COMPARISON OF METHODOLOGIES IN DETERMINING HOST PLANT RESISTANCE TO SOUTHERN CHINCH BUG IN ST. AUGUSTINEGRASS AND INVESTIGATION ON SEED SET RATE OF CARPETGRASS

By

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To my parents

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Abstract of Thesis Presented to the Graduate School
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St. Augustinegrass is used as lawn grass throughout the southern United States for its wide adaptation to varying environmental conditions. The southern chinch bug, *Blissus insularis* Barber, is the plant's most damaging insect pest. Host plant resistance of St. Augustinegrass has been determined in numerous studies using various techniques. However, efficacy of these various procedures in determining St. Augustinegrass resistance to southern chinch bug has not been compared. The objective of this study was to determine the effect of time and methodologies in determining St. Augustinegrass resistance to southern chinch bugs. Four varieties were tested for resistance using four different methods (bag, jar, box, tube) and different time intervals to measure chinch bug mortality. Overall, survival was greater in whole plant methods (box and tube) than excised stolon methods (bag and jar). The bag test gave the most erratic results of the four methods. The effect of time in determining resistance was also very evident. In our tests, it was clear that shorter time intervals in measuring mortality may result in not measuring resistance in a variety. In summary, researchers

should carefully consider method, time and temperature as important variables in determining St. Augustinegrass resistance to southern chinch bugs.

Seed set rate of common carpetgrass was investigated in a greenhouse study. The preliminary results showed that seed set rates ranged from 0.25 to 0.79 when selfed, while open-pollinated flowers had 0.27– 0.70 of seed-set rates. The differences of seed set rates between selfed and open-pollinated flowers varied among genotypes.

CHAPTER 1
LITERATURE REVIEW

Economic Importance of St. Augustinegrass in Florida

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] was the first turfgrass planted in Florida in 1880 with the earliest commercial sod production recorded in the 1920s. It is the most popular turfgrass in Florida due to its competitive characters such as high density, less weed invasion and wide environmental adaptation (Busey and White, 1993). By 1987, about 1 billion square feet of turfgrass was produced annually in Florida, producing about $100 million in annual sales with St. Augustinegrass being the predominant species (White and Busey, 1987). Recent data shows that annual sod production has increased to an estimated 103,923 acres and has created more than $1 billion economic impact, $514 million in-field value and 5,633 jobs. St. Augustinegrass is still the predominant turfgrass in sod production, having 51% of total acreage compared to bahiagrass (ranked second) at 32%. The Floratam variety occupied 81% of total St. Augustinegrass acreage. Both bermudagrass and zoysiagrass accounted for less than 10% (Satterthwaite et al. 2009).

Distribution, Cytotaxonomy, and Genetics of St. Augustinegrass

Origin and Related Species

The genus *Stenotaphrum*, originates from tropical climates, belongs to the tribe Paniceae of the Panicoideae subfamily. It consists of seven species, six out of seven being restricted to the Old World. *Stenotaphrum secundatum* (i.e. St. Augustinegrass) is the only widely distributed species found in both the Old World and New World (Sauer, 1972). This fact strongly suggests the Old World origin of *Stenotaphrum*. But the origin of *S. secundatum* is not clear. Milla-Lewis et al. (2013) suggested a possible dual origin

of St. Augustinegrass based on genetic analysis of existing St. Augustinegrass cultivars and its plant introductions in both the Old world and New world. Sauer (1972) reported possible hybridization of *S. secundatum* with *S. dimidiatum* but had no chromosome information. Milla-Lewis et al. (2011) discovered resistance to *Magnaporthe grisea* (grey leaf spot disease transmission agent) on some members in the Longicaudatus race (Busey, 1995) which was previously only reported in *S. dimidiatum*. A recent molecular analysis investigated the genetic relationships among diploid and polyploid cultivars, plant introductions and pembagrass (*S. dimidiatum*), which will benefit the determination of polyploid and aneuploid formation (Milla-Lewis et al. 2013).

Taxonomy and Geography

Busey (1982) clustered St. Augustinegrass into five groups based on 26 characteristic evaluated using 96 genotypes. Later reassignment into races or groups was based on previous classification. Assignments include the Floratam group, Bitterblue group, Breviflorus race, and Longicaudatus race (Busey, 1986).

The Floratam group is distinguished by long spikelet (>5.2mm) containing genotypes such as Floratam and Floralawn. The Breviflorus race is identified with spikelets less than 5.2mm and short inflorescence branches (≤15mm) including Dwarf Group and Gulf Coast Group which contain the cultivars Seville, Raleigh and Texas Common. Subsequently, the Longicaudatus race is separated by long internodes (80mm on average) containing cultivars Roselawn and Florida common. Lastly, the Bitterblue group contains genotypes that don’t fit the prior descriptions and contains the cultivars Bitterblue and Floratine (Busey, 1986). Milla-Lewis et al. (2013) reported consistent results for previous classification based on principle coordinate analysis (PCO) and cluster analysis.

The Gulf Coast Group, endemic to the southeastern United States, is homogeneous with white stigmata and green stolons including “Texas Common” and Raleigh (Busey, 2003). The Dwarf Group is homogeneous with a reddish stolon, purple stigma and dark green leaves (Busey, 1982). Seville (Riordan et al. 1980), an artificial hybrid between Gulf Coast Group and Dwarf Group, was the first St. Augustinegrass released with a known pedigree (Busey, 2003).

FX-10 St. Augustinegrass was released by Busey (1993) and does not belong to any races or groups mentioned above. It was selected from second generation progenies from African plant introductions (PIs) and clustered by Milla-Lewis et al. (2013) into the same group as those PIs.

Polyploidy and Importance in Sod Production

Chromosome numbers of St. Augustinegrass have been investigated to understand the genetic relationships among the cultivars. The diploid St. Augustinegrass contains 18 chromosomes (n=9). Polyploid St. Augustinegrass was first reported by Long and Bashaw (1961) including both sterile triploids (2n=27) and sterile tetraploids (2n=36). The triploid with purple stigma was later assigned as “Cape deme” which was first collected in 1791 at the Cape of Good Hope (Sauer, 1972). These sterile triploids were generally established via vegetative propagation. Bitterblue, one triploid genotype, was the foundation cultivar for the Florida sod industry and the only suitable cultivar from 1934 to 1973 (Busey and White, 1993).

A historically important sterile polyploid cultivar is Floratam which is an aneuploid having 32 chromosomes. This cultivar was released by Horn et al. (1973) for its combined resistance to southern chinch bug and the St. Augustinegrass Decline strain of Panicum Mosaic Virus (PMV-SAD). However, the chinch bug resistance in Floratam

was later overcome by a polyploid-damaging population of the bugs (PDP) (Busey and Center, 1987), which was confirmed by Cherry and Nagata (1997). Floratam has been widely used in the sod industry since its release and continues to be popular despite the loss of resistance to southern chinch bugs. Busey (1986) reported that Floratam occupied 77 percent of the total sod production. Satterthwaite et al. (2009) reported that 81 percent of St. Augustinegrass sod production was planted in Floratam.

FX-10, another aneuploid (2n=30) cultivar, was released in 1993 for its resistance to PDP southern chinch bugs with other good characters (Busey, 1993). It was selected from the second generation progeny of controlled pollination among African polyploid St. Augustinegrass germplasm with bivalent pairing and regular disjunction bearing normal seeds (Busey, 1990; Busey, 1993).

Milla-Lewis et al. (2013) investigated the use of flow cytometry to determine the ploidy levels of St. Augustinegrass, using chromosome counts from existing cultivars and PIs including Bitterblue, PI300129, PI291594, Floratine, PI290888, PI300130, FX-10, Floratam and Floralawn. It was determined that flow cytometry is a useful tool for ploidy analysis in St. Augustinegrass.

Different ploidy levels in St. Augustinegrass lead to adaptive polymorphism. Polyploid St. Augustinegrass normally have longer, wider, and hicker leaf blades with brighter green color compared with diploid genotypes. Diploids, however, are more highly branched with a stronger rapid cover ability leading to early maturity and harvest (Busey et al. 1982; Busey, 1986; Busey, 2003).

Different ploidy levels also account for the majority of the variations in resistance to biotic stress. Polyploids are more resistant to both PDP and standard southern chinch

bug (Busey, 1990; Busey and Zaenker, 1992; Reinert et al. 1986), but they are more susceptible to grey leaf spot disease than diploids (Atilano and Busey, 1983). But one diploid variety “Captiva” was developed resistant to southern chinch bugs (Nagata and Cherry 2003). The resistant mechanisms also differ in diploid and polyploid varieties. Rangasamy (2006) reported that Captiva showed both antibiosis and antixenosis whereas FX-10 only showed antixenosis. As most variation is between ploidy levels, variations may not be useful until successful hybridization between different ploidy levels can be achieved (Busey, 2003). Genovesi et al. (2009) applied embryo rescue techniques and achieved 10.88% crossability among eight polyploid and six diploid genotypes indicating a possible better use of genetic variation within St. Augustinegrass in the future.

St. Augustinegrass Biology

Plant Characteristics

Leaf blades of St. Augustinegrass are 5-14mm wide and round-tipped with conspicuous midrib. They are normally glabrous and sparsely pubescent in genotypes with suggested introgression with *S. dimidiatum* (Busey, 1986). The leaves arranged strictly distichously along the slightly flattened stolons and their bases attenuated and subsequently delimited by a constricted collar. St. Augustinegrass has a membranous ligule, a laterally compressed leaf sheath and no auricles (Busey, 2003).

The St. Augustinegrass inflorescence is a spike with branches contracted into the rachis and generally reduced to single spikelets. A disarticulated inflorescence rachis can float for 7-10 days which may account for local dispersals (Sauer, 1972). Spike length is free of environmental influences which can be used for cultivar identification (Busey, 1993). The awnless spikelets, 3-6mm long, consist of dissimilar glumes on the

outside and two differently positioned florets inside. The lower glume is scale-like and nerveless while the upper one is similar to nerved lemmas. The lower floret is generally imperfect (staminate or neuter) while the upper perfect floret is perfect with three anthers and two stigmas (Busey, 2003). The stigma color is white, purple or bicolor and the anther color ranges from creamy white to burgundy (Busey et al. 1982).

St. Augustinegrass spreads by branching, above ground stolons. It has relative poor wear tolerance with slow recovery from defoliation (Busey, 2003). Stolon pigmentation ranges from almost green to intense red (Busey et al. 1982).

Reproduction

St. Augustinegrass is generally vegetatively propagated via cutting stolons, plugs or sod. Effort on development of seeded cultivars has not been successful. Some heavily seeded cultivars, for instance, are esthetically unacceptable (Busey, 2003).

Environmental Adaptation

St. Augustinegrass is adapted to wide range of soil conditions with different pH values, and different organic matter content. It is more salt tolerant than other warm-season grasses. The species also has various levels of resistance to drought and shade (Horn et al. 1973).

Different levels of salt tolerance have been noted for some cultivars. Floratam and Floralawn are highly tolerant compared to other cultivars (Dudeck et al. 1993). Seville is more saline tolerant than Floratam (Meyer et al. 1989). Floratine and Hawaii Sel St. Augustinegrass were reported with salt tolerance as well (Marcum and Murdoch, 1990).

St. Augustinegrass is reported to have medium to good drought resistance with a great diversity among cultivars (Beard, 1989). However, it is not as resistant as other C4

grasses like bermudagrass (*Cynodon spp*.) and Zoysiagrass (*Zoysia spp*) (Busey, 1996). Floratam and Floralawn were reported to be drought tolerant by Beard (1989) and further confirmed by Sifers and Beard (1999). FX-10 St. Augustinegrass has a significantly better drought tolerance than floratam and other cultivars for its deep rooting ability (Miller and McCarty, 2001).

St. Augustinegrass can tolerate partial tree shade. Bitterblue, “Jade”, and Seville have better shade tolerance than Floratam (Busey and Davis, 1991). Partial shade tolerance is desired in residential landscapes (Busey, 2003). Smith and Whiteman (1983) reported that St. Augustinegrass had the best response among eight tropical grass species (*Axonopus compressus, Brachiaria decumbens, B. humidicola, B. miliiformis, Dicanthium caricosum, Ischaemum aristatum, Paspalum conjugatum* and *Stenotaphrum secundatum*) under 80% coconut canopy shade.

The effects of nutrients on the growth of St. Augustinegrass have been studied. Cherry et al. (2012) found that nutrition content of leaf tissues varied among varieties indicating that Floratam has a less nutrition requirement for the leaves than Raleigh and Captiva. Proper fertilization is a big concern in turf production. Trenholm et al. (2012) reported that the turf quality of Floratam decreases under extremely low nitrogen application rate (49kgN/ha^{-}1, applied in ~60d intervals). Cherry et al. (2011) found that increased fertilization led to significantly greater gray leaf spot disease in Captiva St. Augustinegrass but did not affect southern chinch bug resistance. Nitrogen leaching and runoff is a common problem related to turfgrass fertilization. However, St. Augustinegrass has significantly less nitrogen leaching than mixed-species landscape

(Erickson et al. 2001). Trenholm et al. (2012) also reported that little nitrate-nitrogen leaching occurred in Floratam St. Augustinegrass.

Biotic Stress

St. Augustinegrass has fewer nematode and disease problems compared with other warm season grasses (Horn et al. 1973). The southern chinch bug, *Blissus insularis* Barber, is the plant's most damaging insect pest. Genotypic variation among cultivars and breeding accessions exists for southern chinch bug resistance (Reinert and Dudeck, 1974; Lu and Cherry, 2012) and other traits such as grey leaf spot disease resistance (Busey et al. 1993, Atilano and Busey, 1983) and PMV-SAD resistance (Horn et al. 1973).

Chinch Bugs

Taxonomy of Southern Chinch Bugs

There are three species of chinch bugs with major economic importance in the United States. They are the western chinch bug, *Blissus occiduus* Barber, the hairy chinch bug, *Blissus leucopterus hirtus* Montandon; and the southern chinch bug, *Blissus insularis* Barber (Vittum et al. 1999). The primary chinch bug in Florida and pest of St. Augustinegrass is the southern chinch bug (Reinert and Kerr, 1973).

Biology and Feeding Habit

The southern chinch bug is a piercing sucking insect that drains sap from St. Augustinegrass leaf tissue (Wilson, 1929). The egg period was reported to vary from 7 d to 14d. The nymph stage lasts for 28 to 30 d containing five to six instars. The adult chinch bug lives from 45 to 56 d. Damage to tissue can occur from both nymph and adult stages (Wilson, 1929; Kuitert and Nutter, 1952; Eden and Self, 1960; Komblas,

1962; Kerr, 1966).Two types of southern chinch bugs (long wings and short wings) exist showing seasonal wing polymorphism (Van Duzee, 1886; Webster, 1907; Wilson, 1929).

Chinch bugs prefer to reside along the edges of the close-fitting leaves of St. Augustinegrass. Their feeding greatly influences the growing condition of the whole plant (Beyer, 1924). The infested turf will turn brown in patches and cause yellowing along the margins of damaged areas and may even completely die out (Watson, 1925). Southern chinch bug infestation also faciliate weed establishment on St. Augustinegrass lawns (Rainbolt et al. 2006). Lawns under drought stress in Florida's sandy soils were more susceptible to southern chinch bug infestation (Wilson, 1929). In neighborhood, chinch bugs can move from lawn to lawn easily covering a long distance in a short time period. In a specific lawn, they tend to be distributed in scattered patches rather than uniformly across the entire lawn (Kerr, 1966). Reinert (1978) found the highest southern chinch bugs population density along the periphery of damaged grass samples. Cherry (2001) concluded that southern chinch bugs were highly concentrated in small areas within light to moderate infestations lawns.

Host Plants

B. leucopterous caused serious damage to St. Augustinegrass in the southeastern United States according to Wilson (1929). Hamilton (1935) observed damage of *B. leucopterous* to lawns and golf courses in the eastern United States.

Barber (1918) reported that the most common chinch bug in Florida was a variation of *B. leucopterous*. Kerr (1966) mentioned that D.E.Leonard identified the common chinch bug in Florida as a separate species *B. insularis*. Kerr (1966) considered *B. insularis* as the second most serious insect pest in Florida just behind the citrus rust mite (*Phyllocoptruta oleivorus*).

In Florida, chinch bugs can feed on many species of lawn grasses but cause the most severe damage in St. Augustinegrass, which is the only species to suffer serious damage from chinch bugs. When the infestation occurred in a mixed lawn containing both St. Augustinegrass and centipedegrass, only the St. Augustinegrass was damaged (Kerr 1966).

Cherry and Nagata (1997) conducted research on southern chinch bugs oviposition on St Augustinegrass varieties and other turf species. St. Augustinegrass was significantly more preferred by the southern chinch bug than other species.

The other three types of chinch bugs can cause damage on different grass species. For instance, *B. occiduus*, the western chinch bug can damage bermudagrass and zoysiagrass lawns causing servere damage. *Blissus leucopterous leucopterous* can also harm these two grass species (Anderson et al. 2006; Eickhoff et al. 2007).

Management and Control

Traditionally, control of southern chinch bug is accomplished through use of chemical insecticides (Congdon and Buss, 2002). Reinert and Kerr (1973) stated that the use of chemical pesticides was the only effective way to control the pest. Reinert (1978) further reported that insecticides may need to be applied to Florida lawns more than six times a year to control the pest. However, Reinert and Niemczyk (1982) reported the occurrence of insecticide resistance in chinch bugs due to the heavy reliance on insecticide use. Lack of rotation among different chemical formulations may increase pesticide resistance of chinch bugs (Congdon, 2004). More recently, combined pest management strategies have been adopted due to the concerns over the repeated use of pesticides (Congdon and Buss, 2002).

Some natural biological control has been noted but has been found to be effective. A Parasite *Eumicrosoma benefica* Gahan (Hymenoptera: Scelionidae) was reported to attack eggs of *B. insularis* (Congdon, 2004). Predators such as *Pagasa pallipes* Stal (Heteroptera: Nabidae) and big-eyed bugs were discovered to feed on *B. insularis*. The density of big-eyed bugs was positively related to the southern chinch bug density (Reinert, 1978; Cherry, 2005). Fire ants were reported to attack *B.insularis* (Reinert, 1978). However, Cherry (2001) found that imported fire ants were not able to suppress the *B. insularis*.

Host Plant Resistance

Reinert and Kerr (1973) indicated that southern chinch bugs could cause great injury and even kill St. Augustinegrass completely and thus should be treated as the most serious insect pest of St. Augustinegrass. Strobel (1971) found that the cost of controlling southern chinch bugs was high, prompting research to find resistant varieties. Reinert and Dudeck (1974) screened eleven accessions along with all available commercial cultivars for their chinch bug response and found that 'FA-108', Floratam, and 'FA-118' were highly resistant. They labeled the type of resistance as antibiosis because of high mortality rates and greatly reduced fertility for surviving adults. Floratam was released as a cultivar jointly by the University of Florida and Texas A&M University for its resistance to SAD and southern chinch bug (Horn et al. 1973). Floratam's attractive color and fast establishment rate made it amenable for sod production. It is also well adapted to the environmental conditions of Florida and Texas (Rangasamy et al. 2006). However, Floratam has some shortcomings such as its coarse-textured leaves and poor cold and shade tolerance (Reinert, 1978). It has also lost its resistance to southern chinch bug (Busey and Center, 1987). Researchers have

attempted to identify the other lines resistant to both SAD and southern chinch bug (Bruton et al. 1979; Reinert et al. 1980; Crocker et al. 1982). ‘Floralawn’ (FA-108) with combined resistance to SAD and southern chinch bug was the only line released (Dudeck et al. 1986). In 2007, Captiva was released as the first diploid cultivar with southern chinch bug and plant hopper resistance (Nagata et al. 2007).

Some studies have been done on other warm-season grasses. Anderson et al. (2006) evaluated both cool-season and warm season grasses for their resistance to multiple chinch bugs. They found that the St. Augustinegrass resistant to *B. insularis* was also resistant to *B. occiduus*. Heng-Moss et al. (2002) observed that some baffalograss (*Buchloe dactyloides*) cultivars were resistant to *B. occiduus*.

Different resistance types and their mechanisms in St. Augustinegrass have been studied. Rangasamy et al. (2006) reported possible antixenosis to southern chinch bugs in both Captiva and FX-10, and antibiosis in Captiva. He further reported possible physical and biochemical mechanisms for host plant resistance. The resistant varieties FX-10 and Captiva were reported to have significantly higher lipoxygenase or polyphenol oxidase activity after infestation. Moreover, resistant varieties had significantly thicker walls of sclerenchyma cells around the vascular bundle but did not differ from susceptible varieties in their total lignin content.

Lab Screening Techniques

Four screening methods using confined insects have been used determine southern chinch bug resistance of St. Augustinegrass. These include: the bag test (Reinert, 1978), the jar test (Crocker et al. 1982), the box test (Nagata and Cherry, 2003), and the tube test (Cherry et al,. 2012). Further information is needed to determine if the different screening methods provide similar results regarding the

responses of St. Augustinegrass lines to southern chinch bug. Because the effectiveness and accuracy of a screening method is important in the determination of resistance, it is desirable to know if the existing screening methods are equally useful in identifying chinch bug resistance in St. Augustinegrass.

Common Carpetgrass

Use and Distribution

Common carpetgrass (*Axonopus fissifolius*, Raddi) is a perennial warm-season grass in the southern United States along coastal areas (Wise, 1961). Common carpetgrass differs from the broadleaf carpetgrass (*Axonopus compressus*) by having a less coarse leaf texture (Smith and Valenzuela, 2002). Common carpetgrass can be used in various locations such as lawns, roadside, cemeteries and similar low-maintenance areas (Greene, 2007).Growing carpetgrass benefits weed control by its mat-like growth habit, which also helps to preserve topsoil on slopes and improves soil structure (Smith and Valenzuela, 2002).

General Biology

Carpetgrass is stoloniferous with narrow glabrous leaves that are 5 to 20cm in length and 2-6mm in width (Martin, 1975). Leave color varies from light green to medium green with folded vernation (Skerman and Riveros, 1990, Turgeon, 2005). It is characterized by compressed two-edged stems with short internodes and small flowers. It has hairy ligule with narrow and continuous collars and no auricles (Turgeon, 2005). Its inflorescence usually contains two to three branches with two rows of alternately arranged spikelets (about 2mm long) (Martin, 1975).

Reproduction and Ploidy Level

Common carpetgrass can be reproduced both sexually via seed and vegetatively via sod, sprigs or plugs. It can be cross-pollinated in the field and also self-pollinated (Heath et al. 1985, Watson and Burson, 1985). Watson and Dallwitz(1992) reported most species of common carpetgrass to be allopolyploid generated from interspecific hybridization within the Axonopus genus. Common carpetgrass has been reported with various ploidy levels including diploid (2n=2x=20) (Gould and Soderstrom, 1967; Norrmann et al. 1994), tetraploid (2n=4x=40) (Gould, 1968; Hickenbick et al. 1975), hexaploid (2n=6x=60) (Davidse and Pohl, 1978) and octoploid (2n=8x=80) (Hickenbick et al. 1975).

Common carpetgrass is long-day plant that flowers normally under 12-14h photoperiod. Cold temperature (<15^{o}C) can inhibit flowering (Knight and Bennett, 1953).

CHAPTER 2
EFFECT OF TIME AND METHODOLOGIES IN DETERMING ST. AUGUSTINEGRASS RESISTANCE TO SOUTHERN CHINCH BUGS

Introduction

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is used as lawn grass throughout the southern United States for its wide adaptation to varying environmental conditions. The southern chinch bug, *Blissus insularis* Barber, is the plant's most damaging insect pest. Insecticidal application was the primary method of control of the southern chinch bug before the release of resistant Floratam St. Augustinegrass in 1973 (Horn et al. 1973). Unfortunately, southern chinch bug damage on Floratam was first reported in Florida in 1985 (Busey and Center, 1987) showing its loss of host plant resistance as was later confirmed by Cherry and Nagata (1997).

Busey (1990) identified several new lines of St. Augustinegrass resistant to southern chinch bugs, which led to development of the variety FX-10 St. Augustinegrass, resistant to southern chinch bug (Busey, 1993). However, FX-10 was never extensively grown due to several negative characteristics including a very course appearance and tough texture (Busey, 1993). More recently, Nagata and Cherry (2003) reported on the resistance of NUF-76 St. Augustinegrass to southern chinch bug. NUF-76 is unique because, for the first time, resistance to southern chinch bug was identified within a diploid line of St. Augustinegrass, unlike polyploids such as Floratam and FX-10. Mechanisms of resistance in NUF-76 have been reported by Rangasamy et al. (2006, 2009a, b). Although NUF-76 has been shown to be widely resistant to southern chinch bug populations in Florida (Nagata and Cherry, 2003), Reinert (2008) and Reinert et al. (2011) reported that it is not resistant to some Texas populations. NUF-76

has been named Captiva for marketing purposes and is currently produced for sale in Florida and Texas..

Most screening methods to measure host plant resistance of St. Augustinegrass to southern chinch bugs have measured nymphal and/or adult survival in no-choice tests. Those tests fall into 4 types with examples as follows. Reinert and Dudeck (1974) used insects and excised stolons enclosed in plastic bags. Crocker et al. (1982) used insects and excised stolons in glass jars. Nagata and Cherry (2003) tested insects in plastic boxes containing a stolon attached to the plant. Lastly, Cherry et al. (2011) tested insects in plastic tubes containing a stolon attached to the plant. Besides using different screening methods, different time intervals have also been used to measure mortality rate. However, efficacy of these various procedures in determining St. Augustinegrass resistance to southern chinch bug has not been compared. The objective of this study was to determine the effect of time and methodologies in determining St. Augustinegrass resistance to southern chinch bugs.

Materials and Methods

Bag Test

St. Augustinegrass grass genotypes used in our tests were Captiva, Floratam, NUF-216 and FX-10. These varieties range in resistance to southern chinch bugs from Captiva (resistant), Floratam (once resistant, now susceptible), NUF-216(resistant) and FX-10(resistant). Plants were planted into 10-cm diam. pots filled with a mixture of 50% sand and 50% Fafard #2 mix, and each pot received 1 g of fertilizer (Scotts 14 -14 -14). Chinch bugs were collected by vacuuming infested lawns in Palm Beach Co., FL. Collected debris were stored in buckets filled with fresh St. Augustinegrass clippings for food at 18°C until testing. Adult chinch bugs (Figure 2-1) were collected by sorting

through debris when needed. Mortality was recorded on 3, 7, 14, 21 and 28 d after insects were placed into bags. This time interval was used to cover the range used in all previous tests from 3 d (Reinert, 1974) to 28 d (Cherry and Nagata, 1997). Fifteen cm of terminal stolons of the 4 varieties were cut from the potted plants and wrapped with a wet cotton ball at the cut end, and each stolon was placed into a 3.78-L clear plastic bag (Figure 2-2). Thereafter, 10 randomly selected adult chinch bugs were placed into each bag, and the bag sealed. Except for 3-d check, stolons were replaced after each reading. Tests were conducted at 28°C and 12d/12l photoperiod.

Jar Test

Chinch bugs, St. Augustinegrass varieties, ambient conditions and time intervals used in this test were as previously described. Fifteen cm terminal stolons of the 4 varieties were cut from the potted plants and inserted into a 26-ml vial containing water and sealed with parafilm. Each vial and 10 adult chinch bugs were placed into a 0.95-L wide-mouth clear glass jar and covered with insect screen cloth secured by a screw-on ring (Figure 2-3). Except for 3-d check, we replaced the stolons after each reading.

Box Test

Chinch bugs, St. Augustinegrass varieties, ambient conditions and time intervals used in this test were as previously described. Polypropylene opaque food storage containers 28 × 16 × 11 cm (l × w × h) were used in this test. The central part of each lid was removed leaving approx. 3cm around the sealing edge. A 6mm diameter hole was drilled half-way up on one of the 16-cm sides. A channel was then cut from the top of the box to the hole. A potted plant was placed beside the box. Strips of Parafilm were wrapped around the stolon where it would pass through the channel. Flaps were bent on each side of the channel to help with the passing and positioning of the stolon within

the hole. A 15-cm stolon attached to the plant was placed in each box. Tape was used to cover the channel from both inside and outside (Figure 2-4). Ten adult chinch bugs were placed into each box. An insect screen cloth was placed on top of the box stabilized by the lid. Plants were watered as needed.

Tube Test

Chinch bugs, St. Augustinegrass varieties, ambient conditions and time intervals used in this test were as previously described. A 15-cm stolon attached to a plant was placed in a 22-cm long, 4 cm diameter, clear plastic tube. A sponge was wrapped around the stolon and wedged into the tube end next to the potted plant (Figure 2-5). Ten adult chinch bugs were placed into the tube. The other end of the tube was covered with insect screen cloth held in place by a rubber band.

Statistical Analysis

The 4 methods using the 4 varieties were conducted at the same time in a replication. Eight replications were conducted from November 2011 to July 2012. Overall survival in whole plant methods (box and tube) versus excised plant methods (bag and jar) was compared at each time interval in contrast tests using analysis of variance (SAS, 2012). Differences in survival among different cultivars, times and different methods were determined using LSD analysis (SAS, 2012).

Results and Discussion

The 4 testing methods fell into 2 general categories. The box and tube tests used stolons attached to plants whereas bag and jar tests used excised stolons. Survival means of box or tube tests were significantly greater than the means of bag or jar tests at night times. Survival means in bag or jar tests were never significantly greater than that of the box or tube tests (Table1). Contrast analysis further showed differences in

survival between the whole plant methods (box and tube) versus excised stolon methods (bag and jar). At all 5 time intervals, survivorship was significantly greater in the whole plant methods than the excised stolon methods.

Chinch bug survival using whole plant methods and excised stolon methods is shown in Tables 2 and 3, respectively. At the 3-d interval, only 1 variety (FX-10) was found to be resistant (i. e. significantly different from Floratam) in one method (jar test). At 7 days, we found 7 times in the 3 resistant varieties in 3 methods (box, tube, jar). At 14 days, resistance was found 9 times in the 3 resistant varieties in all 4 methods. Both at 21 and 28 d, resistance was found 10 times in the 3 resistant varieties in all 4 methods.

As noted earlier, researchers have used different methods, time intervals and ambient temperatures to determine St. Augustinegrass resistance to southern chinch bugs. Overall, our tests confirm earlier tests that show Captiva, NUF-216 and FX-10 are resistant to southern chinch bugs and Floratam is susceptible. However, our data also clearly show that the measurement of resistance may be affected by different factors. For example, overall chinch bug survival was higher in tests using whole plants than in tests using excised stolons. Also, the bag test gave the most erratic results of the 4 methods and never did show Captiva to be resistant which the other three methods demonstrated. The bag test also did not find NUF-216 to be resistant in week 3 and 4. In contrast, the other 3 tests showed NUF-216 consistently resistant in both weeks. Reinert (1978) noted that condensation in plastic bags caused unexpected high mortality, a problem also experienced by Crocker et al. (1982) who switched to glass

jars. We also noted condensation in the bags and also were concerned with stolons drying out infrequently in the bags.

The effect of time in determining resistance was also very evident. In our tests, shorter time intervals in measuring mortality may result in not measuring resistance in a variety. Lastly, the effect of temperature was not measured in our study. However, in previous tests, temperatures have ranged from 25 °C (Cherry et al. 2011) to 35 °C (Crocker et al. 1982). Because insects are poikilothermic, it is probable that increased temperature will reduce time intervals needed to determine resistance because of increased mortality caused by biochemical antibiosis. For example, Rangasamy et al. (2009b) reported increased oxidative responses in resistance St. Augustinegrass varieties to southern chinch bug feeding. In summary, researchers should carefully consider method, time and temperature as important variables in determining St. Augustinegrass resistance to southern chinch bugs.

Conclusions

Both the time and methodologies have a great impact on screening for southern chinch bug resistance in St. Augustinegrass. The effective time for screening should not be as short as 3d and is not necessary for longer than 28d. Different screening methods vary in their ability to detect resistant varieties. The differences need to be considered when using different methods. Future study can determine the influence of temperature and other factors on the use of appropriate time and methodologies for screening southern chinch bug resistance.

Table 2-1. No. of adult southern chinch bugs surviving (out of 10) at different intervals (days) on four varieties using four different methods.

	Days In Test				
Methods	3	7	14	21	28
Tube	8.5±2.3A*	5.3±3.1A	3.2±3.7A	1.9±2.7A	1.2±2.3A
Box	7.8±2.5AB	5.7±2.9A	2.8±2.8AB	1.8±2.6A	1.3±2.1A
Jar	7.5±2.6B	3.5±2.9B	1.9±2.7BC	1.1±2.0A	0.8±1.6AB
Bag	7.8±2.1AB	4.8±2.4A	1.6±2.1C	1.0±1.6A	0.3±0.7B

*Means ± SD within each column followed by the same letter are not significantly different (α=0.05) determined with an LSD test (SAS 2012). Means represent pooled survival of four varieties. Contrast value of whole plant (tube and box) versus excised stolon methods (jar and bag) were F=3.86, p=0.05 at 3 days, F=14.25, p<0.01 at 7 days, F=10.59, p<0.01 at 14 days, F=5.91, p=0.02 at 21 days, F=6.78, p=0.01 at 28 days.

Table 2-2. No. of adult southern chinch bugs surviving (out of 10) at different intervals (days) using whole plants

	Box					Tube				
Variety	3	7	14	21	28	3	7	14	21	28
Floratam	7.5A±2.4*	6.8A±2.4	5.0A±2.7	4.1A±3.1	3.2A±2.9	9.5A±0.9	8.1A±2.2	6.8A±3.2	5.3A±2.8	4.1A±3.0
Captiva	8.6A±2.4	7.0A±2.5	3.0A±2.6	1.3B±2.4	1.1B±2.1	8.3A±2.5	5.1B±2.9	3.1B±3.4	1.1B±1.6	0.6B±1.2
NUF-216	8.4A±1.9	6.5A±2.1	3.1A±2.6	1.6B±1.8	0.9B±1.0	8.1A±3.0	4.8B±3.4	3.1B±4.0	1.1B±2.1	0.0B±0.0
FX-10	6.6A±3.1	2.5B±2.2	0.3B±0.5	0.1B±0.4	0.0B±0.0	8.3A±2.4	3.5B±2.3	0.4B±0.5	0.0B±0.0	0.0B±0.0

*Means ± SD within each column followed by the same letter are not significantly different (α=0.05) determined with an LSD test (SAS, 2012).

Table 2-3. No. of adult southern chinch bugs surviving (out of 10) at different intervals (days) using excised stolons

	Bag					Jar				
Variety	3	7	14	21	28	3	7	14	21	28
Floratam	7.1A±2.9*	5.1A±2.6	3.0A±3.0	1.9A±2.1	0.3A±0.5	9.0A±0.8	6.0A±3.5	5.0A±3.3	3.4A±2.4	2.6A±2.4
Captiva	8.8A±1.3	6.0A±2.5	2.1AB±2.2	1.6A±2.1	0.9AB±1.1	7.4AB±2.1	2.8B±2.0	1.3B±2.2	0.8B±1.8	0.4B±0.7
NUF-216	7.6A±1.6	4.0A±2.2	0.9B±0.6	0.6AB±0.5	0.3AB±0.5	7.1AB±2.6	3.3B±2.6	0.8B±0.9	0.3B±0.5	0.3B±0.5
FX-10	7.5A±2.1	4.0A±1.9	0.5B±0.9	0.0B±0.0	0.0B±0.0	6.4B±3.6	2.1B±2.2	0.5B±0.8	0.1B±0.4	0.0B±0.0

*Means ± SD within each column followed by the same letter are not significantly different (α=0.05) determined with an LSD test (SAS, 2012)

Figure 2-1. Southern chinch bugs. From left to right are nymph, short wing adult and long wing adult. Photo courtesy of Ron Cherry.

Figure 2-2. Bag test. Photo courtesy of Long Ma.

Figure 2-3. Jar test. Photo courtesy of Long Ma.

Figure 2-4. Box test. Photo courtesy of Long Ma.

Figure 2-5. Tube test. Photo courtesy of Long Ma.

CHAPTER 3
TEST ON SEED SET RATE OF COMMON CARPETGRASS

Introduction

Common Carpetgrass (*Axonopus fissifolius*) is a perennial warm-season grass used as low maintenance applications in the southern United States along coastal areas (Wise, 1961). It has a medium to coarse leaf texture compared to tropical carpetgrass (*Axonopus compressus*) with a coarse to very coarse leaf texture (Smith and Valenzuela, 2002). Common carpetgrass can be used in various locations such as lawns, roadside, cemeteries and similar low-maintenance areas (Greene, 2007).Common carpetgrass is competitive against weeds, helps to prevent soil erosion and improves soil quality (Smith and Valenzuela, 2002).

Carpetgrass is stoloniferous with leaves that are 5 to 20cm in length and 2-6mm in width (Martin, 1975). Leave color various from light green to medium green (Skerman and Riveros, 1990). It is characterized by compressed two-edged stems with short internode and small flowers. It has hairy type ligule with narrow and continuous collars but no auricles (Turgeon, 2005). Its inflorescence usually contains two to three branches with spikelets (about 2mm long) arranged in two neat rows (Martin, 1975).

Common carpetgrass can be reproduced both sexually via seed and vegetatively via sod, sprigs or plugs. It can cross-pollinate and self-pollinate (Heath et al. 1985, Watson and Burson, 1985). Watson and Dallwitz (1992) reported most species to be allopolyploid generated from interspecific hybridization within the Axonopus genus. Common carpetgrass has a base chromosome number of ten (x=10) and ploidy levels include: diploid (2n=2x=20) (Gould and Soderstrom, 1967; Norrmann et al. 1994),

tetraploid (2n=4x=40) (Gould, 1968; Hickenbick et al. 1975), hexaploid (2n=6x=60) (Davidse and Pohl, 1978) and octoploid (2n=8x=80) (Hickenbick et al. 1975).

Carpetgrass flowers under long-day conditions with normally 12-14h photoperiod. Cold temperature (<15^{o}C) can inhibit flowering (Knight and Bennett, 1953).

Carpet grass germplasm collection includes both seed-derived and natural collections. Greene et al. (2008a, 2008b) reported the extent of variation among those germplasm collections based on both morphological traits and turf performance traits. They also made comparisons between these two groups and concluded that morphological traits were more effective identifying variation between two groups. Wang et al. (2010) showed that the seed-derived population had less genetic variability than natural collections using AFLP-marker-based molecular analysis.

Because carpet grass primarily propagated through seed, it is desirable to understand if a single elite clone could produce adequate seed or if cross-pollination is needed (i.e. synthetic cultivars) for suitable seed production. The primary objective of the following study was to determine if seed set of carpetgrass differs under selfing or cross pollination conditions.

Materials and Methods

Plants Materials

Common Carpetgrass germplasm collection used in this study was provided by Dr. Kevin Kenworthy in Agronomy Department, University of Florida, Gainesville, FL. This germplasm was previously characterized for many traits by Greene et al. (2008a and 2008b).Ten accessions were randomly selected from the germplasm and vegetatively propagated to produce 4 plants per accession and planted in 10-cm pots using metro-mix 900 potting soil. They were arranged in a randomized complete block

design with four replications in the greenhouse using automatic irrigation. All plants were trimmed to a uniform height after establishment. The experiment started on September 2012 and ended on December 2012. Greene et al. (2008b) grouped 176 accessions within the germplasm collections into 32 clusters based on their morphological traits. The 10 accessions selected in this test fit into 9 separate clusters.

Seed Set Rate Evaluation

During flowering, three inflorescences from each pot were covered with a glassine bag prior to anthesis. The bottom of each bag is folded and stabilized by a paper clip to prevent seed loss. Open pollination among these forty plants was powered by wind in the greenhouse. As the curtains on both sides of the greenhouse were up for most of the time, the environmental condition was almost the same as outside. Upon maturity, the covered inflorescences along with three open pollinated inflorescences from each pot were harvested and dried at 50°C-55°C for 2d. The Number of branches, number of spikelets per branch and number of seed were counted for each inflorescence. Inflorescences were soaked in 50% bleach for 2h for accurate seed counting. Seed set rate per inflorescence was calculated using the formula: seed set rate = (#seed / #spikelets per inflorescence) x 100%.

Statistical Analysis

Variance components for number of branches, spikelets per branch and seed set rate were calculated using SAS PROC VARCOMP procedure (SAS, 2012). Genotypes and replications were considered random effects. Differences in seed set rate among ten genotypes were analyzed using LSD test for self-pollination and open-pollination separately (SAS, 2012).

Broad sense heritabilities (H^2) for the number of branches, spikelets per branch and seed set rate were calculated using the formula below (Hallauer, 1970): $H^2 = \sigma_G^2 / (\sigma_G^2 + \frac{\sigma_E^2}{R})$, where σ_G^2 is variance of genotypes, σ_E^2 represents variance of residuals, and R is the number of replication. Contrast tests were performed using SAS to determine if the differences existed within genotypes for seed set rate between selfing and open pollination (SAS, 2012).

Results and Discussion

Watson and Burson (1985) reported that the common carpetgrass can be cross-pollinated in the field, and single plant can be self-pollinated in the greenhouse when isolated. However a comparison of seed set between self and cross-pollination has not been quantified. Similar tests have been conducted on bermudagrass and the seed set rates for both self and open-pollination are highly variable. Bermudagrass are highly self-incompatible and seed set rate ranged from 0.004 to 0.036 for self-pollination and 0.028 to 0.446 for open-pollination (Richardson et al. 1978; Kenna et al. 1983). Acuña et al. (2007) reported the seed set of sexually reproduced bahiagrass (*Paspalum notatum Flügg*) to be on average 0.12 and 0.39 for self-pollination and open-pollination respectively. In this experiment, we measured seed set rate for both pollination types and also the number of branches along with spikelets per branch.

Number of spikelets per branch and seed set rate for self and cross-pollination had a wide range of variation (Table 3-1). The number of spikelets per branch ranged from 12 to 39, seed set rate under self-pollination from 0.20 to 0.88, and seed set rate for open-pollination from 0.18 to 0.93. The mean seed set rates for self and open-

pollination were 0.54 and 0.53 respectively indicating that carpetgrass sets seed equally well under both pollination conditions.

Estimates of variance (Table 3-2) indicated that the genotype was the primary variance component contributing to observed phenotypes. As this experiment does not have replications over years, the environmental variance component cannot be determined. The relatively high error variance for number of branches and spikelets per branch may probably contain large environmental variance. Considering the low original value, the real genotypic variations among carpetgrass populations is high despite the low genotypic variance showed in the table. Contribution of genotypic effect was also reflected in the broad sense heritability. Number of branches has moderate broad sense heritability while the other three traits have relatively high broad sense of heritability (0.53-0.88). From the breeding perspective, there is a potential to improve the population mean for seed set rate of both self-pollination and open-pollination because of their relatively low variance.

In the contrast test (Table 3-3), three out of ten genotypes had significantly different means for seed set rate between self-pollination and open-pollination. Genotype UFA10 had a higher percent seed set under selfing than when open pollinated; whereas, UFA29 and UFA32 both exhibited greater seed set when open-pollinated. Overall, these results indicate that pollination type does not influence seed set and that carpetgrass is both self and cross compatible. Results from LSD test (Table 3-3) showed that seed set rate for self and open-pollination varies greatly among ten genotypes. Genotype UFA38 had superior self-compatibility; whereas, UFA15 had good cross-compatibility.

Greene (2008b) has previously reported a broad-sense heritability for number of branches per seed head as 0.34. Results from this experiment are very similar (H^2 = 0.32). This indicated that the ten accessions selected are representative of the entire germplasm collection evaluated by Greene et al. (2008b) for branches per seed head. Therefore, it is likely that these ten plants are also representative of the larger collection for number of seeds, seed set rate and compatibility assessments.

Conclusions

Broad-sense heritabilities from this study were similar to those previously reported in carpetgrass for other traits. While all traits indicated good heritability, the variances for seed set rate were low. But comparing to the low original value, real genotypic variations may be high among carpetgrass populations. Therefore, there is a potential to increase seed set through breeding. The genotype variance for the number of branches per inflorescence was high providing good evidence that seed production could also be increased by breeding for greater numbers of branches on seed heads. The results on differences of seed set rates between self- and open pollination have important meanings to breeding programs. Because seed set does not differ between pollination methods different types of breeding methods could be utilized for the improvement of carpetgrass. Of extreme value is the knowledge that an identified superior clone could be commercialized with no effect on seed production versus the extra effort required to produce a synthetic cultivar if self-incompatibility was an issue.

Table 3-1. Minimum, maximum and mean values for number of branches, spikelets per branch and seed set rate under self and open-pollination

			Seed set rate	
	#Branch	Spikelets/Branch	Self-pollinated	Open-pollinated
Min	2	12	0.2	0.18
Max	4	39	0.88	0.93
Mean	3	25.23	0.54	0.53

Table 3-2. Estimate of variance components and broad sense heritabilities for number of branches, spikelets per branch and seed set rate under self and open-pollination

	Variance estimates			
			Seed set rate	
Source	#Branch	Spikelets/Branch	Self-pollinated	Open-pollinated
Genotype(G)	0.03	7.68	0.0207	0.0175
Replication(R)	0	0	0.0007	0.0023
Error	0.25	24.29	0.0108	0.0147
H^2	0.32	0.53	0.88	0.83

Table 3-3. Seed set rates under different pollination methods and comparison of mean values within each genotype

	Self-pollinated		Open-pollinated			
Genotype	Mean	Range	Mean	Range	F value	Pr > F
2	0.63ABC	0.43-0.83	0.65AB	0.59-0.70	0.08	0.8
10	0.25D	0.20-0.30	0.39BC	0.35-0.41	53.34	<0.01**
15	0.67AB	0.60-0.73	0.7A	0.53-0.93	0.11	0.76
21	0.56ABC	0.38-0.69	0.61AB	0.53-0.79	1.09	0.37
29	0.39CD	0.30-0.42	0.27C	0.18-0.32	66.94	<0.01**
32	0.58ABC	0.49-0.68	0.42ABC	0.33-0.52	12.69	0.04*
38	0.79A	0.68-0.88	0.58AB	0.37-0.76	5.89	0.09
60	0.43BCD	0.31-0.60	0.42ABC	0.18-0.66	0.01	0.94
72	0.59ABC	0.52-0.65	0.64AB	0.50-0.74	0.9	0.41
99	0.57ABC	0.42-0.77	0.64AB	0.46-0.87	3.11	0.53

Means within each column followed by the same letter are not significantly different (α=0.05) determined with an LSD test. P value with a * (α=0.05) or ** (α=0.01) within each genotype means significant difference between self-pollination and open-pollination.

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BIOGRAPHICAL SKETCH

Long Ma grew up in Zhejiang, China. He graduated from Hangzhou No.4 High School in 2007. That fall, he began undergraduate study at Guangxi University, Guangxi, China. In the summer of 2011, he received a bachelor's degree in horticultural science. In August, 2011, he began to study at University of Florida for a master's degree in horticulture department.