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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 400 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***.

Editor

Jeff Nus, Ph.D.
1032 Rogers Place
Lawrence, KS 66049
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

USGA Turfgrass and Environmental Research Committee

Zoysiagrass Resistance to the Fall Armyworm

James A. Reinert and M. C. Engelke

SUMMARY

The fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] (Lepidoptera: Noctuidae) is a pest of most turfgrass species in the United States. Twelve cultivars and genotypes of zoysiagrass (*Zoysia* spp.) were evaluated for resistance to both neonate and 4-day-old fall armyworm larvae.

- Three cultivars, 'Cavalier', 'Emerald', and 'Belair', were the most resistant to feeding by neonate larvae with less than 5% of the larvae surviving beyond 4 days of feeding. After 10 days, 10% or less of the confined larvae was alive on these three cultivars along with 'Meyer', 'Korean Common', 'El Toro' and DALZ8501.
- When most of the same genotypes were exposed to 4-day-old larvae that developed on a susceptible zoysiagrass host, approximately 20% of the mortality was eliminated. Survivorship for 7-day-old larvae (after 3 days feeding) was 40% or greater on all genotypes except for 'Cavalier'. Only 'Cavalier', DALZ8501, and 'Korean Common' exceeded 85% mortality after 13 days of feeding.
- 'Meyer' produced 97.6% mortality of neonate larvae, but only 46.7% of the larvae that first fed on a susceptible host were killed.
- The 4-day-old larvae that fed on the resistant genotypes usually weighed less than half the weight of those fed on susceptible 'Palisades' and DALZ8516.

The use of turfgrass with abiotic and biotic stress resistance is economical and environmentally sound and should be a major component of pest management systems in all aspects of turf production and utilization (25). Development of turf cultivars with pest resistance (insect, mite, nematode, or disease) has been widely neglected in turfgrass breeding programs, compared to the emphasis on abiotic traits, including aesthetics. Also, the continued availability of effective insecticides and our reliance on them for pest management has further delayed the development of cultivars with resistance. The tendency to rely on insecticides has led to the development of insecticide resistance in certain turfgrass pests (4, 6, 8, 29). Also, the suspension of many other insecticides due to legislative actions brought on by contaminants in surface runoff and their impact on non-target organisms emphasizes the need for alternate management strategies including pest-resistant cultivars of turfgrasses (17).

Only a few turfgrass cultivars have been developed for their resistance to the major turfgrass pests. Reinert et al. provided a summary of resistance to insects and mites in turfgrasses (31). The limited information on host response to insects and mites in the turfgrass ecosystem suggest greater emphasis is warranted. Host-resistant cultivars, however, have been used successfully as a means of insect control. 'Floratam', a St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] cultivar, was released and widely planted throughout the southern United States for its resistance to the southern chinch bug (*Blissus insularis* Barber) (7, 27). Additionally, 'FLoraTeX' bermudagrass was released for its resistance to the bermudagrass mite (*Eriophyes cynodoniensis* Sayed) and several other abiotic stresses (7).

Fall armyworms move across a turfgrass planting consuming all of the green tissue as the go.

JAMES A. REINERT, Ph.D., Regents Fellow and Professor; and M.C. ENGELKE, Ph.D., AgriLife Research Fellow and Professor; Texas AgriLife Research & Extension Center, Texas A&M System, Dallas, TX.

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A cosmopolitan turfgrass pest, the fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] (Lepidoptera: Noctuidae), is known to feed on most turfgrass species (26, 30) and is damaging to over 50 species of plants, including maize, sorghum, rice, cotton, peanuts, many forage grasses, as well as most turfgrasses (12, 14, 34). Two morphologically indistinguishable host strains have been identified. One was identified from populations feeding on corn and sorghum (corn strain), and the other strain was identified from populations feeding on rice and bermudagrass (rice strain) (16, 18, 20). Both strains attain similar larval and pupal weights when fed bermudagrass or rice, but when reared on maize, the corn strain eats more leaf tissue and attains a larger weight (19).

Leuck et al. first identified resistance to fall armyworms in bermudagrass (13). Additional experiments confirmed the high level of antibiosis and nonpreference in 'Tifton 292' and other bermudagrass genotypes (11, 15, 21). Wiseman et al. (36) and Chang et al. (3) also reported a high level of resistance to fall armyworm in 'Common' centipedegrass, *Eremochloa ophiuroides* (Munro.) Hack. Reinert et al. reported varying levels of resistance in both cool- and warm-season turfgrasses (26, 30). Chang et al. reported zoysiagrass (variety unstated) to be an unsuitable host for fall armyworm because of nonpreference and antibiosis (3). All larvae confined on the tested zoysiagrass in one of their tests were dead within 48 hours, and the few surviving in the second test were much smaller than those developed on susceptible grass hosts.

The purpose of our research was to evaluate several genotypes and cultivars of zoysiagrass and to identify potential resistance to the fall armyworm, which is one of the primary pests of turfgrasses across the South and much of the eastern and midwestern U.S.

Two experiments were set up in the laboratory using 9-cm diameter x 20-mm deep plastic Petri dishes as larvae feeding chambers. Two 7.5-cm diameter filter paper discs saturated with water were placed in each dish before a small amount of fresh leaf tissue (approximately 3 g) of the respective zoysiagrass genotype was added.

Cultivar or Genotype	17-day-old wt (mg) [a]	Pupa wt (mg) [b]	Days to Pupation [c]	Days to adult emergence [d]
Cavalier	-- [e]	--	--	--
Emerald	--	--	--	--
Belair	--	--	--	--
Meyer	14.7 a *	181.6 bc	33.0 a	44.0 a
Korean Common	43.5 a	166.5 abc	30.0 abc	40.0 ab
El Toro	37.7 a	189.8 c	28.3 a-d	38.7 ab
DALZ8501	43.9 a	135.6 a	31.0 ab	41.0 ab
Diamond	193.5 b	165.8 abc	23.4 d	31.7 c
DALZ8508	22.5 a	153.8 ab	31.3 b	41.8 a
Crowne	75.5 a	165.1 abc	28.8 abc	39.6 ab
DALZ8516	125.3 ab	160.2 abc	25.8 cd	35.8 bc
Palisades	97.8 ab	165.3 abc	27.8 bcd	38.3 ab

[a] Mean weight of 17-day-old larvae after 17 days of feeding.

[b] Mean pupa weight taken within 1 day of pupation.

[c] Mean number of days from egg hatch to pupation.

[d] Mean number of days from egg hatch to adult emergence.

[e] No larvae surviving this growth stage for the cultivar.

*Means in a column followed by the same letter are not significantly different by Waller-Duncan k-ratio t test (P = 0.05).

Table 1. Resistance in zoysiagrass (expressed as reduced weight gain and extended development time) to feeding by neonate larvae of fall armyworms.

Materials and Methods

Zoysiagrass (9 cultivars and 3 genotypes) (Tables 1 and 2) were maintained in the greenhouse and grown in 18-cell trays (each cell measured 7.5 x 7.5 cm and 4 cm deep). Clippings from these plants were used to bioassay fall armyworm larvae in no-choice laboratory experiments. Two experiments were set up in the laboratory using 9-cm diameter x 20-mm deep plastic Petri dishes as larvae feeding chambers. Two 7.5-cm diameter filter paper discs saturated with water were placed in each dish before a small amount of fresh leaf tissue (approximately 3 g) of the respective zoysiagrass genotype was added. Water was added to the filter paper as needed to keep it saturated to maintain the grass cuttings.

Grass was added or replaced daily, or every other day, throughout the experiment so that turgid grass was always available to the developing larvae. For these experiments, eggs of the corn strain of fall armyworm were obtained from the lab colony maintained at the USDA-ARS-IBPMRL at Tifton, GA and reared through one generation on 'Laser' rough bluegrass (*Poa trivialis* L.), which served as a very susceptible host (26). Larvae were introduced into the feeding chambers as neonates within a few hours after hatching in Experiment 1. In Experiment 2, they were introduced as 4-day-old larvae that had first fed on fresh tissue of DALZ9516 zoysiagrass (a susceptible genotype). This grass serves as an excellent host, usually with near 100% survival.

For the first experiment, three neonate larvae were randomly selected after egg hatch and placed on each grass in the feeding chambers in each replicate (Table 1), and dishes were arranged in a randomized complete block design with 6

Cultivar or Genotype	17-day-old wt (mg) [a]	Pupa wt (mg) [b]	Days to Pupation [c]	Days to adult emergence [d]
Cavalier	-- [e]	--	--	--
DALZ8501	34.9 a *	151.2 ab	30.0 a	40.0 a
Korean Common	41.2 ab	136.1 ab	30.3 a	40.7 a
Belair	75.5 b	149.8 ab	27.7 ab	37.6 abc
El Toro	58.7 ab	171.1 b	29.0 a	39.0 a
Emerald	52.1 ab	137.5 ab	30.5 a	40.5 a
Meyer	55.4 ab	148.3 ab	28.9 a	38.8 ab
Palisades	131.6 c	122.7 a	25.2 b	35.3 bc

a Mean weight of 17-day-old larvae after 13 days of feeding.
b Mean pupa weight taken within 1 day of pupation.
c Mean number of days from egg hatch to pupation.
d Mean number of days from egg hatch to adult emergence.
e No larvae surviving this growth stage for the cultivar.
*Means in a column followed by the same letter are not significantly different by Waller-Duncan k-ratio t test (P = 0.05).

Table 2. Resistance in zoysiagrass (expressed as reduced weight gain and extended development time) to feeding by larvae of fall armyworm that had fed for 4 days on a susceptible host before transfer to the various grasses below.

replications on the laboratory bench. An additional 8 replications were established at a later date for a total of 14. Survivorship was evaluated when the larvae were 4, 10, 17, and 21 days old, at pupation, and at adult emergence.

Since fall armyworm egg masses are usually laid on some structure or debris adjacent to the turf area and the larvae migrate to the turf to feed, we also evaluated 4-day-old larvae on the test genotypes. For the second experiment, neonate larvae were allowed to develop for 4 days on leaf clipping of DALZ9516 zoysiagrass. When larvae were 4-days-old, three larvae were randomly selected and placed in the feeding chambers with the zoysiagrass genotypes listed in Table 2 in a randomized complete block design with 4 replications. An additional 5 replications were set up at a later date for a total of 9. Survivorship was measured when larvae were at 7-days-old (after 3 days of feeding) and when they were 10-days-old, 17-days-old, 21-days-old, at pupation, and at adult emergence. Also, all surviving larvae were weighed when they were 17-days-old, which was well before any pupation occurred. Days to pupation and adult emergence were recorded, and all pupa were weighed within 1 day of pupation.

Data were analyzed using analysis of variance procedures (ANOVA and PROC GLM) for randomized complete block design and the differences among treatment means were compared at the 5% level by Waller-Duncan k-ratio t test. Percent mortality data were transformed to arcsine (x + 0.001) before each ANOVA was performed, but the actual percentage of mortality is presented (32).

Results

Neonate Larvae

When neonate fall armyworm larvae were confined on the 12 genotypes of zoysiagrass in the no-choice Experiment 1, less than 5% of the larvae survived beyond the 4-day feeding period on ‘Cavalier’, ‘Emerald’, and ‘Belair’ (Figure 1). After 10 days, less than 10% of the confined larvae were alive on these 3 cultivars along with ‘Meyer’, ‘Korean Common’, ‘El Toro’, and

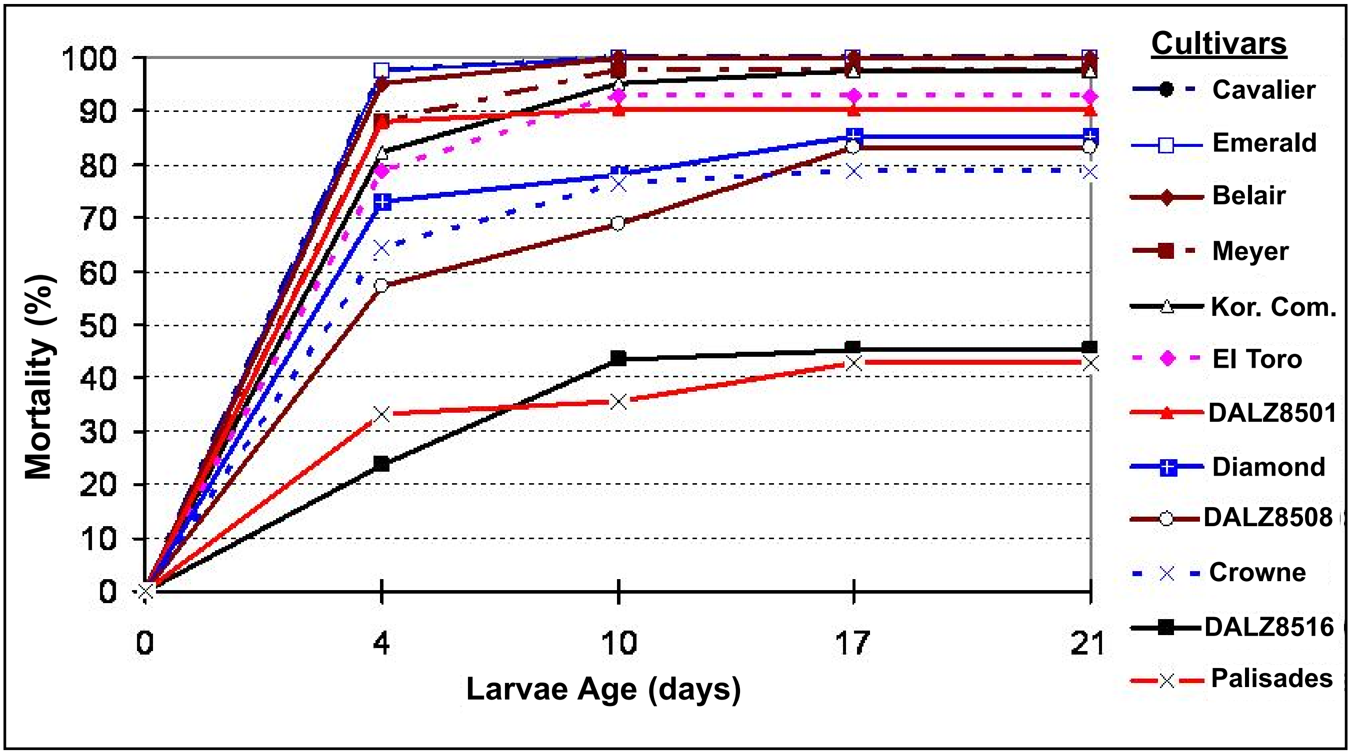

Figure 1. Mortality of fall armyworm larvae (started as neonate larvae) confined on zoysiagrasses in no-choice laboratory experiments (14 reps).

DALZ8501. Mortality on all genotypes appeared to plateau after 10 days of feeding, with less than 70% mortality on 'Diamond', 'Crowne', and DALZ8508. 'Palisades' and DALZ8516 were the most susceptible with 57% and 55% larval survival, respectively, through 21 days. Very little additional mortality was recorded at either pupation and at adult emergence.

For larva that survived, weights at 17 days were lowest on the most resistant genotypes ranging from 14.7 – 53.9 mg (Table 1). Since no larvae survived on 'Cavalier', 'Emerald', and 'Belair', no weights are available. If larvae were able to successfully pupate, only minor weight differences occurred among the genotypes, regardless of resistance level. The largest larvae were produced on the 2 most susceptible genotypes, DALZ8516 and 'Palisades'. Unexpectedly, the largest mean larval weight (193.5 mg) was produced on 'Diamond', even though only 15% of the larvae survived on it.

As expected, days to pupation and days to adult emergence were shortest, but not necessarily significant, for the most susceptible 'Palisades' and DALZ8516. Additionally, larvae developing on 'Diamond' pupated and became adults (23.4 days and 31.7 days, respectively) in a shorter time period than larvae on any of the other genotypes. In other experiments with the tropical sod webworm (*Herpetogramma phaeopteralis* Guenée), 'Diamond' also served as an excellent host and produced some of the largest larvae in the test (24).

<u>*Four-Day-Old Larvae*</u>

When larvae that fed for 4 days on the susceptible DALZ8516 were transferred to most of the same genotypes tested in Experiment 1, about 20% of the mortality expressed for neonate larvae was eliminated. Larval survivorship on several of the grasses that had exhibited high levels of antibiosis to neonate larvae was significantly increased when larvae were first allowed to feed on an acceptable host (Figure 2). Survivorship for 7-day-old larvae (after 3 days of feeding on the test grass) was 40% or greater on all genotypes except for 'Cavalier'. Only 'Cavalier', DALZ8501, and 'Korean Common' caused greater than 85% mortality after 13 days of feeding (17-day-old larvae). A second level of resistance was exhibited by 'Belair', 'El Toro', and 'Emerald' (63.0, 72.3 and 74.1%, respectively). 'Meyer', which had produced 97.6% mortality of neonate larvae, only killed 46.7% of the larvae that had first fed on a susceptible host for 4 days before they were exposed to it. As with the neonate larvae, little or no additional mortality was recorded at either pupation or at adult emergence for these genotypes.

The 4-day-old larval that fed on the resistant genotypes usually weighed less than half the weight of those fed on either of the susceptible genotypes, 'Palisades' or DALZ8516 (Table 2). Also, days to pupation and days to adult emergence were shortest on the two most susceptible genotypes (Table 2). In both experiments, no larvae were able to survive for 17 days on 'Cavalier'. The earlier reported resistance to neonate fall armyworm larvae on the undesignated cultivar of zoysiagrass (3) was probably conducted on either the cultivar 'Emerald' or 'Meyer', since these two cultivars were the most prominently used cultivars at that time. In the present experiment, 'Meyer' expressed a high level of resistance to neonate larvae, but if the larvae were allowed to first feed on a susceptible host to get through the critical early stages of development, they could readily survive on this zoysiagrass host.

Discussion

In these experiments, several genotypes of zoysiagrass have been identified with resistance to the fall armyworm. 'Cavalier' appears to be highly resistant because no larvae survived on it, regardless of the development stage of the larvae. The differences in resistance or susceptibility among the zoysiagrasses may be due to several factors. Leaf toughness and high levels of detergent fiber, lignin, and silica in leaf sheaths have been associated with insect resistance in many crops.

Plant cell walls strengthened by deposition of macromolecules such as cellulose, lignin, suberin, and cellose together with sclerenchyma fibers make a plant resistant to mechanical injury, including the tearing action of mandibles or the penetration of piercing-sucking mouthparts ((22, 33, 35). Detergent fiber, lignin, and silica of plant leaves have been associated with European corn

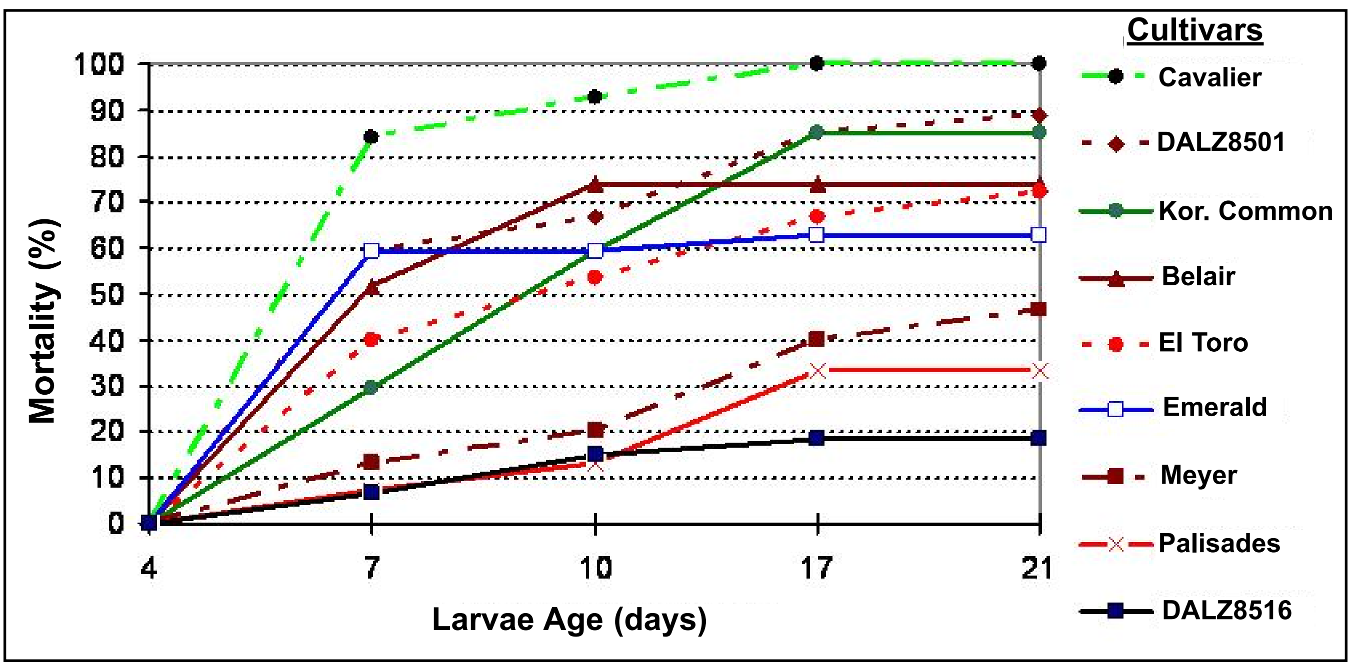

Figure 2. Mortality of fall armyworm larvae (started as 4-day-old larvae) confined on zoysiagrasses in no choice laboratory experiments (9 reps).

borer [*Ostrinia nubilalis* (Hübner)] resistance (5). High levels of indigestible fiber and silica may increase the bulk density of the insect diet to the point that they are unable to consume enough nutrients and water to survive on the host (1).

Studies with six zoysiagrass cultivars showed that lignin concentration and leaf tensile strength in 'Cavalier' and 'Emerald' were positively correlated with fall armyworm host resistance (9). Swain (35) also showed that plant material with high cell wall content is more difficult for insects to chew and could affect mortality, especially among younger chewing insects. Additionally, plants produce a wide array of naturally occurring chemicals that are believed to provide defense against herbivore and pathogen attack. In zoysiagrasses, two unidentified flavonoids (luteolin-glycosides) were consistently associated with fall armyworm mortality (9). An unidentified luteolin 3 exhibited an inverse relationship with mortality, while an unidentified luteolin 9 was positively correlated with mortality (10).

The extended larval development period required on the more resistant zoysiagrasses has an added benefit by allowing a longer time period for increased mortality due to the natural occurring predators, parasites, and pathogens of the fall armyworm in the turf system. During this period of time, larvae are much smaller and therefore eat far less plant material.

'Cavalier', the most resistant cultivar to fall armyworm is these studies, has exhibited high levels of resistance to several other chewing pests. 'Cavalier' has also exhibited resistance to the tropical sod webworm (23), hunting billbug (*Sphenophorus venatus* vestitus Chittenden) (24), the tawny mole cricket, *Scapteriscus vicinus* Scudder (2) and the differential grasshopper [*Melanoplus differentialis* (Thomas)] (28). Cultivars providing multiple pest resistance such as 'Cavalier' should be used extensively for new plantings and for landscape renovations as a major component of IPM programs in landscapes. The benefits in reduced pesticide needs should provide for long-term economic benefits and reduced environment impact from maintenance programs.

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