Effect of Time and Testing Method in Determining St. Augustinegrass Resistance to Southern Chinch Bugs (Hemiptera: Blissidae)[1]

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Abstract 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. 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 determined. The objective of this study was to determine the effect of time and methodologies in assesing St. Augustinegrass resistance to southern chinch bugs. Four varieties were tested for resistance using 4 methods (bag, jar, box, tube) and 5 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 4 methods. The effect of time in determining resistance was also 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.

Key words St. Augustinegrass, southern chinch bugs, *Blissus insularis*

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 way of southern chinch bug control 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 bug, 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.

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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 being sold to the general public in Florida.

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 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 also have 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 varieties 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 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 and Dudeck 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. 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 12 d/12 l 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. 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. 3 cm around the sealing edge. A 6 mm

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. 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 diam. clear plastic tube. A sponge was wrapped around the stolon and wedged into the tube end next to the potted plant. 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 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) were compared at each time interval in contrast tests using analysis of variance (SAS 2012). 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 nine times (Table 1). Survival means in bag or jar tests were never significantly greater than that of the box or tube tests. 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.

Table 1. Survival* of southern chinch bugs at different intervals (days) on four varieties using four different methods.

	Days in test				
Method	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 values of whole plant versus excised stolon methods 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. Survival* of southern chinch bugs at different intervals (days) using whole plants.

	Box					Tube				
Variety	3	7	14	21	28	3	7	14	21	28
Floratam	7.5A	6.8A	5.0A	4.1A	3.2A	9.5A	8.1A	6.8A	5.3A	4.1A
Captiva	8.6A	7.0A	3.0A	1.3B	1.1B	8.3A	5.1B	3.1B	1.1B	0.6B
NUF-216	8.4A	6.5A	3.1A	1.6B	0.9B	8.1A	4.8B	2.6B	1.1B	0B
FX-10	6.6A	2.5B	0.3B	0.1B	0B	8.3A	3.5B	0.4B	0B	0B

* Means within each column followed by the same letter are not significantly different ($\alpha = 0.05$) determined with an LSD test (SAS 2012).

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 1 method (jar test). At 7 d, resistance was 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 showed Captiva to be resistant which the other 3 methods demonstrated. The bag test also did not find NUF-216 to be resistant in weeks 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

Table 3. Survival* of southern chinch bugs at different intervals (days) using excised stolons.

	Bag					Jar				
Variety	3	7	14	21	28	3	7	14	21	28
Floratam	7.1A	5.1A	3.0A	1.9A	0.3A	9.0A	6.0A	5.0A	3.4A	2.6A
Captiva	8.8A	6.0A	2.1AB	1.6A	0.9AB	7.4AB	2.8B	1.3B	0.8B	0.4B
NUF-216	7.6A	4.0A	0.9B	0.6AB	0.3AB	7.1AB	3.3B	0.8B	0.3B	0.3B
FX-10	7.5A	4.0A	0.5B	0B	0B	6.4B	2.1B	0.5B	0.1B	0B

* Means within each column followed by the same letter are not significantly different ($\alpha = 0.05$) determined with an LSD test (SAS 2012).

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 also was evident. In our tests, shorter time intervals in measuring mortality may result in not detecting 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 resistant 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.

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