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A research team of scientists from Texas A&M University initiated work to determine the susceptibility of Texas bluegrass and its hybrids to the bluegrass billbug. The study was conducted in metal livestock water tanks (shown above) used as evaluation cages.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Susceptibility of Bluegrasses to Bluegrass Billbug

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SUMMARY

Researchers with the Texas Agricultural Experiment Station, Texas A&M University Research & Extension Center at Dallas have evaluated at least 15 different turfgrass species for resistance or susceptibility to either one or several of 12 different insect or mite pests associated with each grass. Reported here is the tolerance discovered in 'Reveille' hybrid bluegrass [Texas bluegrass (*P. arachnifera*) X Kentucky bluegrass (*Poa pratensis*)] to the bluegrass billbug (*Sphenophorus parvulus*).

- Before this study, no information was available on the resistance or susceptibility of Texas bluegrass or its hybrid crosses with Kentucky bluegrass to the bluegrass billbug.
- This experiment compared the susceptibility of three Texas bluegrass genotypes with two Kentucky bluegrass genotypes and five F1 hybrids among the tested parents.
- The hybrid, 'Reveille', exhibited a level of tolerance to bluegrass billbug larval damage and ranked in the top statistical grouping for the least leaf-firing (percent of dead or dying leaf and shoot tissue), reduction in tiller number and longest tiller, reduction in number and length of rhizomes, reduction in shoot and total dry weight (<30%) per plants.
- One of the hybrids, TXKY96-66-22, sustained 77% reduction in total dry weight and was the most susceptible genotype in the study.
- No single measured grass trait stood out as being the best for evaluating the effect of bluegrass billbug on the Poa genotypes, but a combination of traits showed that P. arachnifera and its hybrid crosses with P. pratensis produce individual plants that are both tolerant and susceptible.
- The use of self-contained field cages (metal tanks) provided a good test arena to evaluate plant material against soil insects such as the bluegrass billbug. However the limiting factor for this procedure was the small number of genotypes that could be evaluated at one time.
- A resistant or tolerant cultivar has the potential to reduce the amount of pesticides needed for control, and therefore extend the useful life of the pesticide. Other benefits include reduced impact on non-target organisms, reduced human exposure and reduced environmental harm.

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Several species of billbugs (Coleoptera: Curculionidae: Sphenophorus) are serious insect pests of turfgrass, and they cause damage in both cool- and warm-season grasses. Each summer, billbug feeding is often misidentified as damage by other soil insects, chinch bugs, or the result of any of several turf diseases. The bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) is a major pest of Kentucky bluegrass (*Poa pratensis* L.). It is distributed throughout the United States (18) and it was first reported as a pest of Kentucky bluegrass from Nebraska in 1890 (5). This billbug also damages tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.) and several other cool-season grasses (6)

Field trials at several locations in Nebraska, New Jersey, and Oregon have documented resistance or susceptibility to the bluegrass billbug among Kentucky bluegrass cultivars (2, 3, 4, 7, 8, 9, 10, 11, 20, 21). In these studies, injury ratings and billbug densities tended to be highly correlated (9, 10, 11, 20), but there was no correlation between billbug density and thatch accumulation (11).

Selections of Texas bluegrass (*Poa arach-*

Leaf firing or canopy damage was assayed as % yellowing, brown, or dead leaves on the plant.

fira) and its hybrids with Kentucky bluegrass are being developed as cool-season turfgrasses that exhibit many of the qualities of the parent Kentucky bluegrass with the added benefit of being drought tolerant from the Texas bluegrass parent. They can be used in the semi-arid [50-80 cm (20-32 inches) of annual rainfall] regions of the southern United States where most of the cool-season temperate grasses are poorly adapted (12, 13).

This study was intiated to determine the susceptibility of Texas bluegrass and its hybrids to the bluegrass billbug. No information was available for this grass species. In addition, the project was designed to evaluate parents and progeny for potential resistance to bluegrass billbug. Hybrids from these parents have the potential to be used across much of the Kentucky bluegrass region (northern states) as well as the southern states where heat and drought tolerance are needed.

Methods

This study was initiated to determine the feeding preference and damage caused by bluegrass billbugs on 10 *Poa* (bluegrass) genotypes and to develop an indication of the heritability of the billbug resistance. Grasses tested included three Texas bluegrasses ['Tejas 1' (tested as Syn1), 25-11(25-88), and 20-11(3-88)], two Kentucky bluegrasses ('Mystic' and 'Huntsville') and five F_1 hybrids, {'Reveille' [a cross between 20-11(3-88) × Huntsville], TXKY96-66-22, TXKY96-66-25, TXKY96-66-35, and TXKY96-66-51 [four crosses between 25-11(25-88) × 'Mystic']}.

The study was conducted in metal livestock water tanks [0.76 m (2.5 ft) high by 2.44 m (8 ft) in diameter] used as evaluation cages. Cages were filled to an approximate depth of 45 cm (18 inches) with golf course green topdressing sand which provided an easy medium to excavate and separate roots and rhizomes of the different plants which can grow across most of the diameter of the cages during the test period. The top of each cage was fitted with a screened lid (allowing 70% light transmission) to prevent movement into or out of the cages by either billbugs or other insects. A similar field cage has worked well for highly mobile mole crickets (14). The open area between plants allowed the adult bluegrass billbug to move freely from one plant to another to choose a preferred hosts for oviposition. *Poa* plants for the experiment were produced in the greenhouse in 51 cell trays with 4.8 cm diam. x 5.4 cm deep cells until transferred to the field cages.

For physical arrangement of the cages and plants within the cages, a modified randomized complete split-split plot design with four replicates was used. The main plot was billbug treat-

A comparison of the paired tanks in a replicate shows the growth potential for each *Poa* genotypes without billbugs (left) and with bluegrass billbug damage (right) at approximately three months. There was a significant overall reduction in shoot growth due to billbug feeding. Even though 'Reveille' sustained 30% damage, the overall damage was significantly less than for any of the other genotypes tested and it exhibited a good level of tolerance.

ment (e.g., cage), the subplot was location within the cage (north vs. south), and the sub-subplot was grass genotype. Within each replicate (consisting of two cages, one with and one without billbugs), two plants from each genotype were paired by total size and one plant of the pair was planted in the north half of each cage. The exact same randomized arrangement (physical location) of these two paired plants was used for the north half of each cage.

For the south section of each cage, the plants within each genotype were again paired and arranged as described above for the north half of the two cages except that the location of plants from each genotype was re-randomized. The matched arrangement based on plant size minimized the effect of the leaf area and root mass on the treated versus untreated comparison. Likewise, the use of the same randomized placement of genotypes for the north side of each cage helped to minimize any effects due to plants being closer or farther from the edge of the cage than its partner in the other cage (e.g. shading). Because the north vs. south effect (subplot) was inconsequential for all traits measured, the average of the two plants of each genotype per cage was used in the statistical analysis.

Plants were fertilized bi-weekly with Miracle-Gro All Purpose fertilizer [15-30-15 + B (200 ppm), Cu (700 ppm), Fe (1500 ppm), Mn (500 ppm), Mo (5 ppm), Zn (600 ppm)] and watered as needed throughout the test period to maintain good plant growth.

Plants were transplanted on April 23, 2003 and allowed to establish in the cages for five days before billbug adults were introduced. Plants were arranged in two concentric circles with 8 plants in a 3-meter and 12 plants in a 6-meter circumference circle. Plants were spaced approximately 21 cm apart in each quadrant and a minimum of 20 cm from the side of the cage. Treatment cages were infested at a rate of 2 females and 1 male per plant. The adult billbugs were released between the two concentric rows of plants (approximately 50 cm from the center of the cage), and allowed to migrate to the *Poa* plants they preferred as acceptable host. Bluegrass billbug adults for this study were field collected using linear pit-fall traps around a Kentucky bluegrass planting at the Kansas State University's Rocky Ford Turfgrass Farm, Manhattan, KS by Dr. R.J. Bauernfeind.

The *Poa* plants and the resulting damage were evaluated in mid- to late-July, about three months after the adult billbugs were introduced into the cages and after a generation of feeding damage by the billbug larvae. On July 8, 2003, "leaf firing" damage (the percentage of dead or dying leaf and shoot tissue in the surface growth of the plant) was assayed visually. Then all plants were harvested and bagged during mid- to late-July 2003 by excavating the entire plant from the sand. All plants from one replicate were dug and held under refrigeration until they were processed before harvesting the next replicate.

In the laboratory, all tillers were cut at the soil line, washed, and counted. Roots and rhizomes were also washed before measurements were made. Plant traits measured were: total rhizome number; total length, and longest rhizome; total shoot number and longest shoot and the total plant biomass. Shoot and root biomass were collected separately, oven dried, and weighed. Rhizomes from each treated plant were also evaluated for billbug feeding damage.

All data were subjected to appropriate analysis of variance (ANOVA) with the PROC GLM procedure of SAS (19). Two categories of statistical analysis were used. First, variations in plant traits among genotypes from only untreated cages were analyzed. Second, the difference between billbug-infested plants and the corresponding paired non-infested plants was calculated. The percentage reduction of rhizomes, shoots and whole plant dry weights were calculated as: {[(amount in the check plant - amount in the billbug infested plant) / (amount in the check plant)] × (100)} (1).

Results

The percentage of leaf firing or canopy damage for each *Poa* genotype both with and without bluegrass billbug damage was estimated

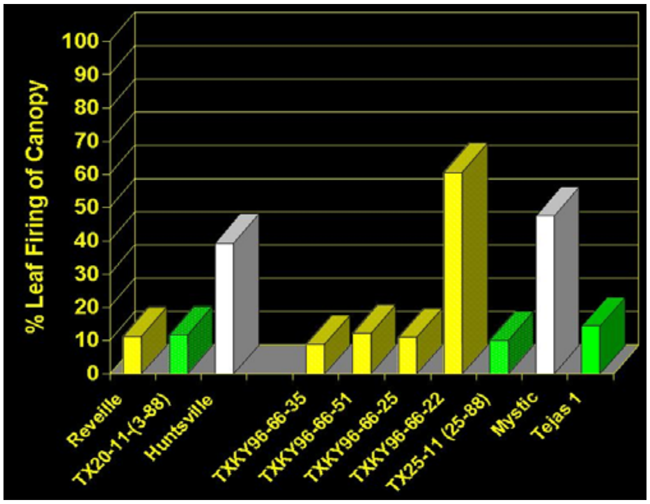

Figure 1. The % leaf-firing or canopy damage (check - treatment) is a good indicator of the resistance/susceptibility of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass, is included for comparison).

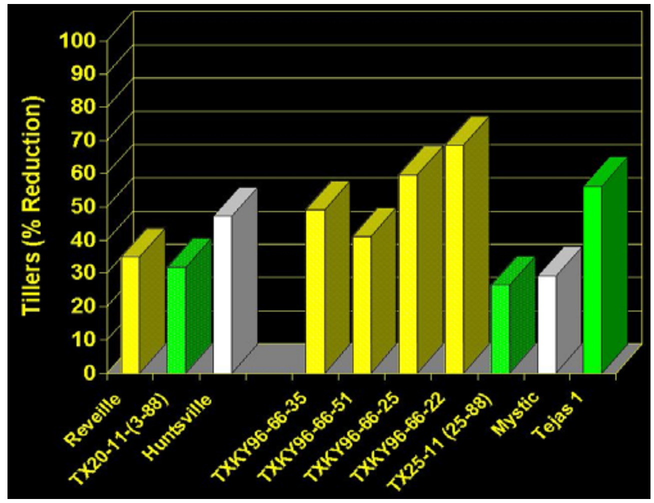

Figure 2. The % reduction in number of tillers per plant (check - treatment) is an indicator of resistance/susceptibility and shows the response of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass is included for comparison).

as the percentage of the leaves and shoots that were yellowing or dead for each test plant. No significant difference was recorded in the level of tolerance for the three *P. arachnifera* genotypes or four of the five hybrids ('Reveille', TXKY 96-66-35, TXKY 96-66-25, or TXKY 96-66-51) (Figure 1). The two *P. pratensis* cultivars, 'Mystic' and 'Huntsville', which sustained 39% and 48% damage, respectively, and one hybrid, TXKY96-66-22, with 61% leaf-firing exhibited significantly greater damage.

Although not significantly different from most of the other genotypes tested, 'Mystic' and 25-11(25-88) expressed the least reduction in tillering (<30%) (Figure 2). In contrast, 'Tejas 1' and two of the hybrids (TXKY96-66-22 and TXKY96-66-25) were the only genotypes exhibiting a significant reduction in the number of tillers

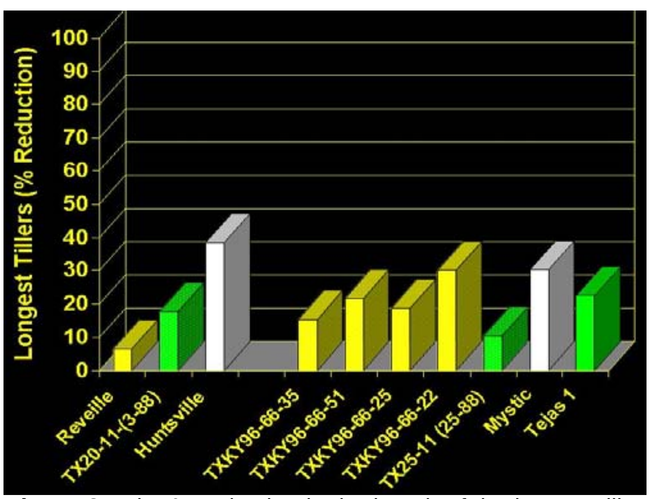

Figure 3. The % reduction in the length of the longest tiller (check - treatment) on each plant is a good indicator of resistance/susceptibility and shows the response of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass included for comparison).

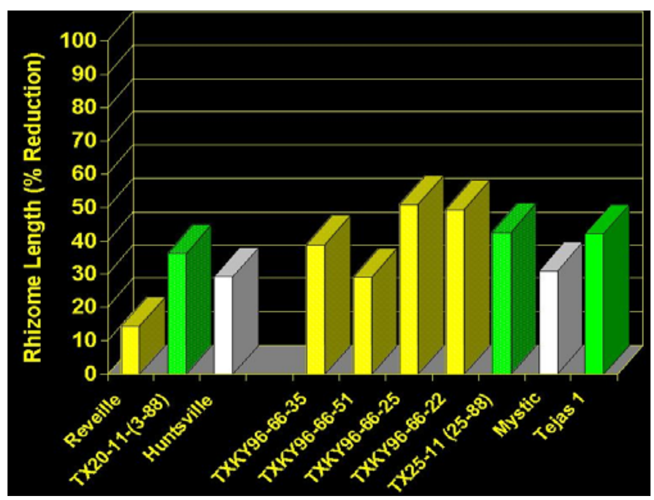

Figure 4. The % reduction in number of rhizomes per plant (check - treatment) is an indicator of resistance/susceptibility and shows the response of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass included for comparison).

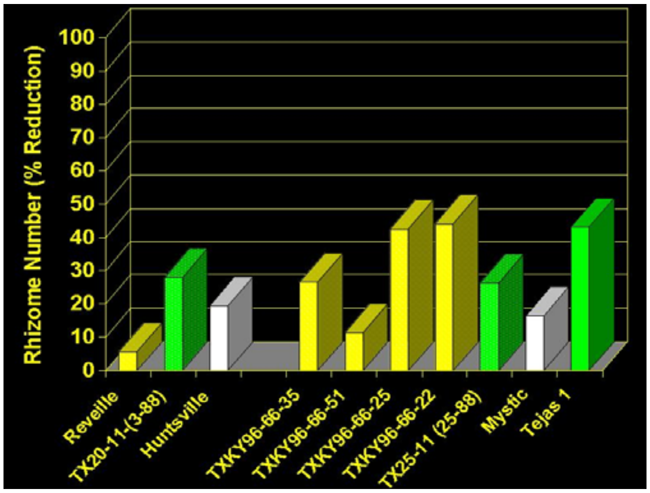

Figure 5. The % reduction in the length of the longest rhizome on each plant (check - treatment) is an indicator of resistance/susceptibility and shows the response of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass included for comparison).

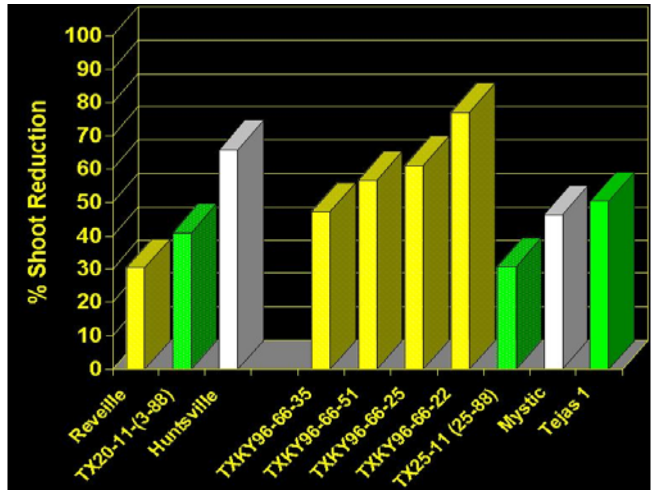

Figure 6. The % reduction in total canopy dry weight (check - treatment) is a good indicator of resistance/susceptibility and shows the response of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass included for comparison).

(>56%) due to larval feeding (Figure 2). A count of the total tillers per plant separated out only the most susceptible genotypes. Also, when the length of the longest tiller per plant was compared, 25-11(25-88), 'Tejas 1', and two hybrids ('Reveille' and TXKY96-66-35) exhibited the least reduction in length. 'Huntsville', 'Mystic' and TXKY96-66-22 expressed the greatest reduction in length of the longest tiller (Figure 3).

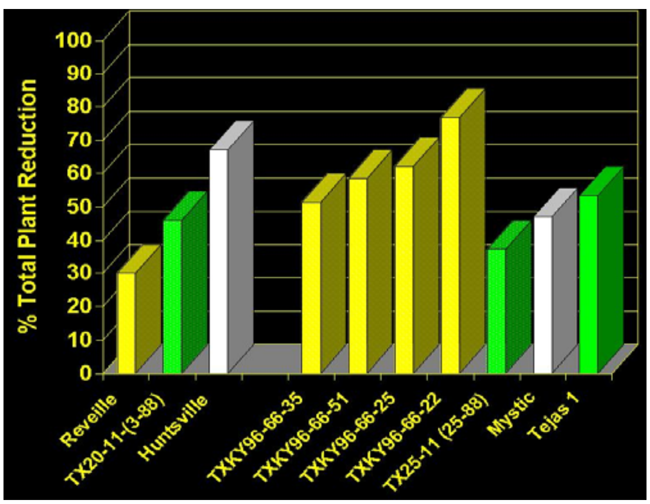

Figure 7. The % reduction in total plant dry weight (check - treatment) is a good indicator of resistance/susceptibility and shows the response of each *Poa* genotype to feeding damage by the bluegrass billbug. (Bluegrass hybrid = yellow; Kentucky bluegrass parent = white; Texas bluegrass parent = green. 'Tejas 1', the only commercial cultivar of Texas bluegrass included for comparison).

Reduction in total length and number of rhizomes was a good indicator of bluegrass billbug larval feeding damage (Figures 4 and 5). The percent reduction in rhizome length, however, did a better job of separating the different genotypes than the number of rhizomes. Five genotypes were identified as being more susceptible based upon rhizome length, whereas only three genotypes ('Tejas 1', TXKY96-66-22 and TXKY96-66-25) were separated as most susceptible using the number of rhizomes. 'Reveille' sustained significantly less damage (14 and 6%) that each of these plants bases upon both number of rhizomes and length, respectively (Figures 4 and 5).

The impact of bluegrass billbug feeding is reported separately for total shoot dry weight (Figure 6), total root dry weight, and for the combined whole plant (Figure 7). Plant dry weights were the most consistent traits measured to separate genotypes for their susceptibility to bluegrass billbug. The shoot and root dry weight separated out the same three genotypes ('Huntsville' and two hybrids, TXKY96-66-22 and TXKY96-66-25), whereas the total dry weight separated these three and one additional hybrid, TXKY96-66-51, as most susceptible to billbug feeding. All three plant weights, however, showed 'Reveille' (< 32%

Poa genotypes	*Poa* type	Morphological Characteristic						
		Leaf Firing	Number of Tillers	Tiller Length	Rhizome Length	Number of Rhizomes	Shoot Weight	Total Weight
25-11(25-88)	Texas	a	a	ab	bc	abc	a	ab
20-11(3-88)	Texas	a	ab	abc	abc	bc	ab	abc
Tejas 1 (Syn1)	Texas	a	bcd	b	bc	c	ab	abc
Mystic	Kentukcy	bc	a	c	abc	ab	ab	abc
Huntsville	Kentukcy	b	abcd	d	ab	ab	bc	cd
Reveille	Hybrid	a	abc	a	a	a	a	a
TXKY 96-66-35	Hybrid	a	abcd	ab	bc	abc	ab	abc
TXKY 96-66-25	Hybrid	a	cd	abc	c	c	bc	cd
TXKY 96-66-51	Hybrid	a	abc	bc	ab	ab	ab	bcd
TXKY 96-66-22	Hybrid	c	d	cd	bc	c	c	d

Table 1. Morphological characteristics of Texas, Kentucky, and hybrid bluegrass associated with bluegrass billbug feeding. Letters within each column indicate which statistical grouping that the *Poa* genotype received for that particular morphological characteristic in response to feeding by bluegrass billbugs.

reduction) was significantly different from these four genotypes and exhibited a significant level of tolerance to bluegrass billbug feeding (Figure 7).

Conclusions

No one trait stands out as being the best for measuring the effect of bluegrass billbug on various bluegrass plants (Table 1). To get a better indication of the ranking of the various bluegrasses tested, the number of times a genotype appeared in the least damaged or most tolerant group (smallest % decrease) was considered. To determine this, we added the number of times a genotype was identified in the top statistical grouping for all the different traits evaluated.

Using this method the grasses separated into four groups:

Group 1 'Reveille' ranked in the top statistical grouping for all parameters evaluated, and it is the least preferred or most tolerant to bluegrass billbug feeding.

Group 2 Two Texas bluegrasses [25-11(25-88) and 20-11(3-88)], and the hybrid, TXKY 96-66-35, each appeared in the top statistical grouping for seven of the eight parameters evaluated.

Group 3 'Mystic' Kentucky bluegrass and TXKY 96-66-51 were assigned to the next group, appearing in the top statistical grouping six times.

Group 4 All the other genotypes appeared in the top statistical grouping fours times or less and were assigned to the most susceptible group. The hybrid, TXKY 96-66-22, was the most susceptible, and at no time did it appear in the top statistical ranking.

'Tejas 1' tested susceptible, but this cultivar is phenotypically very variable because Texas bluegrass is a dioecious species (each plant is genetically different) and only a limited number of plants were tested in our study. To properly evaluate 'Tejas 1', a much larger sample of plants would need to be tested. In fact, the two tested Texas bluegrass genotypes were included as parents in the synthetic which became 'Tejas 1'. This method of testing for bluegrass billbug resistance would be effective for homozygous cultivars and should work well for other plant species that reproduce by apomixes, parental plants to be used in hybridization, or vegetatively-propagated material.

These results show significant bluegrass billbug damage to the two *P. pratensis* cultivars ('Mystic' and 'Huntsville'). In previous reports,

Cultivar	Bluegrass Type[1]	Insects[2] Fall Armyworm	White Grub	Bluegrass Billbug
Reveille	Hybrid	resistant	tolerant	tolerant
Thermablue	Hybrid	ND[3]	ND	ND
Tejas 1	Texas	susceptible	tolerant	susceptible
Syn-2	Texas	susceptible	tolerant	ND
20-11(3-88)	Texas	ND	ND	moderately tolerant
Huntsville	Kentucky	resistant	ND	susceptible
Mystic	Kentucky	resistant	ND	moderately tolerant

[1] Hybrid bluegrass (*Poa arachnifera* X *P. pratensis*); Texas bluegrass (*P. arachnifera*); Kentucky bluegrass (*P. pratensis*).

[2] Fall armyworms (*Spodoptera frugiperda)* ; White grubs [*Phyllophaga congrua*); Bluegrass billbug (*Sphenophorus parvulus*)

[3] ND, no data available

Table 2. Multiple pest resistance in bluegrasses

'Mystic' was reported as resistant to bluegrass billbug with 20% or less visual damage in field plots and 'Huntsville' was reported to sustain 25-40% damage in studies in New Jersey (3, 7, 8, 21). The results reported here reveal these two Kentucky bluegrass cultivars are far more susceptible at the bluegrass billbug densities encountered in our study. However, the contrasting results may be the result of a much higher population pressure in the current study where we were able to provide more equal adult population pressure across all replicates.

These results also show that there are individual genotypes of Texas bluegrass that are resistant while others are very susceptible. To assure resistance in a Texas bluegrass variety, the individual parents that make up the synthetic variety, or that are used to make a hybrid, must be tested before being used in a synthetic variety or in the cross. In our study, both Texas bluegrass parents and both Kentucky bluegrass parents used to develop the hybrids were resistant and susceptible, respectively. It is likely that 'Tejas 1', a susceptible Texas bluegrass, would be a poor parent, but we don't have hybrid data to prove this.

This work provides the first report on the susceptibility of *P. arachnifera* to the bluegrass billbug. Hybrids between the two species exhibited a range of susceptibility to bluegrass billbug, ranging from 'Reveille' which was least preferred and expressed a level of tolerance (the least reduction in tiller length, rhizome number and length, and only 30% reduction in growth), to TXKY96-66-22 which was the most susceptible hybrid in the study and sustained more damage than either of its parents.

In other studies, both 'Reveille' and 'Tejas 1' Texas bluegrass provided good resistance to a natural field infestation of a white grub (*Phyllophaga congrua* Leconte) in north Texas (15). Also, 'Reveille' exhibited high antibiosis of confined fall armyworm (*Spodoptera frugiperda* J.E. Smith) larvae in no-choice laboratory evaluations. However, 'Tejas 1' was susceptible to fall armyworm in that study (16).

Our work showed that the hybrid

'Reveille' was least preferred and expressed moderate tolerance to bluegrass billbug. Also, two hybrids from a totally separate cross expressed levels of tolerance nearly as good as 'Reveille'. Although resistance data from only two crosses are reported here, it appears that bluegrass billbug-resistant hybrids can be developed from a resistant Texas bluegrass and a susceptible Kentucky bluegrass.

Additionally, the use of self-contained field cages (metal tanks) provided a good test arena to evaluate homozygous plant material against soil insects such as the bluegrass billbug. This cage system should work well for other soil insects and even to evaluate plants for foliage feeders. The limiting factor for these cages and procedure is the small number of genotypes that can be evaluated at one time within the cages.

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