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Populations of the hunting billbug (*Sphenophorus venatus vestitus* Chittenden) have increased over the past 10 to 15 years, and this billbug is responsible for extensive turf damage on golf courses and in lawns and other landscapes. In addition, considerable loss of sod production of both bermudagrass and zoysiagrass has occurred due to this insect (shown above). Researchers at Texas A&M University conducted experiments to evaluate cultivars of zoysiagrass for resistance to hunting billbug and to identify potential mechanisms of resistance.

Volume 9, Number 17
September 1, 2010

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 450 projects at a cost of $31 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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Hunting Billbug Resistance Among Zoysiagrass Cultivars

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SUMMARY

Hunting billbugs (*Sphenophorus venatus vestitus*) damages zoysiagrass (*Zoysia spp.*) and bermudagrass (*Cynodon spp*). Damage is often misdiagnosed as drought, disease, or another soil insect. Populations have increased over the past couple of decades causing extensive damage to both of these grasses on golf courses and in lawns and other landscapes. Nine cultivars of zoysiagrass were evaluated for resistance to the hunting billbug in a choice test in field cages. Results include:

- Leaf firing of plant canopy is considered an above ground expression of root feeding damage by larvae. 'Diamond' and 'Zorro' exhibited significantly less leaf firing damage (a reduction of 6.1 and 9.8%, respectively) than 'Palisades', 'Meyer', and 'Crowne' that exhibited greater than 40% canopy damage with insect infestation.
- When root, shoot, and total plant dry weights were compared, 'Diamond', 'Zorro', 'Cavalier', and 'Royal' [all *Z. matrella* (L.) Merr.] sustained less dry weight reduction (<53%) than 'Palisades', 'Meyer', and 'El Toro' (all *Z. japonica* Steud.) with 76, 74, and 70% total dry weight reduction, respectively.
- Cultivars of *Z matrella* appear to be more resistant as a group than the *Z. japonica* cultivars which are susceptible.

Populations of the hunting billbug (*Sphenophorus venatus vestitus* Chittenden) have increased over the past 10 to 15 years, and this billbug is responsible for extensive turf damage on golf courses and in lawns and other landscapes. In addition, considerable loss of sod production of both bermudagrass and zoysiagrass has occurred due to this insect. Hunting billbugs also feed on other turfgrasses including St. Augustinegrass (*Stenotaphrum secundatum* Kuntze), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack], and bahiagrass (*Paspalum notatum* Flugge). Its damage is often misidentified as drought, dormancy, disease, or another root-feeding insect.

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Only one generation of hunting billbug per year was reported in northern Florida (15), Louisiana (18), and Arkansas (31), but Huang (7) and Huang & Buss (8) suggest that it may have at least two or three overlapping generations per year in Florida, and this may also be the case in Southern Texas, Mexico, and throughout the Caribbean Islands. Adult hunting billbugs feed by notching the leaves of both zoysiagrass and bermudagrass (7) and lay eggs in a small feeding scar usually in the crown of the plant. Larvae pass through five instars (6, 7) with the early instars feeding within the crown, larger rhizomes, and stolons before the later instars emerge and continue feeding on the whole root system.

Initial larval damage appears as small pockets of yellowing and dying grass, resembling dollar spot disease infections which increase in size and later coalesce as the larvae continue feeding (29). Infested sod fields often cannot be harvested since many of the roots and rhizomes have been severed and the cut sod will not hold together.

The hunting billbug has been listed as a

Hunting billbugs are responsible for extensive damage in bermudagrass and zoysiagrass and also feed on St. Augustinegrass, centipedegrass, and bahiagrass.

Early instars of hunting billbug feed within the crown, larger rhizomes, and stolons. Later instars emerge from rhizomes (exit shown above) to feed on the full root system.

damaging pest from New Jersey (9), south to Georgia (17) and Florida (14), west to Kansas (4), Texas, California, and Hawaii (5), and throughout the Caribbean Islands (28, 29). It has been identified in Arizona and Idaho, and its total area of distribution across the Western U.S. is not fully established (K. Umeda, University of Arizona; D. J. Shetlar, The Ohio State University; T. Salaiz, University of Idaho, personal communication). It is also listed as a serious turf pest on golf courses in Japan (6). The genus *Sphenophorus* (Coleoptera: Curculionidae) contains 71 species of which 64 occur in the North America. At least nine of these species are known to be pests of turfgrass and cause damage to both cool- and warm-season grasses (12, 17, 28, 29).

Several studies have identified resistance to the bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) which is a primary pest of Kentucky bluegrass (*Poa pratensis* L.) (2, 3, 10, 11, 13, 16, 27, 29). Resistance to the bluegrass billbug was also documented in 'Reveille' and several other hybrids of Kentucky bluegrass × Texas bluegrass (*P. arachnifera* L.) (23, 24). Documented resistance to insect and mite pests in turfgrass has been summarized (22).

This experiment was designed to evaluate cultivars of zoysiagrass for resistance to hunting billbug and to identify potential mechanisms of resistance. When the present experiment was initiated in 2000, no resistance had been identified to hunting billbug in either bermudagrass or zoysiagrass. However, more recent studies in Florida have shown differences in susceptibility among genotypes of these two grasses (7).

Materials and Methods

Nine zoysiagrass cultivars were evaluated (including four *Z. matrella* L. Merr. cultivars and five *Z. japonica* Steud. cultivars) for resistance to the hunting billbug (Table 1). Metal livestock water tanks (0.76 meters high by 2.44 meters in diameter) were used as evaluation cages. Each cage was positioned above the ground on several concrete blocks and set at a slight slant toward a 2.5-cm drain hole to eliminate any excessive water accumulating in the soil profile at the bottom of the cage. Each cage was filled to a depth of approximately 45 cm with a 100% sand rootzone media to facilitate uniform growth and to provide an easy medium to excavate and separate the root systems. The top of each cage was fitted with a screen (allowing 70% light transmission) to prevent movement into or out of the cages by either billbugs or other insects. A similar confined field cage has been used for experiments with bluegrass billbug (23) and mole crickets (19, 20).

Zoysiagrass cultivars used in this experiment were produced in the greenhouse in 18-cell trays (each cell measuring 7.5 x 7.5 cm and 4 cm

Z. mattrella types	*Z. japonica* types
Diamond	Palisade
Zorro	Meyer
Cavalier	El Toro
Royal	De Anza
	Crowne

Table 1. Zoysiagrass cultivars evaluated for resistance to hunting billbugs.

Metal livestock water tanks (0.76 meters high by 2.44 meters in diameter) were used as evaluation cages. Each cage was positioned above the ground on several concrete blocks and set at a slight slant toward a 2.5-cm drain hole to eliminate any excessive water accumulating in the soil profile at the bottom of the cage.

deep). When plants were transferred to the field cages, they were watered and fertilized as needed throughout the test period to maintain good plant growth.

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 treatment (e.g., cage), the subplot was location within the cage (north vs. south), and the sub-subplot was zoysiagrass cultivar. Within each replicate (consisting of two cages, one with and the other without billbugs), two plants from each cultivar were paired by total size and one plant was assigned to the north half of each cage. The exact same randomized arrangement (physical location of cultivar) for each of the paired plants was used for the north half of each cage.

An analogous assignment of varieties was use for the south section of each cage, except that the location of variety 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 cultivars 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., such as shading). Because the north versus south effect (subplot) was insignificant for all traits measured in our study, the average of the two plants of each cultivar per cage was used in the statistical analysis. Using the average of the two plants per cage, the data were subjected to analysis of variance (ANOVA) with the PROC GLM procedure of SAS (25).

Plants were transplanted June 23-24, 2000 and allowed to establish for 5 days in the cages before hunting billbug adults were introduced into the cages. Plants were planted in two concentric circles of 3 and 6 meters circumference with 7 plants in the 3-meter and 11 plants in the 6-meter 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 similar rate of adults and manner of introduction to anoth-

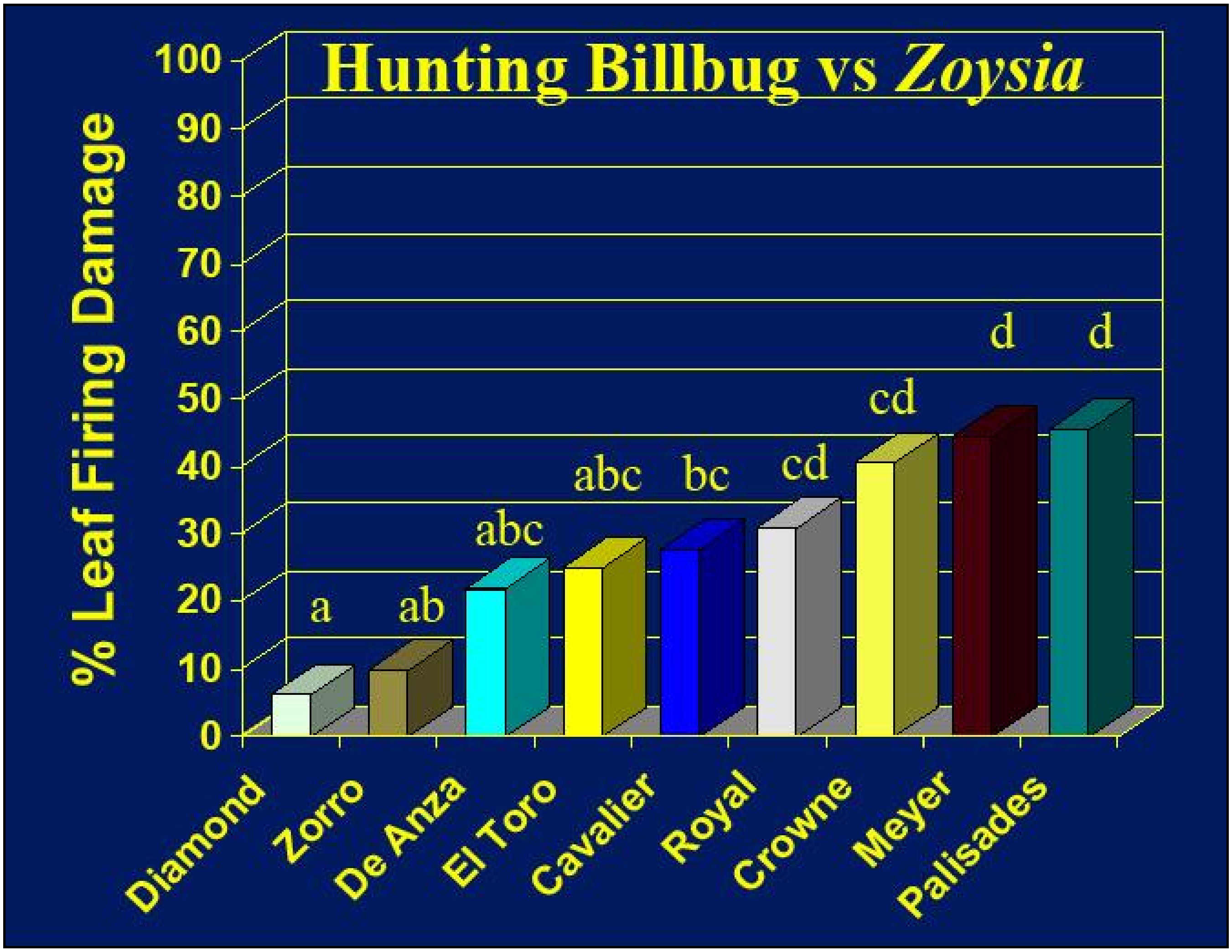

Figure 1. Resistance measured as leaf firing of plant canopy (% canopy damage of billbug infested zoysiagrass compared to uninfested same cultivar control plants) as a result of larval feeding on the root system of nine zoysiagrass cultivars. Bars for each grass with the same letter above them are not significantly different.

er experiment with *Poa spp.* versus bluegrass billbugs (23). One cage of each pair was infested on June 26, 2000 at a rate of 30 female and 15 male hunting billbug adults. The adult billbugs were released between the two concentric rows of plants (appromiately 50 cm from the center of the cage), and allowed to migrate to the zoysiagrass plants they preferred as acceptable hosts. The open area between plants allowed the adult hunting billbug to move freely from one plant to another to choose preferred hosts for egg laying. All hunting billbug adults used in this study were field-collected from a bermudagrass sod farm. Collected hunting billbug adults were held for less than one week in moist soil under refrigeration until released in the study tanks.

On September 21, 2000 leaf firing damage (dead or dying leaf and shoot tissue in the surface growth of the plant) was evaluated by rating each plant on a scale of 1 to 9 (where 1 = 90% leaf firing or dead plants, 9 = no leaf firing). All plants were then harvested and bagged from September 22-29, 2000 (after an evaluation period of 13-14 weeks after adult infestation of the plants) by excavating the entire plant from the sand. All plants from one replicate were dug and held under refrigeration until they were processed before the next replicate was harvested.

In the laboratory, all tillers were cut at the soil line, washed, and counted. Roots and rhizomes were also washed before measurements were made. Traits measured included total rhizome length, longest rhizome, number of rooted nodes on rhizomes, and total plant biomass.

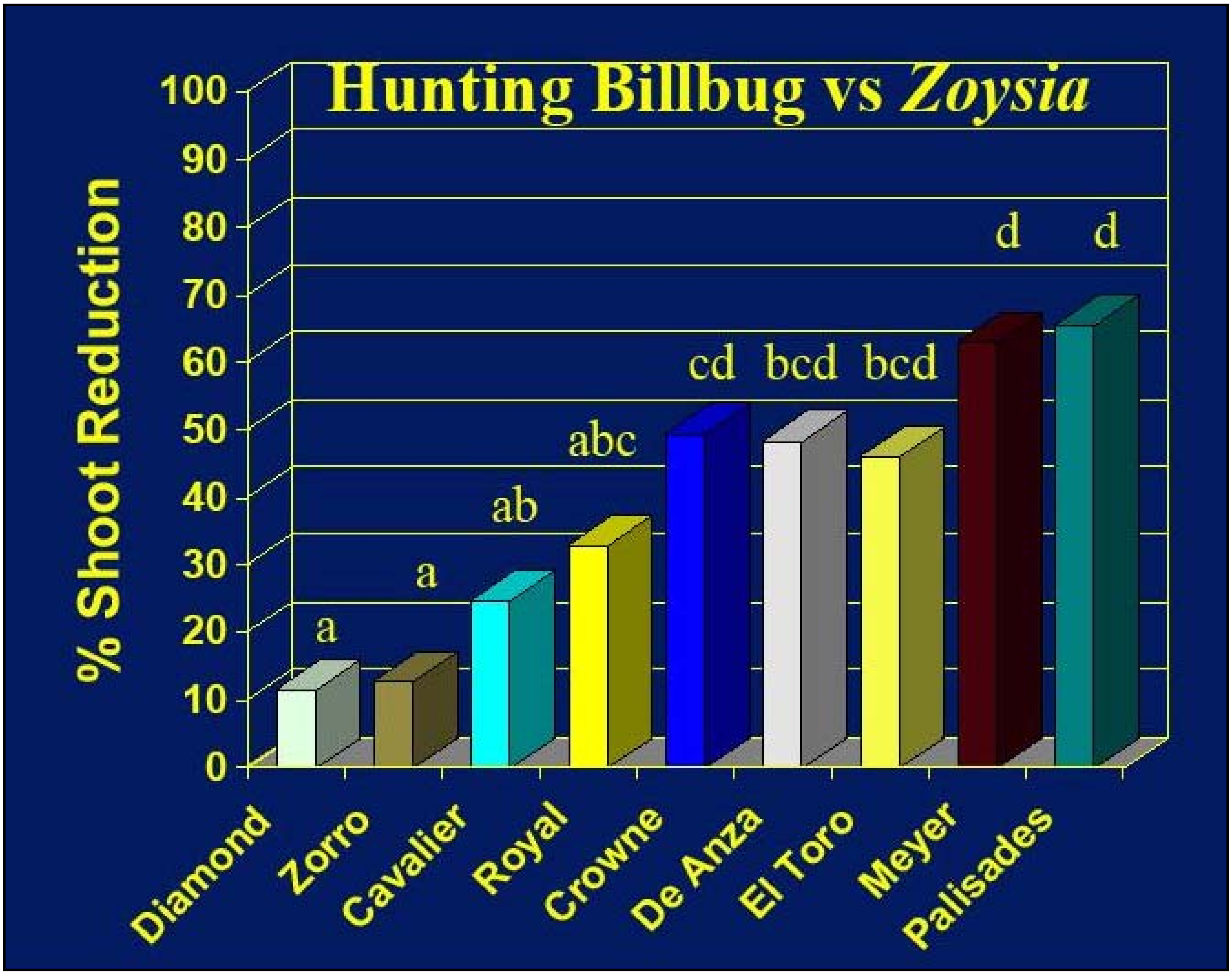

Figure 2. Resistance measured as shoot reduction of plants (% reduction in shoot dry biomass of billbug infested zoysiagrass cultivar compared to biomass produced by unifested same cultivar) as a result of larval feeding on the root system of nine zoysiagrass cultivars. Bars for each grass with the same letter above them are not significantly different.

Shoot and root biomass were collected separately, oven dried (72 hr at 70° C) and weighed. Stolons and rhizomes from each plant were also evaluated for feeding damage.

Two statistical analysis models were used. First, variations in plant traits among genotypes from untreated cages only were analyzed. Second, the percentage reduction of rhizomes, number of rooted nodes, shoots, root, and whole plant dry weights were calculated (1). To analyze these differences, a traditional randomized complete block design analysis with only replicate and cultivar as sources of variation was used. For both analyses (untreated plants only and difference between treatments), F-tests were made using cultivar mean square error as the numerator and residual (error mean square) as denominator. Treatment (billbugs versus no billbugs) was excluded as a source of variation. Comparisons of means for weights and lengths and percent difference in traits between uninfested and infested plants for each genotype were performed using Fisher's protected least significant differences (LSD).

Results

Leaf firing was considered as an above ground symptom expression of the root feeding damage by the billbug larvae. 'Diamond', 'Zorro', 'Cavalier', 'Royal', and 'El Toro' exhibited the

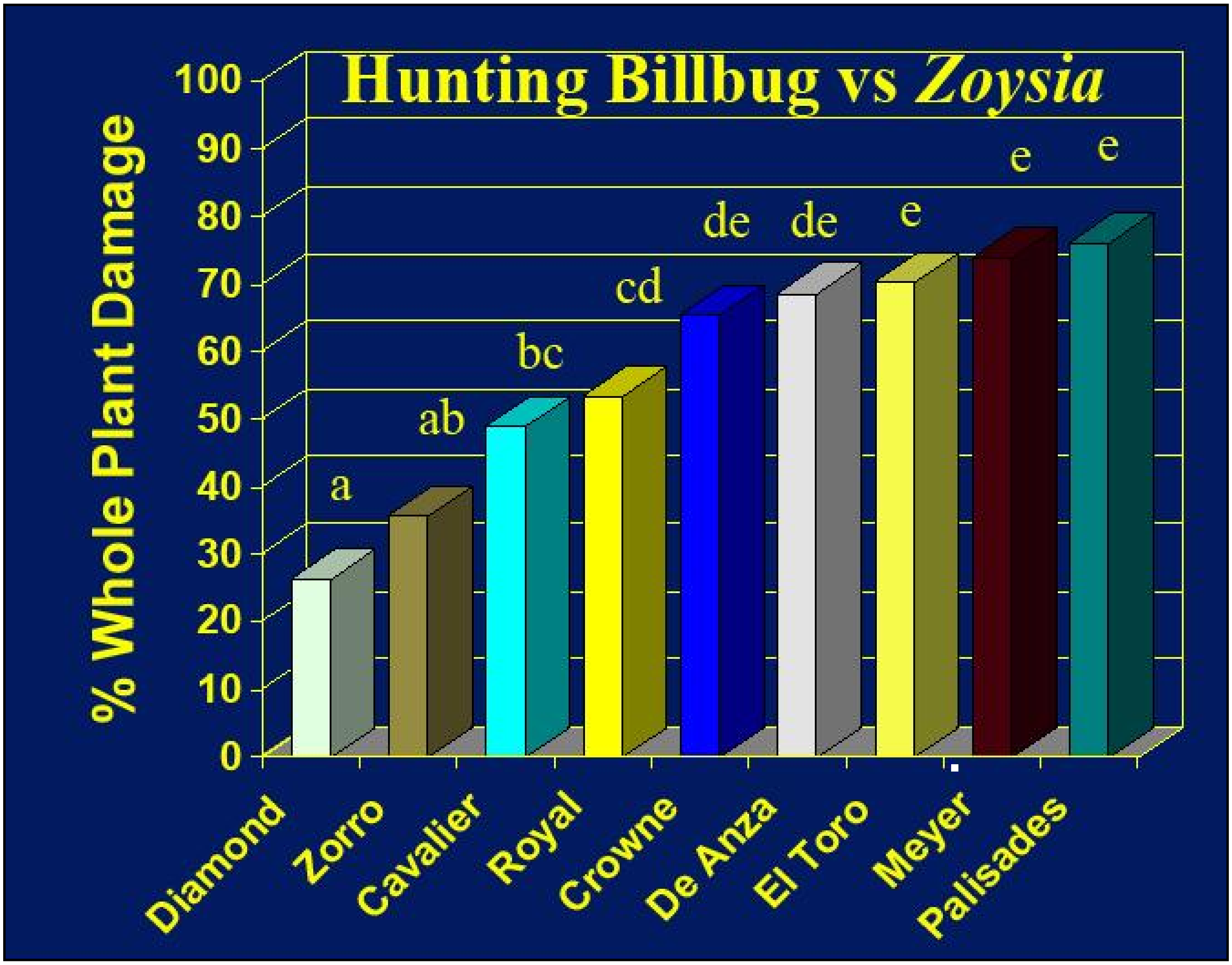

Figure 3. Resistance measured as total dry weight of roots and shoots combined of plants (% reduction in dry biomass of billbug infested zoysiagrass cultivar compared to biomass produced by unifested same cultivar) as a result of larval feeding on nine zoysiagrass cultivars. Bars for each grass with the same letter above them are not significantly different.

least visual damage and were ranked best when plants were exposed to billbugs (Figure 1). 'Meyer', a cultivar which has been the industry standard for years, exhibited the most leaf firing and ranked lowest followed by 'Palisades' = 'Crowne' > 'DeAnza'. Using a modification of Abbott's formula (1), where the treatments are adjusted to the untreated check, 'Diamond' and 'Zorro' exhibited significantly less leaf firing damage (6.1 and 9.8% reduction, respectively). In contrast, 'Palisades', 'Meyer', and 'Crowne' each showed significantly higher canopy reduction (more than 40%) as a result of billbugs feeding on the roots.

After plants were excavated from the sand rootone in the tanks, rhizome length and number of rooted nodes were counted and root, shoot, and total dry plant weights were assayed. 'Diamond' and 'Zorro' sustained only 27.8 and 33.9% reduction, respectively, in total rhizome length, followed by 'Cavalier' with less than 50% reduction. 'Diamond' and 'Zorro' showed the least reduction in total rooted nodes (18.9 and 34.6%, respectively). By contrast, 'Meyer' and 'DeAnza' each sustained more than 70% reduction in total rhizome lengths. 'Diamond' and 'Zorro' expressed a 27.8 and 33.9% reduction difference, respectively, while 'Meyer' and 'DeAnza' each exhibit more than 70% reduction compared to uninfested controls. When the difference in number of rooted nodes on the rhizomes was compared, 'Diamond' and 'Zorro' showed the least reduction with 18.9

Grass plant showing extensive leaf-firing damage as a result of billbug feeding on the root system.

and 34.6%, respectively, while 'Meyer' and 'DeAnza' each produced more than 60% reduction in rooting.

Root, shoot, and total plant weights were assayed on oven-dried plant materials. Differences in root mass were very small in absolute terms (≤13.5 mg) for 'Diamond', 'Zorro', 'Cavalier', and 'Royal' although the percentage differences were more apparent with 33.6, 44.9, 60.8, and 68.1% differences, respectively. Root weight differences exceeded 22.5 mg for 'Crowne', 'El Toro', 'Meyer', and 'Palisades' with percent differences of 70-80% being common for these grasses.

A similar trend was recorded for shoot weights with ≤3 mg difference for 'Diamond', 'Zorro', and 'Cavalier' followed with 5.7 mg difference for 'Royal'. The percentage loss in shoot mass was less than 33.0% for these same cultivars (Figure 2). By comparison, the loss in root mass was greater than 63% for 'Meyer' and 'Palisades'. When the combined shoot and root dry weight or total dry plant mass was compared, differences ranged from 6.2 mg for 'Diamond' to more than 39 mg for 'Palisades' and 'El Toro' . Percentage differences between treatment and check plants ranged from 26.3% for 'Diamond' to more than 65% difference for 'Palisades', 'Meyer', 'El Toro', 'DeAnza', and 'Crowne' (Figure 3). Figure 4 shows the large loss of growth potential for 'Meyer' and 'El Toro' (susceptible), with and without billbug damage, while 'Diamond' and 'Zorro' (resistant) show much less growth loss due to billbug feeding.

Discussion

This experiment provides a controlled study to assay a group of zoysiagrass cultivars for resistance to the hunting billbug, one of the primary limiting pests of zoysiagrass which is used on golf courses and for lawns and other landscapes throughout the world. Based on these results, when the surface damage was assayed as leaf firing of the plant canopy, 'Diamond' and 'Zorro' (*Z. matrella* cultivars), were resistant and sustained minimal loss in plant canopy appearance. The visual appearance of the plant canopy appears to strongly reflect the associated health or damage to the root system. When rhizome length and number of rooted nodes on the rhizome were compared, the plants sustaining the least damage were again all *Z. matrella* cultivars, including 'Diamond', 'Zorro', and 'Cavalier'. Assays of the dry plant mass also showed that the *Z. matrella* cultivars were resistant while the *Z. japonica* cultivars were highly susceptible to damage.

Figure 4 shows marked differences in impact of hunting billbug feeding on the total growth potential of the test plants. 'Diamond' (*Z. matrella*), was the most resistant while 'Meyer' (*Z. japonica*), was highly susceptible and exhibited substantial larval feeding damage. The assays for shoot dry weight and total dry plant weight show that all four cultivars of *Z. matrella* ('Cavalier', 'Diamond', 'Royal', and 'Zorro') sustained the least impact from hunting billbug feeding, while all five cultivars of *Z. japonica* ('Crowne', 'De Anza', 'El Toro', 'Meyer', and 'Palisades') sustained greater than 46 and 65% difference in weights for shoot and total plant mass, respectively.

Experiments by Huang (7) confirm the highest resistance in 'Diamond', 'Zorro', 'Cavalier', and 'Royal' based upon density and quality ratings and that 'El Toro' and 'Palisades' provided lesser quality and density. Additionally, Huang (7) showed no oviposition of eggs on 'Diamond' and 'Zorro', with only an average of

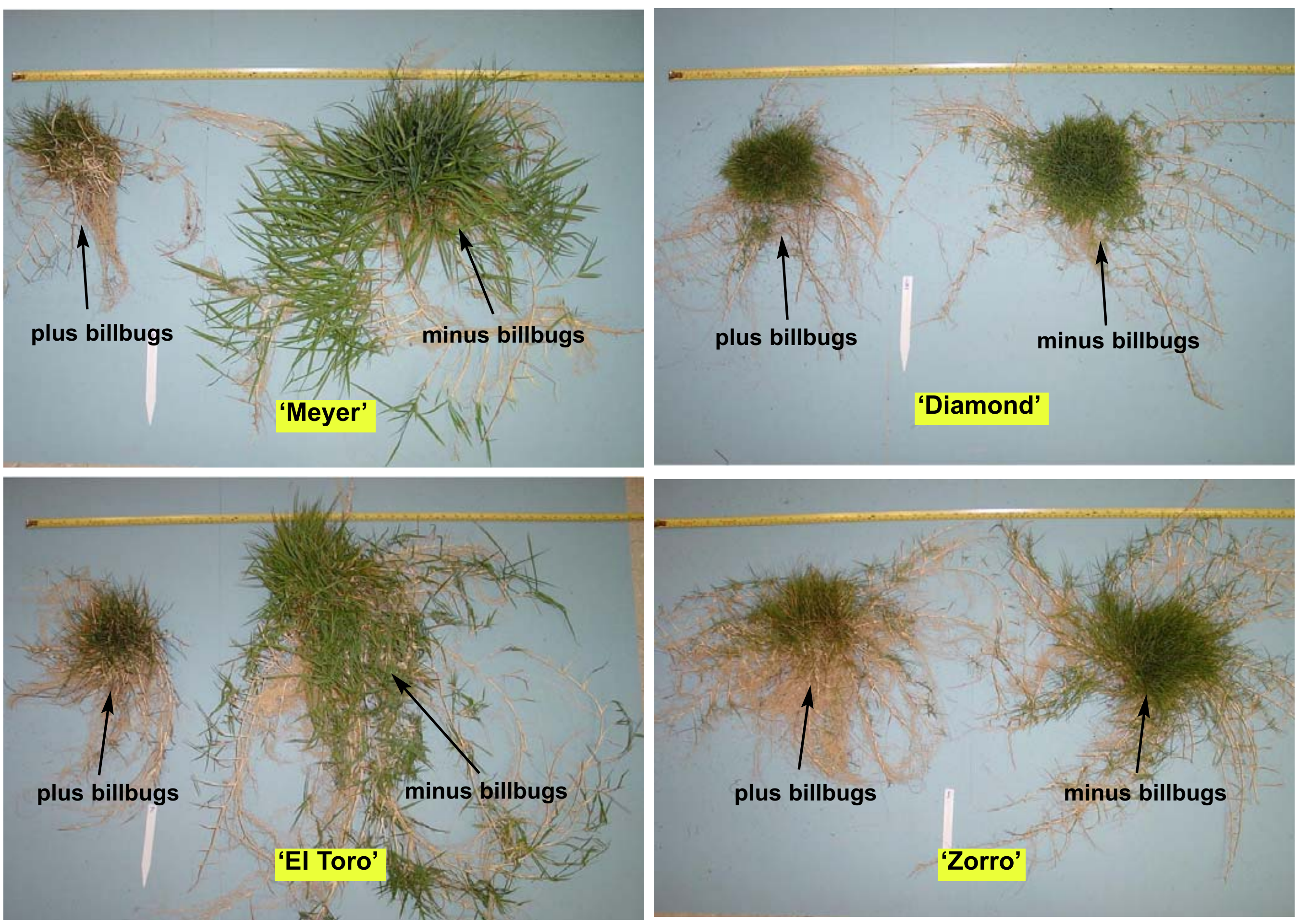

Figure 4. *Zoysia japonica* cultivars ('Meyer' and 'El Toro') were hunting billbug susceptible. They sustained 74 and 70% reduction in growth potential due to billbug larval damage. *Zoysia matrella* cultivars ('Diamond' and 'Zorro') were hunting billbug resistant. They sustained 26 and 36% reduction in growth potential, respectively, due to billbug larval damage

0.2 and 0.4 eggs per plant on 'Royal' and 'Cavalier', respectively, within one month of adult confinement on these grasses. In contrast, the adult hunting billbug had oviposited averages of 1.0, 1.8, 2.6, and 3.0 eggs per plant on 'Palisades', 'El Toro', 'Crowne', and 'Meyer', respectively, in greenhouse studies (7).

Two growth factors associated with *Z. matrella* may be responsible for its resistance to feeding damage by hunting billbug. First, rhizomes developing on this species appear to have much shorter internodes and almost every internode will develop a rooted shoot. Therefore, when the rhizome is severed by larval feeding, the isolated section of the rhizome continues to grow, independently of the parent plant, with only some loss of growth potential. This also results in a much denser root system with many more intertwined rhizomes.

A second mechanism of resistance is the ability of the cultivars of *Z. matrella* to exhibit apical dominance. When a rhizome is severed, it responds by developing new growth points – new stolons and rhizomes with roots and shoots. When a rhizome was severed in this study, new rhizomes were initiated just before the point of larval feeding damaged. It was common to observe three or four, and up to six lateral branches on the billbug infested plants of any of the *Z. matrella* cultivars. Among *Z. japonica* cultivars, it was common to see no lateral branching, occasionally only one, and rarely two lateral branches just before the point of injury. Both the higher number of shoot and root development and the ability of the *Z. matrella* cultivars to compensate for feeding damage to the rhizome through lateral branching is a form of tolerance.

Although there is a range of resistance among the four cultivars of *Z. matrella* and a range of susceptibility among the five *Z. japonica* cultivars, there appears to be a difference in response to this pest and its damage between the two species of zoysiagrass. One may speculate that other cultivars of *Z. matrella* may also carry levels of resistance to this primary pest. The study in Florida by Huang (7) supports this conclusion since several other cultivars of both zoysiagrass species were evaluated, and based upon density, quality, and egg deposition, these cultivars tend to also follow species separations for resistance versus susceptibility, as well.

It is interesting to note that several cultivars of *Z. matrella* have also exhibited good resistance to other insect and mite pests. 'Cavalier'has good levels of resistance to fall armyworm [*Spodoptera frugiperda* (J. E. Smith)], tropical sod webworm (*Herpetogramma phaeopteralis* Guenée), tawny mole cricket (*Scapteriscus vicinus* Scudder), Rhodesgrass mealybug, and differential grasshopper [*Melanoplus differentialis* (Thomas)]. 'Royal' has resistance to the zoysiagrass mite (*Eriophyes zoysiae* Baker, Kono & O'Neill), tropical sod webworm, and Rhodesgrass mealybug. 'Diamond' is resistant to fall armyworm, tawny mole cricket, and Rhodesgrass mealybug, while 'Zorro' is resistant to fall armyworm, tropical sod webworm, and Rhodesgrass mealybug (21). The host resistance responses of these various *Z. matrella* cultivars are summarized by Reinert et al. (22).

Acknowledgements

This study was supported in part by grants from the U.S. Golf Association's Turfgrass and Environmental Research Program and the O. J. Noer Research Foundation, Inc. Appreciation is extended to Dennis Hays and Joe McCoy for technical assistance.

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