Evaluation of Pure Red Pericarp and Eight Selected Maize Accessions for Resistance to Corn Earworm (Lepidoptera: Noctuidae) Silk Feeding

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ABSTRACT New sources of corn earworm, *Helicoverpa zea* (Boddie), silk feeding resistance are needed to protect the maize, *Zea mays* L., crop without increasing the use of pesticides. Previous evaluation of popcorn germplasm with red pericarp color showed a high incidence of resistance. Within the National Plant Germplasm System, there are 15 maize accessions with pure red pericarp color. These 15 accessions were grown in the field at Ames, IA, and Tifton, GA. Fresh silks were collected, dried, and incorporated into a standard pinto bean diet for rearing corn earworms. Diets were infested with neonates, and the larvae were weighed after 8 d. Results of the diet testing identified four plant introduction accessions that produced 8-d larval weights equal to the resistant control, 'Zapalote Chico'. The 15 accessions were analyzed for the presence of maysin, three of its analogues, and chlorogenic acid. One accession, PI 245138, had low levels of maysin but was resistant. In addition, eight maize accessions acquired by the National Plant Germplasm System from 1948 to 1951 were evaluated for corn earworm silk feeding resistance at Ames and Tifton. One accession, PI 172328, was rated resistant at both locations.

KEY WORDS *Helicoverpa zea,* host plant resistance, maysin

THE CORN EARWORM, *Helicoverpa zea* (Boddie), is a major pest of maize, *Zea mays* L., in the United States. Plant resistance by itself or combined with other methods as part of an integrated pest management program is an environmentally safe and viable method of suppressing this insect.

Most sources of silk feeding resistance in maize have been linked to the chemical maysin (Waiss et al. 1979, Elliger et al. 1980, Snook et al. 1994). Styles and Ceska (1989) showed a common link for C-glycosyl flavones (e.g., maysin) and anthocyanins that produce the red color in the pericarp.

Previous evaluation of the popcorn collection of the National Plant Germplasm System (NPGS) identified 21 accessions resistant to corn earworm silk feeding (Wilson et al. 1993). Four of the 21 resistant accessions had pure red colored kernels and several other resistant accessions had partial red colored kernels. We decided to examine all 15 of the pure red pericarp maize germplasm in the NPGS to determine if they possessed resistance to silk feeding by the corn earworm. In addition, eight maize accessions acquired by the NPGS from 1948 to 1951 were evaluated for corn earworm silk feeding resistance. These eight accessions were selected after examining unpublished results of corn earworm evaluations performed at Riverside, CA, by J. W. Cameron in 1954.

Materials and Methods

Ames. Maize accessions (15) with pure red kernels and 8 selected accessions were obtained from the NPGS and planted in the field (15 in one test and 8 in another) in single rows 7.6 m long and 0.9 m apart on 10 June 1993. 'Zapalote Chico' and 'Stowell's Evergreen' sweet corn were also planted as resistant and susceptible controls, respectively, in each of the two tests. Fresh silks were collected when available. Silks were frozen, lyophilized, milled, and placed in a −20°C freezer until used. The milled silks were incorporated into a standard pinto bean diet used to rear the corn earworm. One neonate was placed in a 30-ml plastic cup containing 10 ml of diet. A standard pinto bean diet control was included in each test. The larvae were weighed after 8 d. Each test design was a randomized block with 15 replicates (2 cups per replicate). Data were analyzed with the analysis of variance (ANOVA-2) program of MSTAT-C (MSTAT Development Team 1989). When the F value for treatments was significant ($P < 0.05$), means were separated with the Fisher's protected least significant difference (LSD) test ($\alpha = 0.05$) included in the RANGE program of MSTAT-C.

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Table 1. Flavone chemical determinations and weights of corn earworm larvae after feeding for 8 d on diets containing silks from pure red pericarp maize

Entry	% fresh weight					8-d Larval wt (mg)	
	Chlorogenic acid	Galactolutedin	Maysin	Apimaysin	3′-Methoxy-maysin	Tifton	Ames
213807	0.010	0.003	0.009	0.011	0.007	409.0a	209.2def
Reg BN[a]	0.000	0.000	0.000	0.000	0.000	384.0a	343.2ab
213796	0.007	0.001	0.009	0.002	0.006	370.0a	149.9fgh
303851	0.005	0.000	0.004	0.000	0.006	298.8b	237.9cde
219886	0.006	0.000	0.007	0.001	0.007	274.8bc	246.8cde
303850	0.022	0.001	0.018	0.000	0.018	242.8cd	254.2cde
213757	0.015	0.002	0.072	0.021	0.012	189.4e	284.2bc
SEG[b]	0.012	0.003	0.106	0.000	0.052	182.7e	356.9a
516039	0.015	0.002	0.070	0.003	0.020	80.8f	174.4fg
257626	0.023	0.005	0.127	0.003	0.015	16.9g	203.3efg
516155	0.011	0.011	0.197	0.006	0.021	14.7g	269.0cd
245138	0.012	0.009	0.155	0.008	0.021	13.8g	77.6j
430456	0.010	0.001	0.135	0.009	0.021	11.7g	168.6fg
Z. Chico[c]	0.007	0.015	0.372	0.016	0.052	7.1g	80.1j
516037	0.020	0.001	0.313	0.005	0.031	5.2g	88.8ij
222319	0.024	0.010	0.394	0.009	0.038	4.9g	143.7ghi
217460	0.019	0.011	0.411	0.014	0.085	4.3g	106.9hij
217404	0.020	0.013	0.485	0.018	0.024	3.6g	102.1hij

Means followed by the same letter are not significantly different according to the Waller–Duncan K ratio test ($P < 0.05$). Means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P < 0.05$).
[a] Regular bean diet.
[b] Stowell's Evergreen (susceptible control).
[c] Zapalote Chico (resistant control).

Georgia. The same 15 pure red kernel accessions and the 8 selected accessions were planted in two separate tests in single row plots 3.1 m long and 0.76 m apart in Tifton, GA, on 28 April 1993. Fresh silks were collected, oven dried (41°C), milled (1 mm), and incorporated into a standard pinto bean diet used to rear the corn earworm. One neonate was placed into a 30-ml plastic cup containing 10 ml of diet. The test designs, replications, and controls were the same as used in Ames. The larvae were weighed after feeding for 8 d. Data were analyzed using SAS (SAS Institute 1989). When the F value for treatments was significant ($P < 0.05$), means were separated with the Waller–Duncan k ratio test.

Silks from the 23 accessions from both tests were collected, individually bulked and placed into methanol, and sent to Athens, GA, for chemical analysis (Wiseman et al. 1992). The basic procedure used is outlined in Snook et al.(1993). The chemical analyses for percentage of fresh weight of maysin, chlorogenic acid, apimaysin, 3′-methoxymaysin, and galactolutedin in the silks were determined by reverse-phase, high-performance liquid chromatography (Snook et al. 1989). Correlations were run using the CORR program of MSTAT-C (MSTAT Development Team 1989) to determine relationships between 8-d larval weights and the percentage of fresh weight of the five chemicals analyzed from the silks.

Results and Discussion

Data from the chemical analyses of the silks obtained at Ames, IA, and Tifton, GA, of the 15 pure red kernel accessions and the weights of 8-d-old corn earworm larvae are presented in Table 1. At Ames, silks from four accessions fed to corn earworm larvae produced 8-d larval weights not statistically different ($F = 16.6$; df = 17, 269; $P < 0.01$) from the resistant control, Zapalote Chico. At Tifton, silk diets from eight accessions fed to

Table 2. Correlations of corn earworm 8-d larval weights with percentage of fresh weight of selected chemicals found in maize silks

Larval wt	n[a]	Maysin	Chlorogenic acid	Galactolutedin	Apimaysin	3′-Methoxymaysin
Tifton (8 selected)	10	−0.65*	0.55	−0.22	−0.60	−0.45
Ames (8 selected)	10	−0.60[b]	0.07	−0.13	−0.61	−0.56
Tifton (pure reds)	17	−0.76**	−0.49*	−0.63*	−0.35	−0.53*
Ames (pure reds)	17	−0.62*	−0.18	−0.48*	−0.38	−0.28

*, Level of significance, 5%; **, level of significance, 1%. Correlations run using CORR program of MSTAT-C (MSTAT Development Team 1989).
[a] Includes resistant and susceptible controls.
[b] Level of significance, 6%.

Table 3. Flavone determinations from silks of eight selected maize introductions and 8 d weight of corn earworm larvae

Entry	% fresh wt					8-d larval wt (mg)	
	Chlorogenic acid	Galactolutedin	Maysin	Apimaysin	3′-Methoxy-maysin	Tifton	Ames
165457	0.016	0.004	0.150	0.007	0.058	37.6f	29.5f
172328	0.031	0.004	0.211	0.003	0.016	26.0fg	39.9ef
180359	0.021	0.003	0.105	0.002	0.012	25.7fg	52.2de
181988	0.025	0.005	0.093	0.002	0.018	82.8e	70.3bcd
183753	0.016	0.004	0.080	0.002	0.018	119.6cd	85.6b
184282	0.012	0.004	0.182	0.004	0.012	49.2f	38.7ef
193655	0.023	0.019	0.131	0.003	0.026	88.0e	66.8cd
193658	0.014	0.001	0.028	0.000	0.014	98.3de	55.3de
SEC[a]	0.013	0.003	0.081	0.002	0.016	135.7c	77.2bc
Z. Chico[b]	0.013	0.016	0.678	0.021	0.054	3.7g	27.6f
Reg BN[c]	0.000	0.000	0.000	0.000	0.000	453.3a	146.7a

Means followed by the same letter are not significantly different according to the Waller–Duncan *K* ratio test ($P < 0.001$) at Tifton. Means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P < 0.05$) at Ames.
[a] Stowell's Evergreen (susceptible control).
[b] Zapalote Chico (resistant control).
[c] Regular bean diet.

corn earworm larvae produced 8-d larval weights not statistically different ($F = 131.7$; df = 10, 164; $P < 0.01$) from the resistant control. All four accessions resistant to corn earworm larval feeding at Ames (PI 217404, PI 217460, PI 245138, and PI 516037) were also resistant at Tifton. One of the accessions, PI 217404, is a popcorn, the other three are flint type maizes. We believe the accession × environment interaction is, at least partially, responsible for the different results obtained at Ames and Tifton.

Chemical analyses of the red pericarp maize revealed that three of the four resistant maizes common to both Ames and Tifton had high levels of maysin in their silks (Table 1). Their levels were comparable to the levels found in the resistant control. The fourth resistant accession, PI 245138, is low in all of the flavanoids including maysin. Its level compared to the level in the susceptible control. Thus, the resistance factor(s) for this accession cannot be attributed to the chemicals reported here.

Correlations of 8-d larval weights with the percentage of fresh weight of the chemicals analyzed were run for the 15 pure red pericarp maizes (Table 2). At both locations, there were significant ($P < 0.05$) negative correlations for maysin and galactolutedin. At Tifton, the maysin negative correlation was highly significant ($r = -0.76$, $P < 0.01$). Also at Tifton, chlorogenic acid and 3′-methoxymaysin were significantly negatively correlated with larval weights. These results indicate that other chemicals in addition to maysin are important for the expression of resistance in the maize accessions tested.

The data for the eight selected accessions tested at Ames and Tifton for silk feeding resistance and analyzed at Athens for selected chemicals in the silks are presented in Table 3. At Ames, three accessions fed to corn earworms produced 8-d larval weights that were not statistically different ($F = 23.5$; df = 11, 179; $P < 0.01$) from the larval weights of the resistant control. At Tifton, larvae fed silks of two accessions had 8-d weights not statistically different ($F = 131.7$; df = 11, 179; $P < 0.01$) from the resistant control. One resistant accession, PI 172328, was common to both locations. This accession is a dent maize originally collected in Australia. Chemical analysis of the silks does not indicate high levels of percentage of fresh weight of maysin (0.211) or any of the other chemicals analyzed.

Correlations of 8-d larval weights with the analyzed chemicals were run for the 8 selected accessions at both locations (Table 2) and showed that maysin was negatively correlated at Tifton ($r = -0.65$, $P < 0.05$) and at Ames ($r = -0.60$, $P < 0.06$). Apimaysin was negatively correlated with 8-d larval weights at both locations ($P < 0.06$). The original germplasm of these eight accessions was placed into the NPGS from 1948 to 1951. They have been maintained by sibpollinations since that time and are currently available for use by plant breeders.

The resistance of the red pericarp maizes may be linked to the presence of anthocyanins. If resistance could be associated with the anthocyanins, then a chemical analysis for anthocyanins in the pericarp could provide a rapid method of initial screening for corn earworm resistance in the laboratory. This could save time in conducting initial evaluations of maize added to the NPGS in the future. However, such a technique could fail to discover resistance in an accession whose resistance is not based on flavanoids such as PI 245138. Ultimately, the plants must be exposed to the insect pests to determine their usefulness in the field.

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