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RESISTANCE IN ZOYSIAGRASS (*ZOYSIA* SPP.) TO THE FALL ARMYWORM (*SPODOPTERA FRUGIPERDA*) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

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-d-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 survived beyond 4-d of feeding. After 10 d, 10% or less of the confined larvae were alive on these 3 cultivars along with 'Meyer', 'Korean Common', 'El Toro' and DALZ8501. When most of the same genotypes were exposed to 4-d-old larvae that had developed on a susceptible host, ca. 20% of the mortality was eliminated. Survivorship for 7-d-old larvae (after 3 d feeding) was 40% or greater on all genotypes except for Cavalier. Only Cavalier, DALZ8501, and Korean Common exceeded 85% mortality after 13 d of feeding. Meyer produced 97.6% mortality of neonate larvae, but only 46.7% of larvae that had first fed on a susceptible host. The 4-d-old larval that fed on the resistant genotypes usually weighed less than half the weight of those fed on susceptible Palisades and DALZ8516. Also, days-to-pupation and days-to-adult emergence were shortest on the 2 most susceptible genotypes. No fall armyworm larvae were able to survive more than 17 d on Cavalier. Several other cultivars were identified with resistance.

Key Words: turfgrass pests, host plant resistance, antibiosis, *Zoysia matrella*, *Zoysia japonica*

RESUMEN

El gusano soldado *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), es una plaga de la mayoría de las especies de céspedes en Estados Unidos. La resistencia de doce cultivares y genotipos de zoysiagrass (*Zoysia* spp.) fue evaluada con larvas recién emergidas y larvas de 4 días. Los cultivares 'Cavalier', 'Emerald', and 'Belair' mostraron mayor resistencia al daño por las larvas recién emergidas, con menos del 5% que lograron vivir mas de 4 días alimentándose en ellas. Después de 10 días, 10% o menos de las larvas confinadas permanecieron vivas en los cultivares mencionados anteriormente junto con 'Meyer', 'Korean Common', 'El Toro' y DALZ8501. Cuando la mayoría de los genotipos fueron expuestos a larvas de 4 días de vida, previamente alimentadas en un hospedero susceptible, el 20% de la mortalidad fue eliminada. La sobrevivencia de larvas después de 7 días de vida (después de tres días de alimentación en los materiales) fue mayor en todos los genotipos excepto en 'Cavalier'. Solo en 'Cavalier', DALZ8501, and 'Korean Common' la mortalidad de larvas excedió 85% después de 13 días de alimentación. Meyer presentó 97.6% de mortalidad de larvas recién emergidas, y solo 46.7% con larvas con 4 días alimentadas en hospedero susceptible. Las larvas de 4 días que fueron alimentadas con genotipos resistentes usualmente pesaron menos de la mitad que aquellas alimentadas en materiales susceptibles como 'Palisades' y DALZ8516. Además, el tiempo para alcanzar el estado de pupa y para la emergencia del adulto fue más corto en los dos genotipos más susceptibles. Ninguna larva de gusano soldado vivió más de 17 días en 'Cavalier'. Varios de los otros materiales fueron identificados con resistencia.

The authors provided the translation by Carlos Campos.

The use of host resistance turfgrass is economical and environmentally sound and should be a major component of pest management systems in all aspects of turf production and utilization (Reinert et al. 2004a). 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 (Cherry & Nagata 2007; Cowles et al. 2008; Georghiou & Sanito 1983; Reinert & Portier 1983). 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 host resistant turfgrasses (Nett et al. 2008).

Only a few turfgrass cultivars have been developed for their resistance to the major turfgrass pests. Reinert et al. (2004b) has provided a summary of resistance to insects and mites in turfgrasses. 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, cultivars was released and widely planted throughout the Southern United States for its resistance to the southern chinch bug (*Blissus insularis* Barber) (Reinert et al. 1995; Dudeck et al. 1986). Additionally, 'FLoraTeX™' bermudagrass was released for its resistance to the bermudagrass mite, *Eriophyes cynodoniensis* Sayed, and several other abiotic stresses (Dudeck et al. 1995).

A cosmopolitan turfgrass pest, the fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is known to feed on most turfgrass species (Reinert et al. 1997; 1999b) and is damaging to over 50 species of plants, including maize, sorghum, rice, cotton, peanuts, and many forage grasses (Luginbill 1928; Sparks 1979; Knipling 1980). 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) (Meagher & Gallo-Meagher 2003; Pashley 1986; Prowell 1998). 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 (Pashley et al. 1995).

Leuck et al. (1968) first identified resistance to FAW in bermudagrass. Additional experiments (Lynch et al. 1983; Quisenberry & Wilson 1985; Jamjanyu & Quisenberry 1988), confirmed the high level of antibiosis and non-preference in Tifton 292 and other bermudagrass genotypes. Wiseman et al. (1982) and Chang et al. (1985) reported a high level of resistance to FAW in 'Common' Centipedegrass, *Eremochloa ophiuroides* (Munro.) Hack, and Reinert et al. (1997, 1999c) reported varying levels of resistance in both C3 and C4 (cool- and warm-season) turfgrasses. Chang et al. (1985) reported zoysiagrass (variety unstated) to be an unsuitable host for FAW because of non-preference and antibiosis. All larvae confined on the tested zoysiagrass in one of their tests were dead within 48 h, 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 FAW, which is one of the primary pests of turfgrasses across the Southern Region and much of the Eastern and Midwestern U.S.

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 × 7.5 cm and 4 mm 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 with 9-cm diam × 20-mm deep plastic petri dishes as larvae feeding chambers. Two 7.5-cm diam filter paper discs saturated with water were placed in each dish before a small amount of fresh leaf tissue (ca. 3 g) of the respective 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 fresh grass was always available to the developing larvae. For these experiments, eggs of the corn strain of FAW were obtained from the lab colony maintained at the USDA-ARS-IBPMRL at Tifton, GA and reared through 1 generation on >Laser = rough bluegrass (*Poa trivialis* L.), which serves as a very susceptible host (Reinert et al. 1997).

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-d-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, 3 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 replicates on the laboratory bench. An additional 8 replicates were established at a later date for a total of 14 replicates. Survivorship was evaluated when the larvae were 4-, 10-, 17-, and 21-d-old, at pupation and at adult emergence.

Since FAW egg masses are usually laid on some structure or debris adjacent to the turf area and the larvae then migrate to the turf setting to feed, we evaluated 4-d-old larvae on the test genotypes. For the second experiment, neonate larvae were allowed to develop for 4 d on leaf clipping of DALZ9516 zoysiagrass. When larvae were 4-d-old, 3 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 replicates. An addi-

TABLE 1. RESISTANCE IN ZOYSIAGRASS (EXPRESSED AS REDUCED WEIGHT GAIN AND EXTENDED DEVELOPMENT TIME) TO FEEDING BY NEONATE LARVAE OF FALL ARMYWORM.

Cultivar or Genotype	17-d-old wt (mg)[a]	Pupa wt (mg)[b]	Days to Pupation[c]	Days to a 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-d-old larvae after 17 d of feeding.
[b]Mean pupa weight taken within 1 d of pupation.
[c]Mean number of d from egg hatch to pupation.
[d]Mean number of d 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 ($k = 100$) ($P = 0.05$).

tional 5 replicates were set up at a later date for a total of 9 replicates. Larval survivorship was measured at 7-d-old or after just 3 d of feeding and as 10-, 17-, and 21-d-old larvae, at pupation and at adult emergence. All surviving larvae were weighed when 17-d-old, which was well before any pupation occurred. Days to pupation and adult emergence were calculated and all pupa were weighed within 1 d of pupation.

Data Analysis and Statistics

Data were analyzed by 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 (SAS Institute 2008).

RESULTS

Neonate Larvae

When neonate FAW larvae were confined on the 12 genotypes of zoysiagrass in the no-choice Experiment 1, ≤5% of the larvae survived beyond

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 D ON A SUSCEPTIBLE HOST BEFORE TRANSFER TO THE VARIOUS GRASSES BELOW.

Cultivar or Genotype	17-d-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-d-old larvae after 13 d of feeding.
[b]Mean pupa weight taken within 1 d of pupation.
[c]Mean number of d from egg hatch to pupation.
[d]Mean number of d 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 ($k = 100$) ($P = 0.05$).

the 4-d-feeding period on 'Cavalier', 'Emerald', and 'Belair' (Fig. 1). After 10 d, only ≤ 10% of the confined larvae were alive on these 3 cultivars along with 'Meyer', 'Korean Common', 'El Toro', and DALZ8501. Mortality on all genotypes appeared to plateau after 10 d of feeding, with ≥70% mortality on 'Diamond', 'Crowne', and DALZ8508. 'Palisades' and DALZ8516 were the most susceptible with 57% and 55% larval survival, respectively, through 21 d. Very little additional mortality was recorded at either pupation and at adult emergence.

For larva that survived, weights at 17 d 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 a larva was 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 (23.4 d) and became adults (31.7 d) 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 (Reinert & Engelke 2001).

Four-Day-Old Larvae

When larvae that had fed for 4 d 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 (Fig. 2). 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. Survivorship for 7-d-old larvae (after 3 d 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 d of feeding (17-d-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 d before they were exposed to it. As with the neonate larvae, little or no additional mortality was recorded at either pupation and at adult emergence for these *Zoysia* genotypes.

The 4-d-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 2 most susceptible genotypes. In both experiments, no larvae were able to survive for 17 d on Cavalier. The earlier reported resistance to neonate FAW larvae on the undesignated cultivar of zoysiagrass (Chang et al. 1985) was probably conducted on either the cultivar Emerald or Meyer, since these 2 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 (53.3%) on this host.

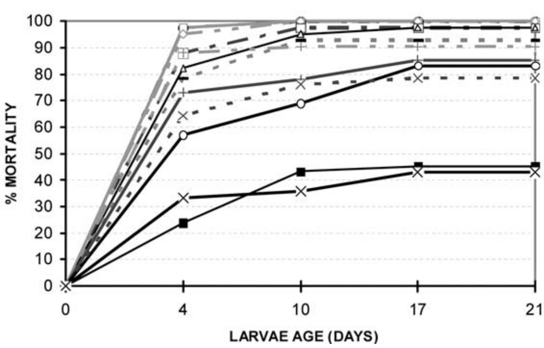

Fig. 1. Mortality of fall armyworm larvae (started as neonate larvae) confined on zoysiagrasses in no-choice laboratory experiments (14 replicates). Cultivars: —• ▪ Cavalier, —□— Emerald, —◊ ▪ Belair, —□ ▪ Meyer, —∆— K Com, ▪— ▪ El Toro, —+▪ DALZ8501, —+— Diamond, —○— DALZ8508, ▪ ▪X▪ ▪ Crowne, — ■ — DALZ8516, —X— Palisades.

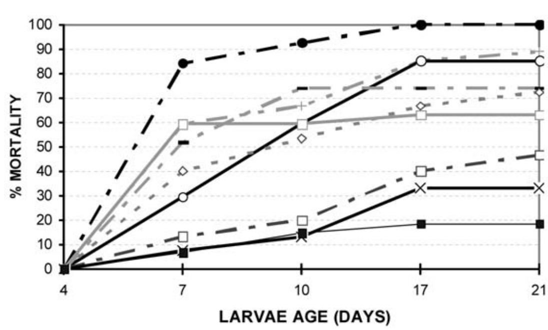

Fig. 2. Mortality of fall armyworm larvae (started as 4-d-old larvae) confined on zoysiagrasses in no-choice laboratory experiments (9 replicates). Cultivars: —• ▪ Cavalier, —+▪ DALZ8501, —○— K Com, — ▪ Belair, ▪ ▪◊▪ ▪ El Toro, —□—Emerald, — □ ▪ Meyer, —X— Palisades, —■—DALZ8516.

Discussion

In these experiments, several genotypes of zoysiagrass have been identified with resistance to the FAW and 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 sclerenchymatous fibers make a plant resistant to mechanical injury as well as to the tearing action of mandibles or the penetration of piercing-sucking mouthparts (Schoonhoven et al. 2005). Detergent fiber, lignin, and silica of plant leaves have been associated with European corn borer, *Ostrinia nubilalis* (Hübner), resistance (Coors 1987). 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 (Bernays 1986).

Studies with 6 zoysiagrass cultivars showed that lignin concentration and leaf tensile strength in Cavalier and Emerald were positively correlated with FAW host resistance (Hale et al. 2009). Swain (1979) 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. Also, lignin has been associated with leaf toughness and resistance to the tearing action of mandibles (Raupp 1985; Swain 1979). Additionally, plants produce a wide array of naturally occurring chemicals that are believed to provide defense against herbivore and pathogen attack. In zoysiagrasses, 2 unidentified flavonoids (luteolin-glycosides) were consistently associated with fall armyworm mortality (Hale et al. 2009). An unidentified luteolin-3 had an inverse relationship with mortality, while an unidentified luteolin-9 was positively correlated with mortality (Hale et al. 2008).

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 FAW 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 FAW is these studies, has exhibited high levels of resistance to several other chewing pests. Cavalier has exhibited resistance to the tropical sod webworm (Reinert & Engelke 2001), hunting billbug, *Sphenophorus venatus vestitus* Chittenden, (Reinert & Engelke, unpublished manuscript), the tawny mole cricket, *Scapteriscus vicinus* Scudder (Braman et al. 1994), and the differential grasshopper, *Melanoplus differentialis* (Thomas), (Reinert et al. 1999a). 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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