Effect of Maysin on Wild-Type, Deltamethrin-Resistant, and Bt-Resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT Larvae of the Old World corn earworm, *Helicoverpa armigera* (Hübner), were fed diets containing lyophilized silks from maize genotypes expressing varying levels of maysin, a flavone glycoside known to be toxic to the New World corn earworm, *Helicoverpa zea* Boddie. Three different *H. armigera* colonies were tested: a wild-type colony (96-S), a colony selected for resistance to deltamethrin (Del-R), and a colony selected for resistance to the Cry1Ac protoxin of *Bacillus thuringiensis* (Bt-R). A colony of *H. zea* was also tested as a control. High-maysin silk diets significantly slowed the growth and arrested the development of larvae from all *H. armigera* colonies compared with low-maysin silk diets, maysin-lacking silk diets, and no-silk control diets. The effects on the *H. armigera* and *H. zea* colonies were similar across maysin levels, although *H. zea* is a larger insect than *H. armigera* and this overall size difference was observed. Among the *H. armigera* colonies, maysin effects were generally similar, although 7-d-old Del-R larvae were significantly smaller than 7-d-old Bt-R and 96-S larvae for one no-silk control and two maysin-containing silk treatments. The toxic effect of maysin on the Bt-R and Del-R colonies suggests that physiological mechanisms of *H. armigera* resistance to Cry1Ac and deltamethrin do not confer cross-resistance to maysin.

KEY WORDS corn earworm, cotton bollworm, cross-resistance, *Helicoverpa*, host plant resistance, *Zea mays*

THE CORN EARWORM (*Helicoverpa zea* Boddie) is a major pest of cotton, maize, and other crops in the United States and throughout the Western Hemisphere. *Helicoverpa armigera* (Hübner) is a major pest of many of the same crops, including cotton and maize, throughout Africa, Asia, Australia, and Europe. *Helicoverpa zea* and *H. armigera* are closely related to each other (Li et al. 2002) and are able to intermate to produce fertile offspring (Laster and Hardee 1995). Both species have developed resistance to pyrethroid insecticides in field populations (Abd-Elghafar et al. 1993, Kranthi et al. 2001). Because of the widespread adoption of transgenic maize, cotton, and other crops expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) in China and the Americas, concerns are being raised about the possible development of insect resistance to Bt proteins (Gahan et al. 2001, Palumbi 2001). As a result of this concern, an insect resistance management program was included in the U.S. Environmental Protection Agency reregistration of Bt transgenic crops (EPA 2001)

Maysin, a flavone glycoside expressed in the silks of some maize genotypes, is toxic when ingested by *H. zea* larvae (Waiss et al. 1979, Snook et al. 1994). Laboratory bioassays have been developed to study this toxic effect (Wiseman and Isenhour 1990, Wiseman et al. 1992) and two breeding populations expressing high levels of maysin, EPM6 and SIM6, have been developed to provide *H. zea*-resistant germplasm for maize breeding (Widstrom and Snook 2001). Maysin-based resistance could be useful as a complement or alternative to Bt-based transgenic resistance in an integrated pest management program designed to delay the development of an *H. zea* biotype that is resistant to Bt.

The objectives of this study were to compare the effects of maysin on the growth and development of *H. zea* and *H. armigera* and to compare maysin effects on wild-type, Bt-resistant, and deltamethrin-resistant laboratory colonies of *H. armigera.*

Materials and Methods

Plant Materials. Lyophilized silks from five maize genotypes were used in the bioassays. Silks were bulk collected on ice from unpollinated, field-grown plants and stored at −20°C before lyophilization at −50°C in a Freezemobile 25ES (VirTis Co., Gardiner, NY). Lyophilized silks were ground in a Cyclotec grinder (Teactor AB, Höganäs, Sweden) using a 1.0-mm screen. After grinding, the silks were stored in airtight containers at −20°C. Zapalote Chico 2451# (P) C3

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Table 1. Means (±SEM) of growth and development parameters for *Helicoverpa armigera* and *H. zea* larvae fed on diets containing maize silks of varying maysin levels

Diet	Maysin level[a]	*H. armigera* (96-S)			*H. zea*		
		7-d larval wt. (mg)	% Pupation	% Emergence	7-d larval wt. (mg)	% Pupation	% Emergence
Water	NA	15.1 ± 1.3a	74 ± 6a	60 ± 4a	748.5 ± 25.3ab	78 ± 5ab	78 ± 5a
Celufil	NA	14.4 ± 2.0a	76 ± 4a	61 ± 4a	757.6 ± 24.1a	88 ± 4a	82 ± 5a
GA209	None	15.4 ± 1.4a	77 ± 6a	64 ± 6a	699.7 ± 40.0b	83 ± 7ab	80 ± 7a
SEG	Low	14.2 ± 1.4a	72 ± 7a	51 ± 6b	731.2 ± 25.5ab	83 ± 5ab	78 ± 5a
ZC	Moderate	3.0 ± 0.5b	10 ± 6b	6 ± 4c	230.9 ± 21.0c	85 ± 5ab	83 ± 5a
SIM6	High	2.2 ± 0.3b	3 ± 2b	2 ± 2c	56.4 ± 15.7d	75 ± 6b	72 ± 6a
EPM6	High	2.0 ± 0.2b	1 ± 1b	0 ± 0c	4.2 ± 0.5d	18 ± 5c	12 ± 4b
F^b		167.37	10.36	4.76	262.71	22.39	22.77
P		<0.0001	0.0059	0.0396	<0.0001	<0.0001	<0.0001

Means within each column followed by the same letter are not significantly different (PROC GLM, $P < 0.05$).
[a] A priori classification from previous studies (data not shown); NA, not applicable.
[b] df = 6, 21 for *H. armigera;* df = 7, 32 for *H. zea.*

(ZC) was known to have moderate maysin content, Stowell's Evergreen (SEG) was known to have low maysin content, GA209 produces no maysin, and EPM6 and SIM6 both produce high levels of maysin (Snook et al. 1994, Widstrom and Snook 2001). Celufil (United States Biochemical Corp., Cleveland, OH) is a neutral cellulose powder that was used as a negative control.

Insect Colonies. Laboratory bioassays on *H. armigera* were performed in Beijing, China using neonate larvae from colonies maintained at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The wild-type (or susceptible) strain of *H. armigera* (96-S) was descended from larvae collected in cotton fields in Henan province, China, in 1996 and has been maintained in the laboratory on artificial diet. The Bt-resistant strain (Bt-R) was selected from the 96-S colony by feeding larvae incrementally larger doses of Cry1Ac protoxin in diet over many generations (Liang et al. 2000). The Bt-R strain had undergone ≈40 generations of selection at the time of this study and has a median weight loss concentration (WLC_{50}) 272 times higher than that of the susceptible 96-S strain (G. Liang, unpublished data). The deltamethrin-resistant strain (Del-R) is descended from larvae collected in 1994 in Henan province cotton fields where *H. armigera* resistance to pyrethroids had been locally reported. They had been selected with deltamethrin in the laboratory (Liang et al. 1999) for approximately 100 generations at the time of this study. The Del-R strain has an LD_{50} for deltamethrin 22,323 times higher than that of the 96-S strain (G. Liang, unpublished data). Laboratory bioassays on *H. zea* were performed in Tifton, GA, using neonate larvae from a colony that has been maintained on artificial diet by the USDA for approximately 250 generations (Young et al. 1976). None of the colonies used in this study have ever had wild germplasm added to them since their inceptions.

Bioassays. The bioassays were conducted by suspending ground, lyophilized silk tissue in water, adding the water/silk suspension to larval diet, and then blending for 1 min. Ten milliliters of silk/water/diet mixture was dispensed into 30-ml containers and allowed to dry before one neonate larva was placed in each container and sealed inside to feed on the diet. The larvae were incubated at 27 ± 2°C and 75 ± 10% RH under a 16:8 (L:D) photoperiod. Three replications of ten larvae (containers) per rep were tested for each treatment in each insect colony. For the *H. zea* experiment, 10 g of silk was suspended in 100 ml of water before being mixed into 300 ml of diet for a final silk concentration of 2.5% (wt:vol). Increasing silk-maysin levels were known to reduce 7-d growth of *H. zea* larvae fed diets with this concentration of silks (B. Rector, unpublished data). Preliminary experiments were conducted on *H. armigera* (96-S) to determine the silk concentration that would result in a similar reduction of larval growth (data not shown). For *H. armigera,* the concentration that provided the most informative data were 1.85% (wt:vol), which was obtained by suspending 10 g of silk in 40 ml of water and adding this mixture to 500 ml of diet. All larvae were weighed after 7 d and returned to their original containers to pupate and subsequently emerge as adults. The number of insects to pupate and to emerge was recorded for each insect colony tested.

Data Analysis. Data were analyzed by analysis of variance and correlations were made using Pearson's correlation coefficient (PROC CORR) (SAS Institute 1989). Means were separated by Waller Duncan k-ratio *t*-test (k-ratio = 100) (Waller and Duncan 1969).

Results and Discussion

Comparison of *H. zea* and *H. armigera* (96-S). Effects of maize silks of varying levels on growth and development of *H. zea* versus *H. armigera* larvae are shown in Table 1. For *H. armigera* larvae from the 96-S colony, there were significant differences between silk diet treatments for 7-d larval weight, percent of larvae that successfully pupated, and percent of insects reaching adulthood. For *H. zea* from the Tifton colony, there were also significant differences for 7-d larval weight, percent pupation, and percent adult emergence. For both species, the diets that contained silks with moderate and high maysin levels significantly reduced 7-d larval growth compared with low-maysin or maysin-lacking diets. For *H. armigera,* the moderate- and high-maysin diets also significantly re-

Table 2. Weights of wild-type (96-S), Bt-resistant (Bt-R), and deltamethrin-resistant (Del-R) *Helicoverpa armigera* larvae after feeding for 7 d on diets supplemented with maize silks of varying maysin levels

Diet	Maysin level[a]	Mean of 7-d larval wt (mg) ± SE			Comparison of the three strains		
		96-S	Bt-R	Del-R	Mean ± SE	$F_{2,6}$	P
Water	NA	18.2 ± 1.6BCa	18.2 ± 2.1ABa	15.3 ± 1.9Ba	17.2 ± 1.1BC	0.82	0.4852
Celufil	NA	24.0 ± 0.8Aa	21.2 ± 2.0Aa	13.3 ± 1.0Cb	19.5 ± 1.7A	16.67	0.0035
GA209	None	19.9 ± 2.3Ba	16.0 ± 0.8Ba	19.3 ± 1.0Aa	18.4 ± 1.0AB	1.88	0.2323
SEG	Low	14.2 ± 1.5Ca	16.6 ± 1.4Ba	16.4 ± 1.2ABa	15.7 ± 0.8C	0.90	0.4535
ZC	Moderate	6.2 ± 1.1Da	4.7 ± 0.9Ca	1.6 ± 0.1Db	4.2 ± 0.8D	8.59	0.0174
SIM6	High	2.2 ± 0.2Ea	2.5 ± 0.2Ca	1.1 ± 0.2Db	1.9 ± 0.2E	14.96	0.0047
EPM6	High	1.6 ± 0.2Ea	2.5 ± 0.5Ca	1.4 ± 0.1Da	1.8 ± 0.2E	3.52	0.0975
F[b]		46.91	38.78	64.40	26.66		
P		0.0001	0.0001	0.0001	0.0004		

Means within each column followed by the same uppercase letter and means in the same row followed by the same lowercase letter are not significantly different (PROC GLM, $P < 0.05$).
[a] A priori classification from previous studies (data not shown); NA, not applicable.
[b] df = 6, 14 for single strains; df = 6, 21 for means of strains.

duced pupation and adult emergence compared with low-maysin or maysin lacking diets. However, only the diet containing EPM6 silks significantly reduced *H. zea* pupation and emergence compared with the other diets. This result was unexpected because EPM6 and SIM6 have similar maysin levels (Widstrom and Snook 2001), and they were expected to have similar effects on *H. zea.* Further studies will be required to determine whether additional factors in EPM6 silks are producing this effect.

Table 1 also shows that after 7 d *H. zea* larvae were much larger than the *H. armigera* larvae, especially on the maysin-lacking diets. It was previously shown that *H. zea* is a larger insect than *H. armigera* (Laster and Hardee 1995), but our observed large difference in growth in the first 7 d was not anticipated. Diet preparation or ingredients, or other conditions that may have differed between the Tifton and Beijing laboratories may have contributed to this disparity, or there may simply be differences in the growth and development characteristics of these two species.

Because the 7-d weights of the *H. armigera* larvae were so much lower than the *H. zea* 7-d weights, it is difficult to compare the effect of the moderate maysin level (ZC) diet on these two species. More precise calibration of silk concentrations for the *H. armigera* bioassay and perhaps collection of larval weight data a few days later in development will be necessary to more fully describe the effects of maysin on *H. armigera* growth and development compared with *H. zea.*

Effects of Maysin on Wild-Type (96-S) and Insecticide-Resistant Strains of *H. armigera.* Maysin effects on the growth and development of Bt-resistant (Bt-R), deltamethrin-resistant (Del-R), and wild type (96-S) *H. armigera* colonies were similar overall. The 7-d larval weights (Table 2), percentage of larvae pupating (Table 3), and percentage of insects reaching adulthood (Table 4) were all significantly reduced with increasing maysin levels for each strain of *H. armigera.* The combined means of these three measures also showed significant differences (Tables 2, 3, and 4), and there were no significant differences between the means of the three strains within each treatment for percent pupated (Table 3) and percent emerged (Table 4). There were significant differences between strains in mean 7-d larval weight gain for the Celufil, Zapalote Chico, and SIM6 treatments (Table 2). For each of these treatments, the Del-R strain had significantly lighter larvae than the 96-S or Bt-R strains. This seems to be unrelated to silk-maysin concentrations because the Celufil treatment contains no

Table 3. Percentage of wild-type (96-S), Bt-resistant (Bt-R), and deltamethrin-resistant (Del-R) *Helicoverpa armigera* larvae that pupated after feeding exclusively on diets supplemented with maize silks of varying maysin levels

Diet	Maysin level[a]	Mean percent pupation ± SE			Comparison of the three strains		
		96-S	Bt-R	Del-R	Means ± SE	$F_{2,6}$	P
Water	NA	63 ± 3A	80 ± 6A	63 ± 3B	69 ± 4A	5.00	0.0527
Celufil	NA	70 ± 6A	87 ± 7A	70 ± 6B	76 ± 4A	2.50	0.1623
GA209	None	70 ± 12A	73 ± 12A	87 ± 3A	77 ± 6A	0.81	0.4891
SEG	Low	70 ± 20A	80 ± 6A	67 ± 9B	72 ± 7A	0.28	0.7633
ZC	Moderate	27 ± 12B	3 ± 3B	0 ± 0C	10 ± 6B	4.07	0.0764
SIM6	High	3 ± 3B	0 ± 0B	7 ± 7C	3 ± 2B	0.60	0.5787
EPM6	High	3 ± 3B	0 ± 0B	0 ± 0C	1 ± 1B	1.00	0.4219
F[b]		9.42	47.10	56.56	9.52		
P-value		0.0003	0.0001	0.0001	0.0074		

Means within each column followed by the same uppercase letter are not significantly different (PROC GLM, $P < 0.05$).
[a] A priori classification from previous studies (data not shown); NA, not applicable.
[b] df = 6, 14 for single strains; df = 6, 21 for means of strains.

Table 4. Percentage of wild-type (96-S), Bt-resistant (Bt-R), and deltamethrin-resistant (Del-R) *Helicoverpa armigera* that pupated and emerged as adults after feeding exclusively on diets supplemented with maize silks of varying maysin levels

Diet	Maysin level[a]	Mean percent adult emergence ± SE			Comparison of the three strains		
		96-S	Bt-R	Del-R	Means ± SE	$F_{2,6}$	P
Water	NA	53 ± 7A	73 ± 7A	53 ± 3B	60 ± 4A	4.00	0.0787
Celufil	NA	60 ± 6A	63 ± 13A	60 ± 6A	61 ± 4A	0.05	0.9492
GA209	None	60 ± 10A	53 ± 9A	80 ± 6A	64 ± 6A	2.74	0.1430
SEG	Low	40 ± 15AB	57 ± 7A	57 ± 7B	51 ± 6B	0.86	0.4687
ZC	Moderate	17 ± 12BC	0 ± 0B	0 ± 0C	6 ± 4C	1.92	0.2263
SIM6	High	0 ± 0C	0 ± 0B	7 ± 7C	2 ± 2C	1.00	0.4219
EPM6	High	0 ± 0C	0 ± 0B	0 ± 0C	0 ± 0C	–	–
F^b		9.75	22.87	46.82	4.76		
P		0.0002	0.0001	0.0001	0.0396		

Means within each column followed by the same uppercase letter are not significantly different (PROC GLM, $P < 0.05$).
[a] A priori classification from previous studies (data not shown); NA, not applicable.
[b] df = 6, 14 for single strains; df = 6, 21 for means of strains.

maysin or plant tissue, whereas the ZC and SIM6 silks have moderate and high levels of maysin, respectively. The observed differences in larval growth in the Del-R strain may be indicative of an overall physiological cost of deltamethrin resistance. Bioassays specific to this issue would be necessary to determine any physiological costs because of deltamethrin resistance in *H. armigera.*

Conclusion

Based on the results of this study, two main conclusions can be drawn. First, the effect of maysin on the growth and development of *H. armigera* is similar to the effect of maysin on *H. zea,* a closely related New World species that attacks many of the same crops as *H. armigera.* This effect was consistent across different stages of *H. armigera* and *H. zea* development, including rate of larval weight gain, ability to pupate, and adult emergence. Second, the effect of maysin on *H. armigera* growth and development seems to be independent of *H. armigera* resistance to either the pyrethroid deltamethrin, or the Bt insecticidal protein Cry1Ac. Thus, for *H. armigera* it is unlikely that cross-resistance would occur between maysin and either deltamethrin or Cry1Ac. This could make maysin-based insect resistance in maize an attractive alternative to transgenic Bt maize for control of *H. armigera* as part of an integrated strategy to manage resistance to Bt toxins in wild insect populations.

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