Foliar Resistance to Fall Armyworm in Corn Germplasm Lines that Confer Resistance to Root- and Ear-Feeding Insects *

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FOLIAR RESISTANCE TO FALL ARMYWORM IN CORN GERMPLASM LINES THAT CONFER RESISTANCE TO ROOT- AND EAR-FEEDING INSECTS*

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ABSTRACT

A holistic approach to developing new corn germplasm that confers multiple insect resistance in various plant tissues at different growth stages was examined. Eight corn germplasm lines were examined for their foliar resistance to fall armyworm [*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)] and natural enemy attraction at V6-V8 (or 6-8 leaf) stages in 2008 and 2009. Four corn germplasm lines with known levels of resistance to root- and ear-feeding insects [‘CRW3(S1)C6’, ‘B37*H84’, ‘SIM6’ and ‘EPM6’], and four germplasm entries with different levels of *S. frugiperda* resistance (‘Mp708’, ‘Ab24E’, ‘FAW7061’ and ‘FAW7111’) were evaluated in the study. All plants were manually infested with 15-20 neonate *S. frugiperda* larvae per plant, and injury was rated 7 and 14 d after infestation. Based on cluster analysis of *S. frugiperda* injury rating and predator survey data, ‘Mp708’ and ‘FAW7061’ were the most resistant, whereas ‘Ab24E’ and ‘EPM6’ were the most susceptible to fall armyworm feeding. The western corn rootworm-resistant ‘CRW3(S1)C6’ showed resistance to *S. frugiperda* feeding. Surveys for the diversity and abundance of predators of *S. frugiperda* in each experimental plot were also conducted 7 d after infestation. ‘CRW3(S1)C6’ and ‘Ab24E’ had the highest and lowest predator abundance, respectively. However, there was no direct correlation between *S. frugiperda* injury ratings and predator abundance. The current study demonstrated the feasibility of developing foliage-, root-, and ear-feeding insect-resistant germplasm covering multiple corn growth stages. In addition, the possibility of utilizing plant volatiles to attract predators, and reduce pest populations and crop damage is discussed.

Key Words: field screening; multiple insect resistance; foliage-, root-, and ear-feeding insect resistance; predator attraction

RESUMEN

Se examinó una aproximación holística para desarrollar nuevo germoplasma de maíz con resistencia múltiple a insectos en varios tejidos y estados de desarrollo de las plantas. Ocho líneas de germoplasma fueron evaluadas para determinar su resistencia foliar a *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) y su atracción de enemigos naturales en las etapas V6-V8 (hojas 6-8) durante los años 2008 y 2009. Se evaluaron cuatro líneas de germoplasma con niveles conocidos de resistencia a insectos que se alimentan de raíces y mazorcas [‘CRW3(S1)C6’, ‘B37*H84’, ‘SIM6’ y ‘EPM6’], y cuatro con diferentes niveles de resistencia a *S. frugiperda* (‘Mp708’, ‘Ab24E’, ‘FAW7061’ y ‘FAW7111’). Las plantas fueron infestadas manualmente con 15-20 larvas neonatas de *S. frugiperda* cada una, y el nivel de daño evaluado 7 y 14 días despúes de la infestación. Basándose en un análisis tipo ‘cluster’ de ‘Mp708’ y ‘FAW7061’ fueron las más resistentes, mientras que ‘Ab24E’ y ‘EPM6’ fueron las más susceptibles a alimentación por parte de *S. frugiperda*. La línea resistente al barrenador de raíz ‘CRW3(S1)C6’ mostró resistencia a alimentación por parte de *S. frugiperda*. Los muestreos de diversidad y abundancia de depredadores de *S. frugiperda* en cada lote experimental también se realizaron 7 días después de la infestación. 'CRW3(S1)C6' y 'Ab24E' tuvieron la más alta y más baja abundancia de depredadores, respectivamente. Sin embargo, no hubo una correlación entre el daño registrado y la abundancia de depredadores. Este estudio demostró que es posible desarrollar germoplasma de maíz resistente al ataque de insectos plaga de follaje, raíz y mazorcas, cubriendo múltiples estadios de crecimiento. Adicionalmente, se discute la posibilidad de utilizar volátiles de plantas para atraer depredadores y reducir poblaciones de plagas y el daño al cultivo.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is an important crop pest in the U.S. where it causes significant economic loss on numerous crops annually (Nagoshi 2009). Across the southeastern states, *S. frugiperda* is an important pest of corn and, in fact, is the most important whorl-feeding insect pest, especially in late-planted corn (Davis et al. 1996). Resistance to *S. frugiperda* has been studied extensively, and a series of corn germplasm lines conferring *S. frugiperda* resistance have been developed at Mississippi State, MS (Brooks et al. 2007), and Tifton, GA (Wiseman et al. 1996) for the southern states.

Recent research efforts have been devoted to identifying and developing corn germplasm that confers resistance to multiple insect pests at various crop growth stages. The possibility of developing resistance to multiple whorl- and ear-feeding insect species has been examined for major pests in the Midwest (Wilson et al. 1995a; Abel et al. 2000a; Abel et al. 2000b). Wilson et al. (1995a) evaluated 11 maize accessions from Peru that were previously found to be resistant to leaf feeding by first-generation European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). That field evaluation identified new genetic resources for multiple insect resistance, including resistance to stalk boring by second-generation *O. nubilalis*, the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), and the southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae); foliar feeding by *S. frugiperda*; root feeding by the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae); and ear-feeding by the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). Abel et al. (2000a) examined another 15 experimental maize lines against 4 lepidopteran pests (*H. zea*, *S. frugiperda*, *D. grandiosella*, and D. Saccharalis) in the midwest and southern states. The 15 lines were developed from crosses between the Peruvian maize lines and the U.S. midwest corn belt adapted inbred lines. Four inbred lines (i.e., '100-R-3', '116-B-10', '81-9-B', and '107-8-7') were identified as new resources for developing breeding populations against each of the 4 insect pests (Abel et al. 2000a). Abel et al. (2000b) also evaluated those same 15 experimental maize lines against *O. nubilalis*, and *D. virgifera virgifera* in the midwest region. All 15 experimental lines showed resistance to leaf feeding by *O. nubilalis*, and 11 of them showed resistance to leaf sheath and collar feeding by *O. nubilalis*. None of the lines showed any antixenosis (or non-preference) with respect to *O. nubilalis* oviposition or *D. virgifera virgifera* root feeding. Although DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is well documented to confer resistance to foliar feeding by first generation *O. nubilalis*, all of these lines had low levels of DIMBOA, suggesting that this compound might not be directly involved in resistance to *O. nubilalis* in these lines.

High levels of corn silk maysin have been considered an important phenotypic trait that confers resistance to ear-feeding corn earworm (Wilson et al. 1995b; Widstrom & Snook 2001; Ni et al. 2008). After examining 94 CIMMYT corn inbred lines with varying levels of silk maysin, Ni et al. (2008) determined that 10 of them conferred resistance to multiple ear-feeding insects in the southeastern Coastal Plain region. In addition, Ni et al. (2007) examined another 10 corn inbreds and 10 experimental hybrids, and identified 2 inbreds and 2 hybrids showing resistance to multiple ear-feeding insects, including *H. zea*, maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), brown stink bug [*Euschistus servus* (Say)], and southern green stink bug [*Nezara viridula* (L.)] (Heteroptera: Pentatomidae).

In general, limited progress has been made on developing corn inbred lines showing resistance to both whorl- and ear-colonizing insects and diseases. After evaluating corn germplasm resistance to multiple ear-feeding insects (Ni et al. 2007, 2008), we expanded recent evaluations to including multiple insect resistance/susceptibility over varying corn plant tissues (root, leaf, and ear) throughout different growth stages (i.e., vegetative versus reproductive growth).

The objective of the present study was to determine whether corn germplasm lines resistant to ear- and root-feeding insects would confer resistance to foliar feeding by *S. frugiperda* at vegetative growth stages. Resistance was assessed by visual ratings of *S. frugiperda* feeding injury and natural enemy profiles at the whorl stage in 8 selected corn inbred lines that possessed known levels of resistance/susceptibility to root-feeding *D. virgifera virgifera* and ear-feeding *H. zea*.

Materials and Methods

Plants and Insects

The 8 corn germplasm lines known to be resistant and/or susceptible to at least 1 of 3 different insects (*D. virgifera virgifera*, *H. zea*, and *S. frugiperda*) are listed in Table 1. The 2 newly-selected inbred lines 'FAW7061' and 'FAW7111' were derived from the 'GT-FAWCC(C5)' population (Wiseman et al. 1996) after being self-pollinated for 6 generations. *Spodoptera frugiperda* neonate larvae used in this study in 2008 were from a laboratory colony maintained in the insectary at the Crop Protection and Management Unit, USDA-ARS, Tifton, Georgia. In 2009, the neonate larvae used for manual infestation were from the Corn Host Plant Resistance Research Unit, USDA-ARS, Mississippi State, Mississippi. The fall armyworm colonies at both locations originated from field-collected insects, and have been maintained on a pinto bean diet (Lynch et al. 1989) for over 10 yr with frequent fusion with field-collected insects.

Manual *S. frugiperda* Infestation and Injury Rating

The experimental plants used in this field study were infested individually with 15-20 *S. frugiperda* neonate larvae when the plants were at the 6-leaf (or V6) stage using the protocol previously described by Davis et al. (1996). The levels of insect injury were rated using the mean injury level of all 15-20 plants per experimental plot (5 × 1 m^2) 7 and 14 d after infestation using a scale of 1-9 (see Davis et al. 1992 and Smith et al. 1994). Briefly, 1 = no damage or few pinholes; 2 = few short holes (also known as shot holes) on several leaves; 3 = short holes on several leaves; 4 = several leaves with short holes and a few long lesions; 5 = several holes with long lesions; 6 = several leaves with lesions < 2.5 cm; 7 = long lesions common on one half of the leaves; 8 = long lesions common on one half to two thirds of leaves; and 9 = most leaves with long lesions. The *S. frugiperda* injury rating was conducted without information about germplasm entry assigned for an experimental plot to avoid biased ratings for any of the germplasm entries. The insect injury ratings were recorded per experimental plot based on overall visual assessment of *S. frugiperda* injury under the field conditions.

Predator Survey Protocols

Both predator types and/or species from each experimental plot were recorded 7 d after *S. frugiperda* infestation, because predators might be differentially attracted to the corn germplasm lines and they were abundant in the corn fields when the plants were at 6-9 leaf stages (V6-V9). Data were collected by careful field counts of all predators on every plant per experimental plot (5 × 1 m^2) with as little disturbance to the predator activities and plants as possible. Field counts of predators have been described as the best sampling method of choice for examining predators in sweet corn fields (Musser at al. 2004). No parasitoids were observed during the visual surveys in both years. The predator survey was conducted within 24 h after the 7 d *S. frugiperda* injury rating in both years. The plant parts examined for predators included whorls, leaf blades, leaf sheaths and stalks to detect accessible fast-moving predators (like *Orius insidiosis* and *Geocoris* spp.) during the survey. The survey of predators in all experimental plots was conducted diurnally between 1000 h and 1700 h EDT when the predators were active and the plants were without dew. As previously described for the *S. frugiperda* injury rating, the predator survey was also conducted without knowledge of the germplasm entry to avoid biasing observations pertaining to a given experimental plot or germplasm entry.

Experimental Design and Data Analysis

The experiment utilized a randomized complete block design with the 8 corn germplasm lines as treatments, and four replications as the blocking factor to minimize the influence of soil and other environmental factors on plant development. The 8 corn germplasm entries were planted adjacent to one another without buffer

Table 1. Eight germplasm lines used in the evaluation of *S. frugiperda* resistance.

Germplasm lines	Traits	References
Ab24E	Fall armyworm susceptible control	Brooks et al. (2007)
B37*H84	Rootworm susceptible	Hibbard et al. (2007)
CRW3(S1)C6	Rootworm resistant	Hibbard et al. (2007)
EPM6	Corn earworm resistant	Widstrom & Snook (2001)
FAW7061	Derived from fall armyworm resistant GT-FAWCC(C5)	Wiseman et al. (1996)
AW7111	Derived from fall armyworm resistant GT-FAWCC(C5)	Wiseman et al. (1996)
Mp708	Fall armyworm resistant control	Brooks et al. (2007)
SIM6	Corn earworm resistant	Widstrom & Snook (2001)

rows (zones) to determine their attraction to predators where choices were provided, and to avoid dilution of predator attraction by planting extra rows of corn plants as buffer areas. Although there were no buffer areas between experimental plots (5 ×1 m^2 with 15-20 plants), the edge of the field was surrounded by a border row of the commercial corn hybrid 'DK6410' to reduce edge effect on natural infestation of fall armyworm and predator distribution in the experimental plots. Each experiment conducted in 2008 and 2009 was considered a separate trial. The data on feeding injury by *S. frugiperda* were analyzed using analysis of variance (PROC MIXED procedure) followed by Fisher's Protected LSD test ($\alpha = 0.05$) (SAS Institute 2003). The data for predator diversity and abundance were analyzed using cluster analysis (PROC CLUSTER procedure) (SAS Institute 2003). Briefly, cluster analysis is a generic term for many techniques that have the common goal to determine whether a multivariate data set contains distinct groups or clusters, and if so, finding which of the observations belong in the same cluster (Der & Everitt 2009). The correlation between *S. frugiperda* injury ratings and predator profiles was also assessed using the PROC CORR procedure (SAS Institute 2003).

Results

Fall Armyworm Injury Ratings

Leaf injury ratings were significantly different among the 8 germplasm lines 7 d and 14 d after the infestation (Table 2). The 7 d ratings were not different between 2008 and 2009, but the 14 d injury ratings varied significantly between the 2 years. The germplasm entry × year interaction significantly affected both 7 d and 14 d injury ratings (Table 2), which reflected the variation caused by weather conditions from year to year for such field studies. Because the entry × year interaction was significant, the 7 d and 14 d rating data from 2008 and 2009 were presented separately (Figs. 1A to 1D). When compared to the *S. frugiperda* injury ratings of the susceptible control 'Ab24E', the resistant control (Mp708') showed lower ratings in all evaluations, except at 7 d post-infestation in 2009 (Fig. 1C).

While *S. frugiperda* injury ratings varied between 2008 and 2009 at the 7 d post-infestation evaluations (Figs. 1A and 1C), the 14 d post infestation ratings were relatively consistent (Figs. 1B and 1D). Four germplasm lines, i.e., 'CRW3(S1)C6', 'FAW7061', 'FAW7111', and 'Mp708' had significantly lower *S. frugiperda* injury ratings than the susceptible control, 'Ab24E'. In particular, the lower *S. frugiperda* injury ratings were recorded on 'CRW3(S1)C6', 'FAW7061', and 'FAW7111' 14 d post-infestation than the susceptible control, 'Ab24E' in 2008 (Fig. 1B), but not at the 7 d post-infestation assessment in 2008 (Fig. 1A). Also, *S. frugiperda* injury ratings on 'CRW3(S1)C6', 'FAW7111', and 'Mp708' at the 14 d post-infestation evaluation in 2009 (Fig. 1D) were lower than the 7 d post-infestation evaluation (Fig. 1C). The reduced *S. frugiperda* injury ratings on the 14 d evaluation compared to the 7

TABLE 2. ANALYSIS OF VARIANCE TABLE OF *S. FRUGIPERDA* INJURY RATINGS AND PREDATOR ABUNDANCE ON EIGHT CORN GERMPLASM LINES.*

	Germplasm	Year	Germplasm*year interaction
FAW7d	$F = 2.56$; df = 7, 44; $P = 0.03$	$F = 0.04$; df = 1, 44; $P = 0.85$	$F = 2.56$; df = 7, 44; $P = 0.05$
FAW14d	$F = 10.85$; df = 7, 44; $P = 0.0001$	$F = 93.82$; df = 1, 44; $P = 0.0001$	$F = 3.26$; df = 7, 44; $P = 0.007$
Hippo	$F = 0.49$; df = 7, 44; $P = 0.83$	$F = 3.97$; df = 1, 44; $P = 0.05$	$F = 0.35$; df = 7, 44; $P = 0.92$
Cmac	$F = 1.82$; df = 7, 44; $P = 0.11$	$F = 26.26$; df = 1, 44; $P = 0.0001$	$F = 0.83$; df = 7, 44; $P = 0.57$
C7	$F = 2.56$; df = 7, 44; $P = 0.03$	$F = 2.86$; df = 1, 44; $P = 0.1$	$F = 2.56$; df = 7, 44; $P = 0.03$
Harmonia	$F = 1.09$; df = 7, 44; $P = 0.38$	$F = 5.58$; df = 1, 44; $P = 0.02$	$F = 1.00$; df = 7, 44; $P = 0.45$
Geop	$F = 1.14$; df = 7, 44; $P = 0.35$	$F = 17.43$; df = 1, 44; $P = 0.0001$	$F = 1.25$; df = 7, 44; $P = 0.30$
Scymnus	$F = 0.83$; df = 7, 44; $P = 0.57$	$F = 1.90$; df = 1, 44; $P = 0.17$	$F = 0.83$; df = 7, 44; $P = 0.57$
Orius	$F = 0.48$; df = 7, 44; $P = 0.85$	$F =2.00$; df = 1, 44; $P = 0.16$	$F = 1.12$; df = 7, 44; $P = 0.37$
Nabidae	$F = 0.87$; df = 7, 44; $P = 0.54$	$F =1.71$; df = 1, 44; $P = 0.20$	$F = 0.87$; df = 7, 44; $P = 0.54$
Hdbeetle	$F = 1.54$; df = 7, 44; $P = 0.18$	$F = 0.52$; df = 1, 44; $P = 0.48$	$F = 2.39$; df = 7, 44; $P = 0.04$
Earwigs	$F = 2.72$; df = 7, 44; $P = 0.02$	$F = 0.31$; df = 1, 44; $P = 0.58$	$F = 0.68$; df = 7, 44; $P = 0.69$
Pdtotal	$F = 1.49$; df = 7, 44; $P = 0.19$	$F = 19.83$; df = 1, 44; $P = 0.0001$	$F = 0.63$; df = 7, 44; $P = 0.73$

*Natural enemy names are abbreviated as follows: Hippo = Hippodamia convergens (Coleoptera: Coccinellidae); Cmac = the pink spotted lady beetle, Coleomegilla maculata (Coleoptera: Coccinellidae); C7 = the seven-spotted lade beetle, Coccinella septempunctata (Coleoptera: Coccinellidae); Harmonia = the multicolored Asian lady beetle, Harmonia axyridis (Coleoptera: Coccinellidae); Geo = big-eyed bugs, Geocoris spp. (Heteroptera: Geocoridae); Scymnus = Scymnus spp. (Coleoptera: Coccinellidae); Nabid = damsel bugs, Nabis spp. (Heteroptera: Nabidae); Orius = the insidious flower bug, Orius insidiosus (Heteroptera: Anthocoridae); hdbeetle = hooded (or flower) beetles, Notoxus spp. (Coleoptera: Anthicidae); earwigs = Dermapteran taxa identified as Labidura riparia (Labiduridae), and Doru taeniatum (Forficulidae); and Pdtotal = total number of predators.

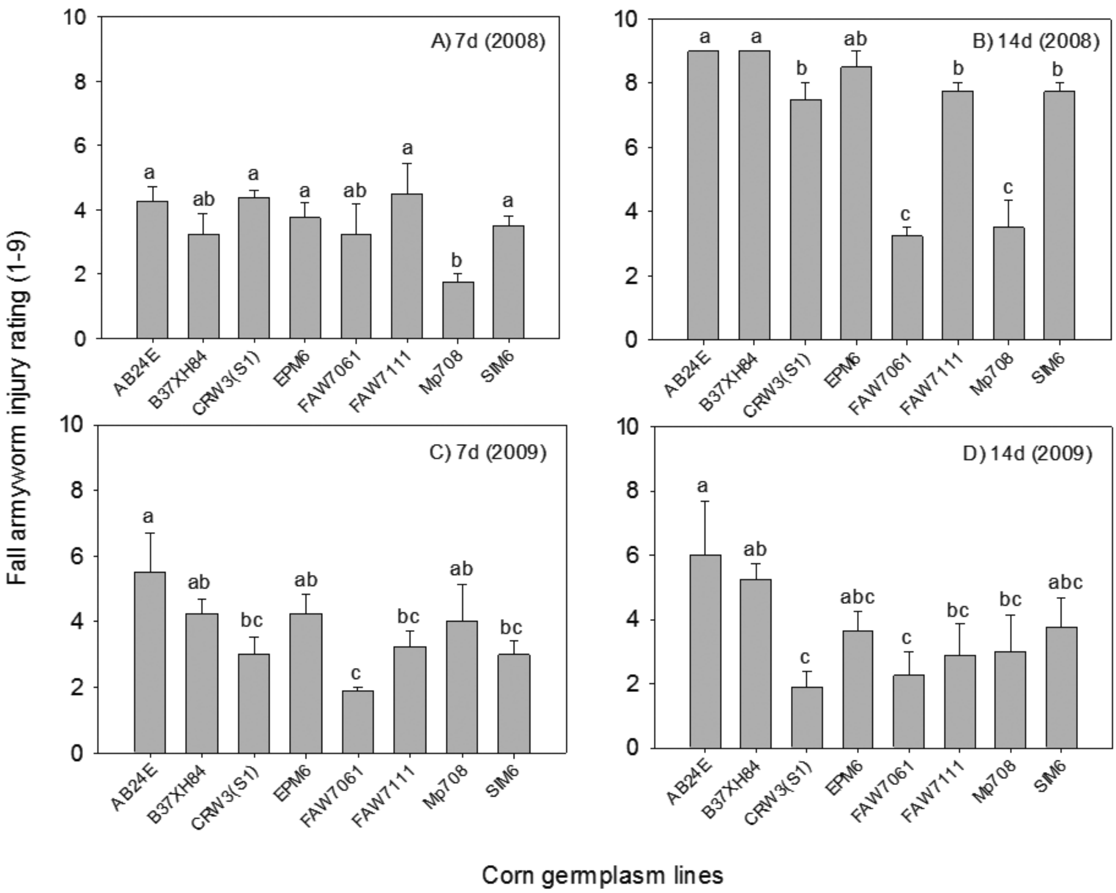

Fig. 1. Mean injury ratings after *S. frugiperda* infestation on 8 corn germplasm lines in the field seasons of 2008 and 2009. A) 7 d post-infestation injury rating data in 2008; B) 14 d post-infestation injury rating data in 2008; C) 7 d post-infestation injury rating data in 2009; D) 14 d post-infestation injury rating data in 2009. Bar graphs (mean ± SEM) with the same letters were not different ($P > 0.05$, Fisher's Protected LSD). Insect resistance and susceptibility of these 8 germplasm lines are described in Table 1.

d evaluation may have been related to predation of the *S. frugiperda* larvae by predators in the experimental plots. Thus, the predator diversity and abundance were further examined in these experimental plots 7d after the infestation.

Predator Survey

Ten predator species were recorded in the experimental plots (Table 3). The 5 lady beetle species (Coleoptera: Coccinellidae) included the convergent lady beetle, *Hippodamia convergens* Guérin-Meneville, the pink spotted lady beetle, *Coleomegilla maculata* (De Geer), the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), the seven-spotted lady beetle, *Coccinella septempunctata* L., and a *Scymnus* sp. In addition, hooded (or flower) beetles, *Notoxus* spp. (Coleoptera: Anthicidae) was also recorded in the experimental plots. Earwigs (Dermaptera) were not differentiated by species when recorded during the surveys, but later identified as *Labidura riparia* (Pallas) (Labiduridae) and *Doru taeniatum* (Dohrn) (Forficulidae). Three taxa of heteropteran predators were also recorded in the experimental plots including the insidious flower bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae), the big-eyed bug, *Geocoris* spp. (Heteroptera: Geocoridae), and the damsel bugs, *Nabis* spp. (Heteroptera: Nabidae).

Coleomegilla maculata was the most abundant predator observed, whereas *C. septempunctata* was the least abundant species (Table 3). Because both predator taxa and the number of each taxon were equally important in evaluating the attraction of the corn germplasm lines to different types of predators, cluster analysis was utilized for assessing predator diversity and abundance on the 8 corn germplasm lines. The number of *C. septempunctata*, and earwigs were significantly differ-

TABLE 3. PREDATOR ABUNDANCE (MEAN ± SEM) AND DIVERSITY PER 5 M^2 (15-20 PLANTS) FROM EIGHT CORN GERMPLASM LINES IN 2008 AND 2009 (N = 4).*

		Predator profile[1]										
Year	Corn	Hippo	Cmac	C7	Harmonia	Geo	Scymnus	Nabid	Orius	Hdbeetle	Earwigs	[2]Taxa
2008	Ab24E	0	1 ± 0.41	0	0.25 ± 0.25	0.5 ± 0.5	0	0	0	0	0	3
2008	B37*H84	0	3.25 ± 0.75	0	0	1.25 ± 0.25	0	0.5 ± 0.5	0.5 ± 0.29	0	0	4
2008	CRW3(S1)C6	0	3.25 ± 1.11	0.5 ± 0.29	0.25 ± 0.25	0.5 ± 0.50	0	0	2.25 ± 1.60	0	0.5 ± 0.29	6
2008	EPM6	0	2 ± 0.71	0	1.25 ± 0.95	0.25 ± 0.25	0	0	1 ± 0.41	0	0.25 ± 0.25	5
2008	FAW7061	0	1.75 ± 0.48	0	0	1 ± 0.41	0.25 ± 0.25	0	1.75 ± 0.63	0.25 ± 0.25	0	5
2008	FAW7111	0.25 ± 0.25	1.75 ± 0.75	0	0.5 0± 0.29	0.25 ± 0.25	0.25 ± 0.25	0	1.25 ± 0.95	0	0	6
2008	Mp708	0	1.5 ± 0.65	0	0.25 ± 0.25	0.5 ± 0.29	0	0	2 ± 1.35	0.25 ± 0.25	0	5
2008	SIM6	0	2.5 ± 0.65	0	0.50 ± 0.29	0.25 ± 0.25	0	0.25 ± 0.25	1 ± 0.71	0	0	5
2009	Ab24E	0.25 ± 0.25	0	0	0	0.25 ± 0.25	0	0	0.25 ± 0.25	0	0	3
2009	B37*H84	0.25 ± 0.25	1.5 ± 0.87	0	0	0	0	0	2.5 ± 2.18	0	0	3
2009	CRW3(S1)C6	0	0.5 ± 0.50	0	0	0	0	0	0.25 ± 0.25	0.75 ± 0.25	1 ± 1	4
2009	EPM6	0.25 ± 0.25	0	0	0	0	0	0	0.25 ± 0.25	0	0	2
2009	FAW7061	0.25 ± 0.25	1 ± 0.58	0	0	0	0	0	0.5 ± 0.50	0	0	3
2009	FAW7111	0.25 ± 0.25	1 ± 0.71	0	0.25 ± 0.25	0	0	0	1 ± 0.71	0	0	4
2009	Mp708	0.33 ± 0.33	0	0	0	0	0	0	0	0	0	1
2009	SIM6	0	0.5 ± 0.29	0	0	0	0	0	0	0	0	1

[1]Natural enemy names are abbreviated as follows: Hippo = *Hippodamia convergens* (Coleoptera: Coccinellidae); Cmac = the pink spotted lady beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae); C7 = the seven-spotted lade beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae); *Harmonia* = the multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae); Geo = big-eyed bugs, *Geocoris* spp. (Heteroptera: Geocoridae); Scymnus = *Scymnus* spp. (Coleoptera: Coccinellidae); Nabid = damsel bugs, *Nabis* spp. (Heteroptera: Nabidae); Orius = the insidious flower bug, *Orius insidiosus* (Heteroptera: Anthocoridae); hdbeetle = hooded (or flower) beetles, *Notoxus* spp. (Coleoptera: Anthicidae); and earwigs = Dermapteran taxa identified as *Labidura riparia* (Labiduridae), and *Doru taeniatum* (Forficulidae);

[2]Taxa denote the diversity of predators using the number of predator taxonomic groups recorded per germplasm entry.

ent among the 8 germplasm lines, whereas other predators were equally abundant among the germplasm lines (Table 2). Three predators (C. *maculata, H. axyridis,* and *Geocoris* spp.) and the total number of predators were significantly different between 2008 and 2009 (Table 2). Also, the number of *C. septempunctata* (C7) and *Notoxus* spp. was influenced by the corn germplasm line × year interaction (Table 2).

Identification of *Spodoptera frugiperda* Resistance Using Injury Rating and Predator Survey Data

From cluster analysis of the corn germplasm lines, 4 clusters were extracted each with an eigenvalue >1 (ranging between 1.2 and 3.8), which contributed to 87% of the total variance. Cluster analysis using the combined *S. frugiperda* injury rating and predator data (Fig. 2) aligned with previous identification of *S. frugiperda* resistance using only *S. frugiperda* injury rating data as shown in Figs. 1A to 1D. 'Mp708' and 'FAW7061' were in the same cluster (Fig. 2), which were *S. frugiperda* resistant. Rootworm-resistant 'CRW3(S1)C6' showed *S. frugiperda* resistance (Figs. 1A to 1D). In addition, 'CRW3(S1)C6' was separated from the other 7 germplasm lines (Fig. 2) because the most predators were observed on 'CRW3(S1)C6', as shown in Table 3, and 'CRW3(S1)C6' also showed *S. frugiperda* injury (Figs. 1A to 1D). In particular, more earwigs and *C. septempunctata* were also recorded on the western corn rootworm resistant line,'CRW3(S1)C6', than on the other 7 germplasm lines (Tables 2, and 3). In contrast, 'EPM6' and the susceptible control, 'Ab24E', had the highest *S. frugiperda* injury ratings (Figs. 1A to 1D) and the fewest predators were recorded in this cluster in both years (Table 3 and Fig. 2).

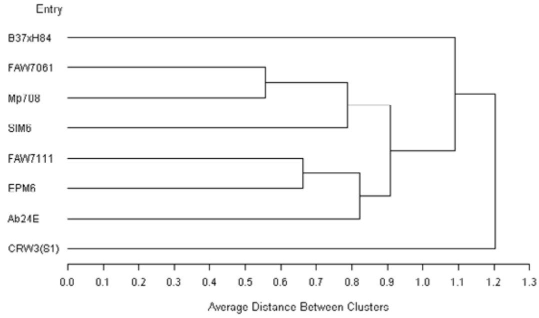

Fig. 2. *Spodoptera frugiperda* resistance in 8 corn germplasm lines based on cluster analysis of injury rating and predator abundance data recorded in 2008 and 2009. 'Mp708' was used as the insect-resistant control, while 'Ab24E' was used as insect susceptible control.

Correlation between *S. frugiperda* Injury Ratings and Predator Abundance

Based on the combined two-year data of the 8 germplasm lines, the 2 (7d and 14d) ratings of *S. frugiperda* injury was positively correlated, whereas the *S. frugiperda* injury ratings were negatively correlated to the number of hooded beetles (Table 4). The 14 d *S. frugiperda* injury ratings were positively correlated to *C. maculata*. The correlation coefficients among the 10 predator species varied (Table 4). The total number of predators was positively correlated to *C. maculata, C. septempunctata, H. axyridis, Geocoris* spp., and *O. insidiosus* (Table 4), but not to the others. These 5 species were the most common predators in the experimental plots.

Positive and negative correlations were detected among predators (Table 4). *Coleomegilla maculata* abundance was positively correlated to *C. septempunctata, Nabids*, and *Geocoris* spp. In addition, *C. septempunctata* was also positively correlated with *O. insidiosus. Notoxus* spp. were positively correlated with *Scymnus* spp. and earwigs. Cluster analysis among the diversity and abundance of predators showed one main cluster with an eigenvalue >1 (i.e., 6.8) that contributed to 85% of the variation. This cluster analysis showed that the most abundant predators across the 8 corn germplasm lines at 7 d after infestation were *C. maculata* and *O. insidiosus* in the same cluster (Fig. 3), whereas the least abundant predators in the same cluster were *H. convergens, C. septempunctata, Scymnus* sp., *Nabis* spp., and earwigs (Fig. 3). The abundance of the other 8 predator species varied significantly between the 2 years. In particular, *O. insidiosus* was abundant in 2008, but less so in 2009 (Table 3). In contrast, all species of earwigs and the hooded beetles were more abundant in 2009 than in 2008 (Table 3).

Discussion

The current study demonstrated that the *D. virgifera virgifera*-resistant corn germplasm line, 'CRW3(S1)C6' conferred fall armyworm resistance. Previous reports on multiple insect resistance were mainly limited to similar plant tissues, such as multiple leaf-feeding insects (Wilson et al. 1995a; Abel 2000a), and multiple ear-feeding insects and ear-colonizing diseases (Ni et al. 2007; Ni et al. 2008). The present study also showed that the 2 newly-developed partial inbred lines, i.e., 'FAW7061' and 'FAW7111' derived from a previously released population, 'GT-FAWCC(C5)', were resistant to *S. frugiperda* feeding compared to the resistant 'Mp708' and the susceptible control, 'Ab24E', although 'FAW7061, had less *S. frugiperda* injury than 'FAW7111'. In particular, the rootworm resistance, i.e., 'CRW3(S1)C6' will be useful in developing *S. fru-*

TABLE 4. CORRELATION COEFFICIENT BETWEEN *S. FRUGIPERDA* INJURY RATINGS AND PREDATOR ABUNDANCE ON EIGHT CORN GERMPLASM LINES (N = 63)*.

	FAW7d	FAW14d	Hippo	Cmac	C7	Harmonia	Geop	Scymnus	Orius	Nabidae	Hdbeetle	Earwigs
FAW14d	0.48											
	0.0001											
Hippo	0.15	-0.13										
	0.24	0.3										
Cmac	0.02	0.32	-0.22									
	0.88	0.01	0.08									
C7	0.14	0.17	-0.06	0.32								
	0.26	0.18	0.62	0.01								
Harmonia	0.03	0.21	0.05	0.2	0.09							
	0.81	0.11	0.71	0.11	0.49							
Geop	0.14	0.24	-0.18	0.3	-0.09	-0.09						
	0.26	0.06	0.15	0.02	0.46	0.49						
Scymnus	-0.01	0.01	-0.06	0.14	-0.03	-0.06	0.06					
	0.92	0.94	0.62	0.28	0.8	0.63	0.63					
Orius	-0.09	-0.003	-0.1	0.22	0.27	0.2	0.06	0.01				
	0.47	0.98	0.43	0.09	0.03	0.11	0.66	0.95				
Nabidae	-0.07	0.18	-0.06	0.38	-0.03	-0.06	0.21	-0.03	-0.06			
	0.58	0.15	0.64	0.002	0.81	0.64	0.11	0.81	0.65			
Hdbeetle	-0.31	-0.36	-0.1	-0.03	-0.05	-0.003	0.05	0.28	0.15	-0.05		
	0.01	0.004	0.42	0.8	0.68	0.98	0.7	0.03	0.24	0.7		
Earwigs	-0.12	-0.09	-0.07	-0.03	0.13	-0.02	-0.06	-0.04	0.04	-0.04	0.38	
	0.36	0.49	0.57	0.81	0.31	0.86	0.66	0.77	0.73	0.78	0.002	
Tpredators	-0.04	0.2	-0.12	0.73	0.37	0.39	0.34	0.14	0.74	0.25	0.23	0.2
	0.75	0.11	0.34	0.0001	0.003	0.002	0.01	0.29	0.0001	0.05	0.07	0.12

*Of the paired values in a table cell, the top value is the Pearson Correlation Coefficient value (r), while the bottom value is P value from PROC CORR procedure of the SAS software; Please refer to the legend of Table 2 for all abbreviations in Table 4.

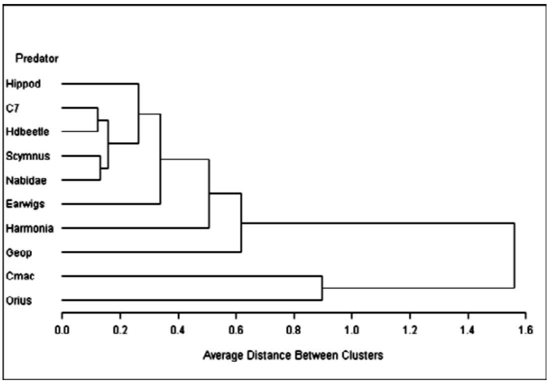

Fig. 3. Predator profiles recorded in 2008 and 2009 on corn plants across 8 corn germplasm lines evaluated for resistance to multiple insect species. The 10 predator species (in the order listed) were: Hippo = *Hippodamia convergens* (Coleoptera: Coccinellidae); C7 = the seven-spotted lade beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae); Hdbeetle = the hooded (or flower) beetle, *Notoxus* spp. (Coleoptera: Anthicidae); Scymnus = *Scymnus* spp. (Coleoptera: Coccinellidae); Nabid = the damsel bug, *Nabis* spp. (Heteroptera: Nabidae); Earwigs = Dermapteran taxa identified as *Labidura riparia* (Labiduridae), and *Doru taeniatum* (Forficulidae); Harmonia = the multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae); Geop = the big-eyed bug, *Geocoris* spp. (Heteroptera: Geocoridae); Cmac = the pink spotted lady beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae), and Orius = the insidious flower bug, *Orius insidiosus* (Heteroptera: Anthocoridae).

giperda and multiple insect pest resistance in new corn germplasm adapted to the southern U.S. states, although the phenotypic traits and underlying resistance mechanisms need further elucidation.

The predator profiles varied among the 8 germplasm entries at whorl stage under the field conditions in the present study. 'CRW3(S1)C6' had the largest number of predators at the whorl stage, whereas 'Ab24E' had the fewest. Phenotypic traits, e.g., flowering time, leaf color, and leaf trichome density, may interfere in plant attraction to predators, but the impact might be limited in this study, which was only conducted at the V6-9 (6-9 leaf) stages of vegetative growth. For instance, although 'CRW3(S1)C6' was phenologically an early-flowering line compared to the other entries in Tifton (Ni, unpublished data), any influence of flowering time would be minimal because the ratings and predator survey was conducted before tasseling.

Prey species of the observed predators were not apparent. A total of 10 different predator species were recorded without any apparent infestations of either aphids or spider mites. Besides the manual infestation of corn plants with *S. frugiperda* neonate larvae, the only abundant herbivores in the experimental plots were thrips (Ni, personal observation). Predation efficacies of all 10 predator species on thrips are not well known, although some are noteworthy thrips predators, e.g., *O. insidiosus* (Dicke & Jarvis 1962). *Coleomegilla maculata* and *O. insidiosus* were the most abundant species observed in 2008 and 2009, while *C. septempunctata* was the least common species on the 8 corn germplasm lines. Similarly, Hoballah et al. (2004) noted that both *C. maculata* and *O. insidiosus* were abundant on corn plants at 4-5 leaf stages (V4 to V5) between Jan and Feb 2000 in Mexico, and Sueldo et al. (2010) reported that earwigs were effective predators for fall armyworm larvae in Argentina. Our findings indicated that predators are common at the whorl stage in corn fields. The abundant natural enemies recorded on the corn plants might have been attracted to either constitutive corn plant volatiles or to the corn plant volatiles synthesized in response to *S. frugiperda*-injury, because our sampling was conducted 7 d after the manual insect infestation with the *S. frugiperda* neonates. Several natural enemies, i.e., C. septempunctata and earwigs, exhibited differential responses to the corn germplasm lines, suggesting possible germplasm-specific interactions. The chemical ecology and general significance of these phenomena observed in the field should be further elucidated.

Differential responses of natural enemies to corn plants could be further examined and utilized as a favorable trait in corn breeding programs intended to reduce foliar injury by *S. frugiperda* and other pests (particularly aphids) in corn at vegetative growth stages. This ecologically-based resistance has been termed pseudo-resistance (Painter 1951; Panda & Khush 1995). In recent years, a number of studies have demonstrated host plant volatile-mediated insect herbivore-natural enemy interactions (De Moraes et al. 2001; Ryan 2001; Ode 2006; Smith 2010; Hare 2011). Diel pattern of plant volatile profiles may differentially serve to recruit natural enemies diurnally and repel pest oviposition nocturnally (Ryan 2001). The utilization of natural enemies as an extension of conventional (or constitutive) plant defenses against insect herbivory still needs to be further examined and elaborated (Ode 2006). At the same time, Hare (2011) also pointed out that variations in plant volatile blends might be influenced by both abiotic and biotic factors under field conditions, which would in turn alter the tri-trophic interactions among host plants, herbivores, and natural enemies. It is necessary to utilize the techniques from evolutionary quantitative genetics to test the hypotheses related to volatile production of plants in response to herbivory damage under natural or field conditions (Hare 2011).

Predators in this study were surveyed on only 1 date each year, and during a restricted time of the day, using methods similar to those described by Musser et al. (2003). Additional samples over multiple dates and times of day and night would add additional insights into the plant-pest-natural enemy relationships. Further in-depth ecological studies are needed to decipher the roles of predators in crop pest suppression in agricultural ecosystems (Furlong & Zalucki 2010). Understanding these ecologically-based dynamics in host plant-pest-natural enemy interactions could lead to the utilization of plant volatile-mediated insect ecology (or natural enemy attraction) to reduce pest populations and, in turn, to reduce crop losses by insect herbivory and mycotoxin contamination. It is likely that the similarity of *S. frugiperda* injury ratings on 'CRW3(S1)C6' between 7 d and 14 d was the result of predation of *S. frugiperda* larvae by the predators that were abundant on this line. Utilizing ecological genetics of corn plants to reduce yield and quality losses from and insects and diseases by reducing insect herbivory and attracting natural enemies could be one of the effective tactics of corn breeding program in the long-term. The present study serves as a baseline for our corn breeding program to further examine multiple insect resistance, including foliar-, root-, and ear-feeding insects at various growth stages, and mycotoxin reduction in the southeastern Coastal Plain region of the U.S.

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