. J. Stout. 1996.** Antinutritive and toxic components of plant defense against insects. *Arch. Insect Biochem. Physiol.* 32: 3-37.
- Duffy, S. S., and G. W. Felton. 1991.** Enzymatic anutritive defenses of the tomato plants against insects, pp. 167-197. *In* P. A. Hedin (ed.), Naturally occurring pest bioregulators. ACS symposium Series 449, Dallas, 1989. American Chemical Society, Washington, DC.
- Eden, W. G., and R. L. Self. 1960.** Controlling chinch bug on St. Augustinegrass lawns Auburn Uni. Agric. Exp. Sta. Prog. Rprt. No.7.
- Ehrman, T. 1996.** Determination of acid-soluble lignin in biomass. NREL-LAP-004. Golden, CO: 1-7.
- Eschrich, W. 1975.** Sealing systems in phloem. , pp. 39-56. *In* M. H. Zimmerman and J. A. Milburn (eds.), Encyclopedia of plant physiology, transport in plants I: phloem transport Springer-Verlag, Berlin, Germany.
- Faccioli, G. 1979.** Relation of peroxidase, catalase, and polyphenoloxidase to acquired resistance in plants of *Chenopodium amaranticolor* locally infected by tobacco necrosis virus. *Phytopathology* 95: 237-249.
- Falkenberg-Borland, D. F., and J. D. Butler. 1982.** Buffalograss - a new turfgrass for golf course. *USGA Green Sec. Rec.* 20: 6-8.
- Farstad, C. W. 1951.** Insects of the season in Alberta, 1950. *Can. Insect Pest Rev.* 29: 18.
- Feeny, P. 1970.** Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51 565-81.
- Felton, G. W., R. J. Donato, D. Vecchio, and S. S. Duffey. 1989.** Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. *J. Chem. Ecol.* 15: 2667-2694.

- Felton, G. W., Workman J, Duffey SS. 1992.** Avoidance of antinutritive plant defense: Role of midgut pH in Colorado potato beetle. *J. Chem. Ecol.* 18: 571-583.
- Felton, G. W., K. K. Donato, R. M. Broadway, and S. S. Duffey. 1992.** Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. *J. Insect Physiol.* 38: 277-285.
- Felton, G. W., Summers CB, Mueller AJ. 1994a.** Oxidative responses in soybean foliage to herbivory by bean leaf beetle and three-corned alfalfa leafhopper. *J. Chem. Ecol.* 20: 639-650.
- Felton, G. W., J. Bi, C. B. Summers, A. J. Mueller, and S. S. Duffey. 1994b.** Potential role of lipoxygenases in defense against insect herbivory. *J. Chem. Ecol.* 20: 651-666.
- Felton, G. W. 2005.** Indigestion is a plant's best defense. *Proc. Natl. Acad. Sci.* 102: 18771-18772.
- Fereres, A., J. A. Shukle, J. A. Araya, and J. E. Foster. 1990.** Probing and feeding behaviour of *Sitobion avenae* (F.) (Hom. Aphididae) on three wheat cultivars *J. Appl. Entomol.* 109: 29-36.
- Ferris, G. F. 1920.** Insects of economic importance in the cape region of lower California, Mexico. *J. Econ. Entomol.* 13: 463-467.
- Fidantsef, A. L., M. J. Stout, J. S. Thaler, S. S. Duffey, and R. M. Bostock. 1999.** Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. *Physiol. Mol. Plant Pathol.* 54: 97-114.
- Fitch, A. 1856.** Chinch bug. *Trans. N. Y. State Agr. Soc.* 15: 277-297.
- Fujisaki, K. 2000.** Seasonal adaptations in subtropical insects: wing polymorphisms and egg diapause in the oriental chinch bug *Cavelerius saccharivorus* Okajima (Heteroptera: Lygaeidae). *Entomol. Sci.* 3: 177-186.
- Garcia-Lara, S., J. T. Arnason, D. Diaz-Pontones, E. Gonzalez, and D. J. Bergvinson. 2007.** Soluble peroxidase activity in maize endosperm associated with maize weevil resistance. *Crop Sci.* 47: 1125-1130.
- Gardner, H. W. 1979.** Lipid hydroperoxide reactivity with proteins and amino acids: a review. *J. Agri. Food Chem.* 27: 220-228.
- Gardner, H. W. 1991.** Recent investigations into the lipoxygenase pathway of plants. *Biochimi. Biophys. Acta* 1084: 221-239.
- Gaspar, T., C. Priel, T. A. Thorpe, and H. Greppin. 1981.** Peroxidases, 1970-1981. Geneva Press, University of Geneva, Switzerland.

- Gasper, T., C. Peenel, T. Thorpe, and H. Greppin. 1982.** Peroxidase: A survey of their biochemical and physiological roles in higher plants. University of Geneva, Geneva Switzerland.
- Green, T. R., and C. A. Ryan. 1972.** Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* 175: 776-777.
- Hamer, J. L. 1985.** Southern branch insect detection, evaluation and prediction report 1983. Southeastern Branch of the Entomological Society of America. vol. 8.
- Hamilton, C. C. 1935.** The control of insect pests of lawn and golf courses. *N. J. Agric. Exp. Sta. Circ.* 34.
- Hartley, S. E. 1988.** The inhibition of phenolic biosynthesis in damaged and undamaged birch foliage and its effect on insect herbivores. *Oecologia* 76: 65-70.
- Haruta, M., J. A. Pedersen, and C. P. Constabel. 2001.** Polyphenol oxidase and herbivore defense in trembling aspen (*Populus tremuloides*): cDNA cloning, expression, and potential substrates. *Physiol. Plantarum* 112: 552-558.
- Haydu, J. J., L. N. Satterthwaite, and J. L. Cisar. 2005.** An economic and agronomic profile of Florida's sod Industry in 2003. Institute of Food and Agricultural Sciences: University of Florida, Gainesville. pp 38: (<http://edis.ifas.ufl.edu/pdffiles/FE/FE56100.pdf>).
- Headlee, T. J. 1910.** Burning chinch bugs. *Kansas State Agric. College Exp. Sta. Circ.* No. 16.
- Heinisch, O., Kowalski E, Ludwig H, Tauscher B. 1996.** Staining for soybean lipoxygenase activity in electrophoretic gels. *Fett/Lipid* 98: 183-184.
- Hellman, L., and J. L. Mathias. 2002.** IPM control options for turfgrass insect pest- chinch bugs. (http://iaa.umd.edu/umturf/Insects/chinch_Bugs.html).
- Heng-Moss, T. M., F. P. Baxendale, T. P. Riordan, and J. E. Foster. 2002.** Evaluation of buffalograss germplasm for gesistance to *Blissus occiduus* (Hemiptera: Lygaeidae). *J. Econ. Entomol.* 95: 1054-1058.
- Heng-Moss, T. M., F. P. Baxendale, T. P. Riordan, L. Young, and K. Lee. 2003.** Chinch bug-resistant buffalograss: an investigation of tolerance, antixenosis, and antibiosis. *J. Econ. Entomol.* 96: 1942-1951.
- Heng-Moss, T. M., G. Sarath, F. Baxendale, D. Novak, S. Bose, X. Ni, and S. Quisenberry. 2004.** Characterization of oxidative enzyme changes in buffalograsses challenged by *Blissus occiduus*. *J. Econ. Entomol.* 97: 1086-1095.
- Hildebrand, D. F., J. G. Rodriguez , G. C. Brown, K. T. Luu, and C. S. Volden. 1986.** Peroxidative responses of leaves in two soybean genotypes injured by twospotted spider mites (Acari: Tetranychidae). *J. Econ. Entomol.*: 1459-1465.

- Hodges, A. W., J. J. Haydu, P. J. van Blokland, and A. P. Bell. 1994.** Contribution of the turfgrass industry to Florida's economy, 1991/92: A value added approach. Economics Report pp. ER94-1, Fla. Agric. Exp. Sta. Circ., Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.
- Holcomb, G. E., K. S. Derrick, R. B. Carver, and R. W. Toler. 1972.** St. Augustinegrass decline virus found in Louisiana. *Plant Dis. Rep.* 45: 69-70.
- Hori, K. 1973.** Studies on the feeding habits of *Lygus disponi* Linnavuori (Hemiptera : Miridae) and the injury to its host plant IV. amino acids and sugars in the injured tissue of sugar beet leaf. *Appl. Entomol. Zool.* 8: 138-142.
- Hori, K., A. Wada, and T. Shibuta. 1997.** Changes in phenoloxidase activities of the galls on leaves of *Ulmus davidana* formed by *Tetraneura fusiformis* (Homoptera: Eriosomatidae). *Appl. Entomol. Zool.* 32: 365-371.
- Horn, G. C., A. E. Dudeck, and R. W. Toler. 1973.** 'Floritam' St. Augustinegrass: a fast growing new variety for ornamental turf resistant to decline and chinch bugs. Fla. Agric. Exp. Sta. Circ. S-224.
- Howard, L. O. 1888.** . The chinch bug. U. S. Dept. of Agric. Circ.17.
- Iiyama, K., T. B. T. Lam, and B. A. Stone. 1990.** Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochemistry* 29: 733-737.
- Janssen, J. A. M., W. F. Tjallingii, and J. C. van Lenteren. 1989.** Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomol. Exp. Appl* 52: 69-81.
- Jerez, M. I. 1998.** Response of two maize inbred lines to chinch bug feeding. M.S. thesis. Mississippi State University, Mississippi State.
- Jiang, Y. X., N. Zareh, G. P. Walker, and L. R. Teuber. 2003.** Characterization of alfalfa germplasm expressing resistance to silverleaf whitefly, *Bemisia argentifolii*. *J. Appl. Entomol.* 127: 447.
- Jiang, Y. X., and G. P. Walker. 2007.** Identification of phloem sieve elements as the site of resistance to silverleaf whitefly in resistant alfalfa genotypes. *Entomol. Exp. Appl.* 125: 307-320.
- Johnson-Cicalese, J. M., F. P. Baxendale, T. P. Riordan, and T. M. Heng-Moss. 1998.** Identification of mealy bug (Hemiptera: Pseudococcidae) resistant turf-type buffalograss germplasm. *J. Econ. Entomol.* 91: 340-346.
- Judd, B. I. 1975.** New World tropical forage grasses and their management. 4. Bermudagrass, giant stargrass, St. Augustinegrass and jaraguagrass. . *World Crops* 27: 69-72.

- Kaloshian, I., M. G. Kinsey, V. M. Williamson, and D. E. Ullman. 2000.** Mi-mediated resistance against the potato aphid *Macrosiphum euphorbiae* (Hemiptera : Aphididae) limits sieve element ingestion. *Environ. Entomol* 29: 690-695.
- Kaloshian, I., and L. L. Walling. 2005.** Hemipterans as plant pathogens. *Annu. Rev. Phytopathol.* 43: 491-521.
- Kanofsky, J. R., and B. Axelrod. 1986.** Singlet oxygen production by soybean lipoxygenase isozymes. *J. Biol. Chem.* 261: 1099-1104.
- Kawabe, S., and D. L. McLean. 1980.** Electronic measurement of the probing activities of the green leafhopper of rice. *Entomol. Exp. Appl.* 27: 77-82.
- Kelsheimer, E. G., and S. H. Kerr. 1957.** Insects and other pests of lawns and turf. *Fla. Agric. Exp. Sta. Circ.* S-96.
- Kerr, S. H. 1958.** Tests on the chinch bugs and current status of control. *Fla. State Hort. Soc. Proc.* 71: 400-402.
- Kerr, S. H., and F. A. Robinson. 1958.** Chinch control tests, 1956-57. *Fla. Entomol.* 41: 97-101.
- Kerr, S. H. 1960.** Insect control. *Proc. Uni. Fla. Turf-Grass Manag. Conf.* 8: 116-118.
- Kerr, S. H. 1966.** Biology of the lawn chinch bug, *Blissus insularis*. *Fla. Entomol* 49: 9-18.
- Kessler, A., and I. T. Baldwin. 2002.** Plant responses to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* 53: 299-328.
- Kessler, A., R. Halitschke, and I. T. Baldwin. 2004.** Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science* 305: 665-668.
- Khan, Z. R., and R. C. Saxena. 1984.** Electronic device to record feeding behavior of whitebacked planthopper on susceptible and resistant rice varieties. *Int. Rice Res. Newsl.* 9: 8 - 9.
- Khatab, H. 2007.** The defense mechanism of cabbage plant against phloem-sucking aphid (*Brevicoryne brassicae* L.). *Aust. J. Basic Appl. Sci.* 1: 56-62.
- Kirk, T. K., and J. R. Obst. 1988.** Lignin determination. *Methods Enzymol.* 161: 87-100.
- Kimsey, R. B., and D. L. McLean. 1987.** Versatile electronic measurement system for studying probing and feeding behavior of piercing sucking insects. *Ann. Entomol. Soc. Am.* 80: 118 -129.
- Klingauf, F. A. 1987.** Host plant finding and acceptance, pp. 209 -23. *In* A. K. Minks and P. Harrewijn (eds.), *Aphids their biology, natural enemies and control*. Elsevier, Amsterdam, Netherlands

- Klingler, J., G. Powell, G. A. Thompson, and R. Isaacs. 1998.** Phloem specific aphid resistance in *Cucumis melo* line AR 5: effects on feeding behaviour and performance of *Aphis gossypii*. Entomol. Exp. Appl. 86: 79-88.
- Koch, E., B. M. Meier, H.-G. Eiben, and A. Slusarenko. 1992.** A lipoxygenase from leaves of tomato (*Lycopersicon esculentum* Mill.) is induced in response to plant pathogenic *Pseudomonads*. Plant Physiol. 99: 571-576.
- Kogan, M., and E. F. Ortman. 1978.** Antixenosis - a new term proposed to define Painter's non-preference modality of resistance. Bull. Ent. Soc. Am. 24.
- Kogan, M. 1994.** Plant resistance in pest management, pp. 73-128. In R. L. Metcalf and W. H. Luckman (eds.), Introduction to insect pest management. Wiley, New York, USA.
- Komblas, K. N. 1962.** Biology and control of the lawn chinch bug, *Blissus leucopterosus insularis* Barber, MSc. Thesis. Louisiana State University, Baton Rouge, LA. .
- Kowalski, S. P., N. T. Eannetta, A. T. Hirzel, and J. C. Steffens. 1992.** Purification and characterization of polyphenol oxidase from glandular trichomes of *Solanum berthaultii*. Plant Physiol. 100: 677-684.
- Kranthi, S., K. R. Kranthi, and R. R. Wanjari. 2003.** Influence of semilooper damage on cotton host-plant resistance to *Helicoverpa armigera* (Hub). Plant Sci. 164: 157-163.
- Kutiart, L. C., and G. C. Nutter. 1952.** Chinch bug control and subsequent renovation of St. Augustinegrass lawn. Fla. Agric. Exp. Sta. Circ. S-50: 1-10.
- Lagrimini, L. M., W. Burkhart, M. Moyer, and S. Rothstein. 1987.** Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissuespecific expression. Proc. Natl. Acad. Sci. USA 84: 7542-7546.
- Lee, T. A., and R. W. Toler. 1973.** Purifications and characterization of virus causing St. Augustinegrass decline (SAD). Phytopathology 63: 444.
- Leonard, D. 1966.** Biosystematics of the *Leucopterosus* complex of the genus *Blissus* (Heteroptera: Lygaeidae). Conn. Agric. Expt. Sta. Bull.: 677.
- Lintner, J. A. 1883.** The chinch bug in New York. Science 2: 540.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951.** Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265-275.
- Lucarini, A. C., and B. V. Kilikian. 1999.** Comparative study of Lowry and Bradford methods: interfering substances. Biotech. Tech. 13: 149-154.
- Ludlum, C. T., G. W. Felton, and S. S. Duffey. 1991.** Plant defenses: chlorogenic acid and polyphenol oxidase enhance toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to *Heliothis zea*. J. Chem. Ecol. 17: 217-237.

- Mao, J., A. J. Burt, A. I. Ramputh, J. Simmonds, L. Cass, K. Hubbard, S. Miller, I. Altosaar, and J. T. Arnason. 2007.** Diverted secondary metabolism and improved resistance to European corn borer (*Ostrinia nubilalis*) in maize (*Zea mays* L.) transformed with wheat oxalate oxidase. *J. Agri. Food Chem.* 55: 2582-2589.
- Marion-Poll, F., W. Della Giustina, and B. Mauchamp. 1987.** Changes of electric patterns related to feeding in amesophyll-feeding leafhopper. *Entomol. Exp. Appl.* 39.
- Matkovics, B., Szabo, L., and Varga, S. I. 1981.** Study of host-parasite interaction in tomato plants. *Acta Univ. Szegediensi Acta Biolog.* 27: 17-23.
- Mauseth, J. D. 1988.** Plant anatomy. The Benjamin/Cummings Publish. Co, Menlo Park, California.
- Maxwell, K. E., and G. F. Macleod. 1936.** Experimental studies of the hairy chinch bug. *J. Econ. Entomol.* 29: 339-343.
- McGregor, R. A. 1976.** Florida turfgrass survey, 1974. Fla. Crop and Livestock Rep. Serv. Orlando, Fla.
- McLean, D. L., and M. G. Kinsey. 1964.** A technique for electronically recording aphid feeding and salivation. *Nature* 202: 1358-1359.
- Meehan, M., and J. Wilde. 1989.** Screening for sorghum line and hybrid resistance to chinch bug (Hemiptera: Lygaeidae) in the greenhouse and growth chamber. *J. Econ. Entomol.* 82: 616-620.
- Menden, B., M. Kohlhoff, and B. M. Moerschbacher. 2007.** Wheat cells accumulate a syringyl-rich lignin during the hypersensitive resistance response. *Phytochemistry* 68: 513-520.
- Merchant, M. E., and R. L. Crocker. 1998.** Chinch bugs in St. Augustinegrass lawns, pp. 4. Texas Agricultural Extension Service publication No: L-1766. .
- Miller, H., D. R. Porter, J. D. Burd , D. W. Mornhinweg, and R. L. Burton. 1994.** Physiological effects of Russian wheat aphid (Homoptera: Aphididae) on resistant and susceptible barley. *J. Econ. Entomol.* 87: 493-499.
- Mitchell, H. J., S. A. Hall, R. Stratford, J. L. Hall, and M. S. Barber. 1999.** Differential induction of cinnamyl alcohol dehydrogenase during defensive lignification in wheat (*Triticum aestivum* L.): characterisation of the major inducible form. *Planta* 208: 31-37.
- Mize, T. W. 1980.** Chinch bug, *Blissus leucopterus leucopterus* (Say): screening of sorghum germplasm for resistance and evaluation of insecticides at planting time for control. M.S. thesis. Kansas State University, Manhattan, Kansas.

- Mize, T. W., and G. Wilde. 1986.** New resistant germplasm to the chinch bug (Heteroptera: Lygaeidae) in grain sorghum contribution of tolerance and nixenosis as resistance mechanisms. *J. Econ. Entomol.* 79: 42-45.
- Mohri, S., Endo, Y., Matsuda, K., Kitamura, K., and Fujimoto, K. 1990.** Physiological effects of soybean seed lipoxygenases on insects. *Agri. Biol. Chem.* 54: 2265 - 2270.
- Montandon, A. E. 1893.** Lygaeids exotiques. Notes et descriptions d'especes nouvelles *Ann. Ent. Soc. Belg.* 37: 399-406.
- Montllor, C. B., and W. F. Tjallingii. 1989.** Stylet penetration by two aphid species on susceptible and resistant lettuce. *Entomol. Exp. Appl.* 52: 103-111.
- Nagata, R. T., and R. H. Cherry. 1999.** Survival of different life stages of the southern chinch bug (Hemiptera: Lygaeidae) following insecticidal applications. *J. Entomol. Sci.* 34: 126-131.
- Nagata, R. T., and R. H. Cherry. 2003.** New source of chinch bug (Hemiptera: Lygaeidae) resistance in a diploid selection of St. Augustinegrass. *J. Entomol. Sci.* 38: 654-659.
- Nault, L. R., and W. E. Styer. 1972.** Effects of sinigrin on host selection by aphids. *Entomol. Exp. Appl.* 15: 423-437.
- Newell, W., and E. W. Berger. 1922.** Insects injurious to the principal crops of South. Fla. Sta. Plant Board. Q. Bull. 6.
- Ni, X., and S. Quisenberry. 1997.** Distribution of Russian wheat aphid (Homoptera: Aphididae) salivary sheaths in resistant and susceptible wheat leaves. *J. Econ. Entomol.* 90: 848 -853.
- Ni, X., S. S. Quisenberry, T. Heng-Moss, J. Markwell, G. Sarath, R. Klucas, and F. Baxendale. 2001.** Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. *J. Econ. Entomol.* 94: 743-751.
- Niblett, C. L., A. Q. Paulsen, and R. W. Toler. 1977.** Panicum mosaic virus. CMI/AAB Descript. Plant Viruses No.177.
- Nuland, D. S., P. E. Reece, and E. J. Kinbacher. 1981.** Buffalograss: energy efficient turf for lawns. *Coop. Ext. Uni. Nebraska-Lincoln.* G 81-564.
- Painter, R. H. 1928.** Notes on the injury to plant cell by chinch bug feeding. . *Ann. Entomol. Soc. Am.* 21: 232-242.
- Park, S.-J., Y. Huang, and P. Ayoubi. 2006.** Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. *Planta* 223: 932-947.

- Parker, J. R. 1920.** The chinch bug in Montana. *J. Econ Entomol* 13: 318-322.
- Paul, T. A., P. Darby, C. P. Green, C. J. Hodgson, and J. T. Rossiter. 1996.** Electrical penetration graphs of the damson-hop aphid, *Phorodon humuli* on resistant and susceptible hops (*Humulus lupulus*). *Entomol. Exp. Appl.* 80: 335-342.
- Pickles, A. 1940.** Measure for the control of chinch bug infesting lawns in Trinidad. *Trop. Agric.* 17: 236-237.
- Pierpoint, W. S. 1983.** Reactions of phenolic compounds with proteins, and their relevance to the production of leaf protein, pp. 235-267. *In* L. Telek and H. Graham (eds.), *Leaf protein concentrates*. Avi Publishing, Westport, Connecticut.
- Rafi, M. M., R. S. Zemetra, and S. S. Quisenberry. 1996.** Interaction between Russian wheat aphid (Homoptera: Aphididae) and resistant and susceptible genotypes of wheat. *J. Econ. Entomol.* 89: 239-246.
- Raman, K. V., W. M. Tingey, and P. Gregory. 1979.** Potato glycoalkaloids: effect on survival and feeding behavior of the potato leaf hopper. *J. Econ. Entomol.* 72: 337- 341.
- Ramiro, D., O. Guerreiro-Filho, and P. Mazzafera. 2006.** Phenol contents, oxidase activities, and the resistance of coffee to the leaf miner, *Leucoptera coffeella*. *J. Chem. Ecol.* 32: 1977-1988.
- Rangasamy, M., H. J. McAuslane, R. H. Cherry, and R. T. Nagata. 2006.** Categories of resistance in St. Augustinegrass lines to Southern chinch bug (Hemiptera: Blissidae). *J. Econ. Entomol.* 99: 1446-1451.
- Rastogi, S., and U. N. Dwivedi. 2006.** Down-regulation of lignin biosynthesis in transgenic *Leucaena leucocephala* harboring *o*-methyltransferase gene. *Biotechnol.* 22: 609-616.
- Raupp, M. J. 1985.** Effects of leaf toughness on mandibular wear of the leaf beetle, *Plagioderma versicolor*. *Ecol. Entomol.* 10: 73-79.
- Rayapuram, C., and I. T. Baldwin. 2006.** Using nutritional indices to study LOX3-dependent insect resistance. *Plant Cell Environ.* 29: 1585 -1594.
- Reinert, J. A. 1972.** Turf-grass insect research. *Fla. Turf-Grass Manag. Conf. Proc.* 20: 79-84.
- Reinert, J. A., and A. E. Dudeck. 1974.** Southern chinch bug resistance in St. Augustinegrass. *J. Econ. Entomol.* 67: 275-277.
- Reinert, J. A. 1978a.** Antibiosis to the southern chinch bug by St. Augustinegrass accessions. *J. Econ. Entomol* 71: 21-24.
- Reinert, J. A. 1978b.** Natural enemy complex of the southern chinch bug in Florida. *Ann. Entomol. Soc. Am.* 71: 728-731.

- Reinert, J. A. 1982.** Insecticide resistance in epigeal insect pests of turfgrass, I. Southern chinch bug resistance to organophosphates in Florida. pp. 71-76. *In* H. D. Niemczyk and B. G. Joyner (eds.), *Advances in turfgrass entomology*. Hammer Graphics, Inc., Piqua, Ohio.
- Reinert, J. A., and K. Portier. 1983.** Distribution and characterization of organophosphate-resistant southern chinch bugs (Heteroptera: Lygaeidae) in Florida. *J. Econ. Entomol.* 76: 1187-1190.
- Reinert, J. A., B. D. Bruton, and R. W. Toler. 1980.** Resistance of St. Augustinegrass lines for resistance to southern chinch bug (Hemiptera: Lygaeidae) and to St. Augustinegrass decline strain of *Panicum mosaic virus*. *J. Econ. Entomol.* 73: 602-604.
- Reinert, J. A., P. Busey, and G. B. Frank. 1986.** Old World St. Augustinegrass resistant to southern chinch bug (Heteroptera: Lygaeidae). *J. Econ. Entomol.* 79: 1073-1075.
- Reinert, J. A., P. R. Heller, and R. C. Crocker. 1995.** Chinch bugs, pp. 38-42. *In* R. L. Brandenburg and M. G. Villani (eds.), *Handbook of turfgrass insect pests*. Entomological Society of America, Annapolis, MD.
- Riordan, T. P., F. P. Baxendale, R. E. Gaussoin, and J. E. Watkins. 1996.** Buffalograss: an alternative native grass for turf. *Coop. Ext. Uni. Nebraska-Lincoln* G96-1297-A.
- Ruuhola, T., and S. Yang. 2006.** Wound-induced oxidative responses in mountain birch leaves. *Ann. Bot.* 97: 29-37.
- Ryan, J. D., K. W. Dorschner, M. Girma, R. C. Johnson, and R. D. Eikenbary. 1987.** Feeding-behavior, fecundity, and honeydew production of 2 biotypes of greenbug (Homoptera, Aphididae) on resistant and susceptible wheat. *Environ. Entomol.* 16: 757-763.
- SAS. 1997.** SAS/STAT user's guide. SAS Institute, Cary, NC.
- SAS. 1999.** SAS/STAT user's guide. SAS Institute, Cary, NC.
- Sauer, J. D. 1972.** Revision of *Stenotaphrum* (Gramineae: Paniceae) with attention to its historical geography. *Brittonia* 24: 202-222.
- Sauge, M.-H., J. Kervella, and Y. Rahbe. 1998.** Probing behaviour of the green peach aphid *Myzus persicae* on resistant *Prunus* genotypes. *Entomol. Exp. Appl.* 89: 223-232.
- Shanks, C. H., and D. Chase. 1976.** Electric measurement of feeding by the strawberry aphid on susceptible and resistant strawberries and nonhost plants. *Ann. Entomol. Soc. Am.* 69: 784 -786.
- Shimer, H. 1867.** Notes on *Micropus (Lygaeus) leucopterosus* Say, ("The chinch bug") with an account of the great epidemic disease of 1865 among insects. *Proc. Acad. Nat. Philadelphia* 19.

- Short, D. E., R. J. Black, and L. B. McCarty. 1997.** Management of insects in lawn. EDIS (<http://edis.ifas.ufl.edu/LH034>).
- Shukle, R. H., and L. L. Murdock. 1983.** Lipoxygenase, trypsin inhibitor, and lectin from soybeans: effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). Environ. Entomol. 12: 787-791.
- Skorzynska-Polit, E., and Z. Krupa. 2003.** The activity of lipoxygenase in *Arabidopsis thaliana* (L.) Heynh - a preliminary study. Cell. Mol. Biol. Lett. 8: 279-84.
- Slater, J. A. 1964.** A catalogue of the Lygaeidae of the world. University of Connecticut, Storrs, Connecticut, USA
- Snelling, R. O., R. H. Painter, J. H. Parker, and W. M. Osborn. 1937.** Resistance of sorghum to the chinch bug. U.S. Dep. Agric. Bull. 585.
- Spurr, A. R. 1969.** A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 21.
- Stacy, W. 2001.** Southern chinch bug, *Blissus insularis* Barber, (http://creatures.ifas.ufl.edu/orn/turf/Southern_chinch_bug.htm).
- Staswick, P., and C. Lehman. 1999.** Jasmonic acid-signaled responses in plants, pp. 117-136. In A. A. Agrawal, S. Tuzun and E. Bent (eds.), Inducible plant defenses against pathogens and herbivores: biochemistry, ecology, and agriculture. American Phytopathological Society, St. Paul, Minnesota, USA.
- Stout, M. J., A. L. Fidantsef, S. S. Duffey, and R. M. Bostock. 1999.** Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiol. Mol. Plant Pathol. 54: 115-130.
- Strobel, J. 1971.** Turfgrass. Fla. Turf-Grass Manag. Conf. Proc. 19: 19-28.
- Tarn, T. R., and J. B. Adams. 1982.** Aphid probing and feeding, electronic monitoring, and plant breeding. pp. 221-246. In K. F. Harris and K. Maramorosch (eds.), Pathogens, vectors, and plant diseases Academic Press., New York, NY, USA.
- Thaler, J. S. 1999.** Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399: 686-688.
- Thipyapong, P., S. Mahanil, A. Bhonwong, J. Attajarusit, M. J. Stout, and J. C. Steffens. 2006.** Increasing resistance of tomato to lepidopteran insects by overexpression of polyphenol oxidase. Acta Hort. 724: 29-38.
- Tjallingii, W. 1978.** Electronic recording of penetration behaviour by aphids. Entomol. Exp. Appl. 24: 721-730.

- Tjallingii, W. F. 1985.** Electric nature of recorded signals during stylet penetration by aphids. *Entomol. Exp. Appl* 38: 177-186.
- Tjallingii, W. F. 2006.** Salivary secretions by aphids interacting with proteins of phloem wound responses. *J. Exp. Bot.* 57: 739-745.
- Thompson, G. A., and F. L. Goggin. 2006.** Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *J. Exp. Bot.* 57: 755-766.
- Toler, R. W., and W. J. Walla. 1976.** Turf disease. *Tex. Agric. Stn. Prog. Rep.* PR: 3365C.
- Triplehorn, B. W., L. R. Nault, and D. J. Horn. 1984.** Feeding behavior of *Graminella nigrifrons* (Forbes). *Ann. Entomol. Soc. Am.* 77: 102 -107.
- Tsudoku, M., T. Takano, F. Nakatsubo, K. Yoshida, A. Shinmyo, and H. Asao. 2006.** Resistance to insects in transgenic *Solanum* plants expressing a peroxidase gene from horseradish. *Plant Biotech.* 23: 71-74.
- Ullman, D. E., and D. L. McLean. 1988.** The probing behavior of the summer-form pear psylla. *Entomol. Exp. Appl.* 47: 115-125.
- Ullman, D. E., C. O. Qualset, and D. L. McLean. 1988.** Feeding responses of *Rhopalosiphum padi* (Homoptera: Aphididae) to barley yellow dwarf virus resistant and susceptible barley varieties. *Environ. Entomol.* 17 988-991.
- Vallejos, C. E. 1983.** Enzyme activity staining, pp. 469-516. *In* S. D. Tanksley and T. J. Orton (eds.), *Isozymes in plant genetics and breeding, part A.* Elsevier, Amsterdam, Netherlands.
- van Bel, A. J. E. 2003.** The phloem, a miracle of ingenuity. *Plant Cell Environ* 26: 125-149.
- van der Westhuizen, A. J., and Z. Pretorius. 1995.** Biochemical and physiological responses of resistant and susceptible wheat to Russian wheat aphid infestation. *Cereal Res. Comm.* 23: 305-313.
- van der Westhuizen, A. J., X. M. Qian, and A. M. Botha. 1998.** Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Rep.* 18: 132-137.
- van Duzee, E. P. 1886.** Occurrence of the chinch bug (*Blissus leucopteros* (Say)) at Buffalo. N.Y. *Can. Entomol.* 18: 209-210.
- van Giessen, W. A., and D. M. Jackson. 1998.** Rapid analysis of electronically monitored homopteran feeding behavior. *Ann. Entomol. Soc. Am.* 91: 145-154.
- van Helden, M., and W. Tjallingii. 1993.** Tissue localisation of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. *Entomol. Exp. Appl.* 68: 269-278.

- Voelckel, C., W. W. Weisser, and I. T. Baldwin. 2004.** An analysis of plant-aphid interactions by different microarray hybridization strategies. *Mol. Ecol.* 13: 3187-3195.
- Wainhouse, D., D. J. Cross, and R. S. Howell. 1990.** The role of lignin as a defence against the spruce bark beetle *Dendroctonus micans*: effect on larvae and adults. *Oecologia* 85: 257-265.
- Wainhouse, D., R. Ashburner, E. Ward, and R. Boswell. 1998.** The Effect of lignin and bark wounding on susceptibility of spruce trees to *Dendroctonus micans*. *J. Chem. Ecol.* 24: 1551-1561.
- Walden, B. H. 1936.** Notes on the hairy chinch bug, a pest of lawns. *Conn. Agric. Exp. Sta. Bull.* : 383.
- Walker, G. P. 2000.** A beginner's guide to electric monitoring of homopteran probing behaviour *In* G. P. Walker and E. A. Backus [eds.], Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say, Lanham, MD.
- Wang, J., and C. P. Constabel. 2004.** Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). *Planta* 220: 87-96.
- Watschke, T. L., P. H. Dernoeden, and D. J. Shelter. 1995.** Managing the turfgrass pests. CRC, Boca Raton, FL.
- Watson, J. R. 1925.** The chinch bug on St. Augustinegrass lawns. *Fla. Agric. Exp. Sta. Circ. Bull.*: 371.
- Webster, F. M. 1907.** The chinch bug. U.S. Dept. Agric. Circ. 69.
- Wenger, L. E. 1943.** Buffalo grass. *Kans. Agric. Exp. Stn. Bull.* 321.
- Wilde, G., T. Mize, and J. Stewart. 1982.** Chinch bug resistance in commercial grain and forage sorghum hybrids. *Kans. Agric. Exp. Stn. Bull.* 56.
- Wilson, R. N. 1929.** The chinch bug in relation to St. Augustinegrass. U.S. Dept. of Agric. Circ. 51.
- Wolfenbarger, D. O. 1953.** Insect and mite control problems on lawn and golf grasses. *Fla. Entomol.* 36.
- Works Progress Administration. 1939.** Dairy of A. M. Reed, 1848-1899, and a Portion of 1900 by others. Works Progress Administration, State Office, Historical Records Survey. St. Augustine Historical Society Library, St. Augustine, Florida.

Zhu-Salzman, K., R. A. Salmon, J.-E. Ahn, and H. Koiwa. 2004. Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. *Plant Physiol.* 134: 420-431.

BIOGRAPHICAL SKETCH

Murugesan Rangasamy was born in Erugalur, a village 20 miles south of Salem, India in 1975 and is the only child of his parents. He graduated from Mallasamudram higher secondary school in 1992 and earned his Bachelor's degree in Agriculture in 1999 from the Tamil Nadu Agricultural University, Coimbatore India. After graduating with the bachelor degree in agriculture, Murugesan worked as a Marketing Development Trainee with EID Parry (India) Ltd. Farm Input Division for eight months and involved in promoting an environmentally friendly insecticide, Neemazal, among cotton growers. He was the recipient of a prestigious Junior Research Fellowship awarded by Indian Council of Agricultural Research for doing graduate studies in Entomology at the Punjab Agricultural University, Ludhiana, India, where he earned his master's in 2001. Upon graduation with Master's, he worked as a Senior Research Fellow on the World Bank sponsored National Agricultural Technology at the Indian Agricultural Research Institute, New Delhi, India. His research project aimed to develop insect and disease resistant cole crops such as cauliflower and cabbage. His undaunted zealous to work on insects, especially on host plant resistance, landed him at the University of Florida with an opportunity to do PhD in Entomology. Murugesan investigated mechanisms of southern chinch bug resistance in St. Augustinegrasses. After earning his PhD in 2007, he is planning to continue his career in research on insect plant interactions. Murugesan has always been a meritorious student throughout his carrier and recipient of many scholarships and fellowships for his studies which otherwise would have been extremely difficult.

Murugesan has been married to Shunmuga Priya Jeevaratchagan, an Accounting Coordinator at the University of Florida Financial Services, for 2½ years and the couple are blessed with a baby girl, Sudhiksha Murugesan, who will be turning one year on February 20, 2008.