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Title: Evaluating Southern Chinch Bug Resistance of Interploid and Inter-specific Hybrids of St. Augustinegrass

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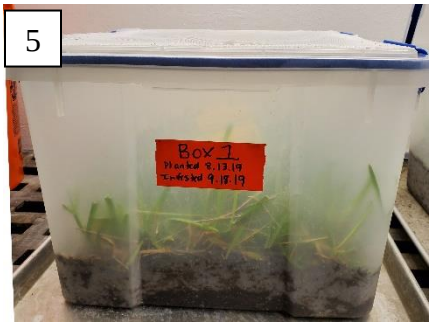
Statement of Problem: St. Augustinegrass (*Stenotaphrum secundatum* Walt. (Kuntze)) is one of the most prominent species of warm-season turfgrass grown in Texas and across the southeast. Commercially available cultivars have been shown to express varying degrees of resistance to drought and southern chinch bugs (SCB). 'Floratam', an aneuploid ($2n = 2x = 30$), is one of the oldest cultivars of St. Augustinegrass that has served as an industry standard for resistance to both drought and certain biotypes of SCB (1). However, polyploid damaging populations (PDP) of SCBs have been shown to overcome resistance in Floratam (2, 3). Additionally, extensive damage from SCB is most often seen in summer when turf is stressed from drought. For these reasons, the turfgrass industry is seeking new alternatives to Floratam that are both drought resistant and tolerant to biotypes of SCB while maintaining high turfgrass quality.

Project Objective:

1) Collect one SCB populations in Texas (Dallas and/or College Station) and begin rearing these populations at Texas A&M AgriLife-Research - Dallas, TX.

   Status: Completed

   Poplulations of southern chinch bugs were collected from four sites, Houston, College Station, Garland, and Dallas TX (Fig. 1, 2, and 3). The Houston population collected on 18 Sept 2019 did not survive using referenced methods due to the presence of predator insects on host sod. The rearing methods were therefore modified for future populations (Fig. 4, 5, 6). The College Station population collected on 27 Sept 2019 has been kept alive using this method but has not produced enough 5th instars for a replicated study. Researchers have contacted county agents and turfgrass professionals in the DFW area to locate more populations. On 7 Aug 2020 a new population was acquired in Garland from a homeowners yard (unknown cultivar) and is being reared similarly to the College Station population. Chinch bugs from the same yard were collected again on October 5th and added to the existing population. On 1 Oct 2020, a population was identified and collected from Raleigh field plots at the Dallas Center and is being reared separately.



2) Use the whole-plant attached single-stolon box method (6) to evaluate responses to no-choice feeding in replicated trials.

<u>Status:</u> Incomplete

In anticipation of starting this study, two replicates of potted plants were propagated on 30 Sept 2019 and 17 Jan 2020 from stolons rooted in water for 7 d (Fig. 7). Population growth was monitored weekly from October 2019 through February 2020. Although the College Station population is alive, a shortage of 5th instars prevented the initiation of the study as expected. The whole-plant attached single-stolon box method requires a much larger and sizable population to conduct statistically robust replicated experiments. We will continue to monitor the development of the College Station, Garland and Dallas populations for use in future studies.

During the rearing phase, time was spent determining how to best anesthetize and sex the chinch bugs in preparation for infestation. The best method would allow for a longer anesthetization time but no mortality. Vials of bugs were tested with 4 methods: 1) Carbon dioxide, 2) Freezing at -1.2°C, 3) Trimethyamine (FlyNap), and 4) Chilling at 2 to 3°C. Chinch bugs recovered within 2 min using carbon dioxide regardless of exposure time with no mortality but the short time was not long enough to sex a large proportion of adults. Freezing for 1 hr resulted in complete mortality, and trimethylamine caused partial or complete mortality. No mortality occurred with chilling overnight for 16 hours and bugs recovered within 2 to 5 min. therefore chilling was the preferred method.

3) Evaluate for evidence of antixenosis (feeding behavior), antibiosis (adult and nymph mortality and excrement production), and fecundity (oviposition) using established methods (6, 7).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Status: Completed

A detached stolon assay was initiated on 18 Aug 2020 using newly molted adult chinch bugs from the Garland population (Trial 1) and on 6 Oct 2020 from the Dallas population (Trial 2). Two pairs of adults (4 bugs) were infested onto three replicates (Trial 1) and six replicates (Trial 2) for each of the susceptible (Seville), resistant (Captiva), and experimental line (DALSA 1618) (Fig. 8). Adult mortality (antibiosis) was recorded weekly, and the number of eggs, nymphs and excrement production were recorded at 28 d.

Results:

Trial 1: Despite replenishing water in the testtubes weekly, Captiva stolons had completely dehydrated and began dying by 7 d due to failure to produce roots. Seville produced few short roots and DALSA 1618 had an ample production of long roots. No significant differences in percent mortality were detected between genotypes until 22d after infestation, which did not increase at 28d (Table 1). Total adult mortality was 100% for Captiva the resistant check. We are unclear whether mortality on Captiva represents resistance to southern chinch bugs or mortality resulting from starvation due to lack to healthy stolons. To remedy this situation, modifications were made in Trial 2 where only pre-rooted stolons were used in the bioassay. Seville, the susceptible check, and DALSA 1618 were not significantly different on day 22 and 28 with 33.3 % and 25.0 % mortality, respectively. However, DALSA 1618 had significantly greater mortality of females than Seville (Table 2). Male mortality for DALSA 1618 was significantly less than both Captiva and Seville. No significant differences were detected for egg count, nymphs, or excrement production. More replications may have detected greater differences and increase reliability.

Table 1. Mean percent adult mortality by day of evaluation after infestation with the Garland Population.

	% Mortality						
Genotype	**1d**	**2d**	**3d**	**6d**	**14d**	**22d**	**28d**
Captiva	0.0	0.0	0.0	0.0	16.7	100.0 a	100.0 a
DALSA 1618	8.3	8.3	8.3	8.3	8.3	25.0 b	25.0 b
Seville	8.3	16.7	16.7	16.7	25.0	33.3 b	33.3 b
$LSD_{0.05}$	NS	NS	NS	NS	NS	25.3	25.3
Genotype	0.6467	0.323	0.323	0.323	0.7449	0.0012	0.0012
Rep	0.4366	0.4366	0.4366	0.4366	1.0000	0.0522	0.0522

Table 2. Mean mortality of female and male chinch bugs, and egg, nymph and excrement counts at 28d.

Genotype	% Female mortality	% Male mortality	# Eggs	# Nymphs	# Excrement
Captiva	100.0 a	100.0 a	0.7	6.7	56.7
DALSA 1618	33.3 b	16.7 b	5.3	12.3	52.3
Seville	0.0 c	66.7 a	8.7	7.3	20.3
$LSD_{0.05}$	33.2	38.3	NS	NS	NS
Genotype	0.0015	0.0069	0.5901	0.5574	0.1872
Rep	0.2532	0.0756	0.5301	0.6023	0.7941

Trial 2: Modifications were made to the protocol such that 7 to 9 stolons per genotype were placed in cups of water 5 d before initiating the study to promote rooting. The number of replications was increased to 6 using only rooted stolons and data was only collected every 7d. Because the Garland population was not large enough to replicate Trial 1, the Dallas population was used. After 7d adult chinch bugs began dying but no significant differences were detected between genotypes (Table 3). Female mortality was similar between all 3 genotypes (Table 4). Male mortality was statistically highest for Captiva (66.7%) compared to Seville (8.3%), and was intermediate for DALSA 1618 (25.0%). Trial 2 had a noticeable amount of spider mites which are predatory on younger instars and likely reduced the egg and nymph population. No significant differences were detected for egg count or excrement production. Seville had the highest nymphal development comprising only first instars, whereas DALSA 1618 was similar to Captiva with 0.0%.

Table 3. Mean percent adult mortality by day of evaluation after infestation with the Dallas Population.

	% Mortality			
Genotype	**7d**	**14d**	**21d**	**28d**
Captiva	25.0	41.7	41.7	50.0
DALSA 1618	4.2	16.7	16.7	29.2
Seville	4.2	4.2	4.2	12.5
$LSD_{0.05}$	NS	NS	NS	NS
Genotype	0.2624	0.1805	0.1805	0.2826
Rep	0.4888	0.7245	0.7245	0.8625

Table 4. Mean mortality of female and male chinch bugs, and egg, nymph and excrement counts at 28d.

Genotype	% Female mortality	% Male mortality	# Eggs	# Nymphs	# Excrement
Captiva	33.3	66.7 a	0.0	0.0 b	38.5
DALSA 1618	33.3	25.0 ab	0.5	0.0 b	41.8
Seville	16.7	8.3 b	3.2	3.2 a	54.0
$LSD_{0.05}$	NS	64.7	NS	3.6	NS
Genotype	0.7724	0.0432	0.3361	0.0289	0.5555
Rep	0.6593	0.8545	0.1626	0.3533	0.3901

Conclusions: The purpose of this study was to test the level of resistance/tolerance of elite interploid St. Augustinegrass hybrids as compard to commercial checks. Due to a shortage of chinch bug populations and difficulty in rearing, only one interploid hybrid, DALSA 1618, could be tested against Captiva (resistant) and Seville (susceptible) using two different chinch bug populations in the detached stolon assay. Female mortality of DALSA 1618 was lower than Captiva but higer than Seville in Trial 1. Male mortality was lower than Captiva and Seveille in Trial 1 but similar to Captiva and Seville in Trial 2. In both trials, number of eggs produced, excrement counts and nymphal development data were not statistically different among entries, and therefore not reliable in understanding their tolerance levels to southern chich bugs. Overall, these results were inconclusive. Future studies with larger population size of southern chich bugs would allow us to include more replications and more genotypes which would help shed more light in to the resistance/tolerance levels of these interploid hybrids.

References:

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