CUMULATIVE EFFECTS OF ANTIBIOSIS ON FIVE BIOLOGICAL PARAMETERS OF THE FALL ARMYWORM

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ABSTRACT

Results of a laboratory study showed that even low levels of resistant maize, *Zea mays* L., silks reduced fall armyworm, *Spodoptera frugiperda* (J. E. Smith) growth, extended the life cycle, and reduced fecundity in four of five generations. An intermediate level of resistance reduced growth, extended the life cycle by an average of about 4 d and significantly reduced fecundity in each of the five generations. A high level of resistant silks reduced growth significantly, extended the life cycle on an average of about 10 d per generation and reduced fecundity by almost 50% over that for the laboratory control in generations four and five. Though the estimated fecundity was not greatly reduced in all generations, there were no apparent adjustments to the stress of various levels of resistance after five generations. Thus, fall armyworm fed on various levels of resistant silk-diets did not appear to adjust to the resistance in any of the five parameters measured after five generations.

Key Words: Plant Resistance; maize; corn silks; meridic diets

RESUMEN

Los resultados de un estudio de laboratorio demostraron que aún niveles bajos de resistencia en estigmas de maíz, *Zea mays* L., redujeron el crecimiento del gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), extendieron su ciclo de vida, y redujeron su fecundidad en cuatro de cinco generaciones. Una reducción de crecimiento causada por un nivel intermedio de resistencia extendió el ciclo de vida por un promedio de aproximadamente 4 d y redujo la fecundidad significativamente en cada una de las cinco generaciones. Un nivel alto de resistencia en estigmas redujo el crecimiento significativamente, extendió el ciclo de vida por un promedio de aproximadamente 10 d por generación y redujo la fecundidad por casi un 50% sobre la del control en el laboratorio en las generaciones cuatro y cinco. Aunque la fecundidad estimada no se redujo grandemente en todas las generaciones, no hubo ningún ajuste evidente a tensión causada por varios niveles de resistencia despues de cinco generaciones. Así que, aparentemente, el gusano cogollero del maíz alimentado con dietas de estigmas de varios niveles de resistencia no se ajustó a la resistencia en ninguno de los cinco parámetros medidos después de cinco generaciones.

Maize, *Zea mays* L., is one of the major sources of animal and human foods in the Americas. Maize is attacked by a variety of insect pests, but possibly the most destructive has been the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Maize is susceptible in all plant growth stages to the fall armyworm, but production is most often limited because of severe injury or complete destruction of whorl-stage plants (Wiseman et al. 1967).

Resistance in maize to larvae of the fall armyworm was reviewed by Wiseman & Davis (1979). The resistance mechanisms of antibiosis and nonpreference associated with leaf-feeding have been identified (Wiseman et al. 1981, 1983). High levels of antibiosis and nonpreference resistance mechanisms in the silks of 'Zapalote Chico', a maize cultivar from the state of Oaxaca, Mexico have since been identified (Wiseman & Widstrom 1986). A laboratory study is reported herein to demonstrate the effects of different levels of silk resistance on fall armyworm growth, development, and fecundity over five generations.

MATERIALS AND METHODS

The fall armyworm used in this study were obtained from a colony maintained at the Insect Biology and Population Management Research Laboratory, Tifton, GA. (Burton & Perkins 1989).

The maize silks were produced from a maize genotype: a resistant field maize type, 'Zapalote Chico 2451# (PC3)' (ZC) (Wiseman et al. 1983). The entry was grown in a bulk planting at Tifton, GA using agronomic practices common to the area. Open-pollinated silks of the cultivar were harvested when the silks had emerged for 2 d. Silks were excised to the ear tip, removed from the husk channel, and bulked. Silks were oven dried at 41°C. The dried silks were finely ground (1-mm screen) using a Cyclotec

TC1093 sample mill (Fisher Scientific, Atlanta). The ground silks were stored in a standard freezer (-10°C) until needed for bioassaying.

Meridic pinto bean diet (Burton & Perkins 1989) was obtained in bulk from the insect rearing section of this laboratory. For each silk treatment, the regular pinto bean diet was diluted 3ml diet: 2 ml water. Treatments for each generation were: susceptible = control, no silk/diet (no dilution); low resistance, 12.5 mg ZC silk/ml diet; intermediate resistance, 25 mg ZC silk/ml diet; high resistance, 50 mg ZC silk/ml diet; and control (regular pinto bean diet without silk) as a check for the additional generations and as a check for the original laboratory colony. Each silk treatment was mixed separately in the diluted pinto bean diet, and the diet mixtures were dispensed into 30 ml plastic cups. In addition, a new pinto bean diet control was added after the first generation and each subsequent generation from the normal laboratory fall armyworm culture (Lab Control) to detect any possible changes that might be occurring in the susceptible control from generation to generation. Larvae for generation two and subsequent generations were obtained from eggs produced by moths from each respective specific treatment. One neonate was placed in each treatment cup after the diet had cooled and solidified for about 2 h. The cups were capped, and the tests were placed in an incubator maintained at 26.7 ± 2%C, 75 ± 5% RH, and a photoperiod of 14:10 (L:D).

Treatments for each generation were arranged as a randomized complete block with 15 replications and two cups per replicate. Biological measurements recorded were weight of larvae at 9 d, days to pupation, weight of pupae (24 h old), and days to adult eclosion and estimated fecundity. Standard statistical analyses were applied to each measured parameter as a combined analysis over generations (SAS Institute 1989); means were separated by Waller-Duncan *k* ratio *t* test at *k* ratio = 100 and $P \leq 0.05$ (Waller & Duncan 1969).

Fecundity of fall armyworm produced from the various treatments in each generation was calculated by the regression equation developed by Leuck & Perkins (1972); where mean egg production (Y) was estimated from pupal weight (X)/replicate by the regression formula of $Y = 5.33X - 423.23$. Then a standard statistical analysis was applied to the treatments over generations. Means were separated by Waller-Duncan *k* ratio *t* test at *k* ratio = 100 and $P \leq 0.05$ (Waller & Duncan 1969).

Results and Discussion

A significant ($\underline{P} \leq 0.05$) level of resistance (treatment) by generation interaction occurred for each measured parameter. Therefore each measured parameter and generation will be discussed separately.

Weight of larvae for the first generation was significantly ($P \leq 0.05$) (df = 14; F = 107.1) different at each treatment level (Table 1). The larvae on the high resistant silk-diet weighed less than one-tenth the weight of those on the intermediate resistant silk-diet. Likewise, the development time of larvae to pupation was significantly ($P \leq 0.05$; F = 400.5) different for each treatment level with larvae that were fed the high resistant silk-diet requiring more than a week longer to pupate than the larvae fed the intermediate resistant silk-diet. Weight of pupae from larvae fed the various silk-diets showed a gradual decline ($P \leq 0.05$; F = 3.00) with increased levels of resistance, but were not distinctly separable as were 9-d weight of larvae and development times. Thus, it appears that larvae on the resistant silk-diets were able to compensate for the reduced weights at 9-d by feeding longer and attaining almost equivalent pupal weights. However, time to adult eclosion among the various diet treatments were distinctly separable ($P \leq 0.05$; F = 160.0)at each treatment level. Larvae that were fed the high resistant silk-diet required more than a week longer to reach adult eclosion than larvae that were fed the intermediate resistant silk-diet.

TABLE 1. MEAN GROWTH AND DEVELOPMENTAL TIME OF FALL ARMYWORM AFTER HAVING FED ON SUSCEPTIBLE, LOW-RESISTANCE, INTERMEDIATE AND HIGH-RESISTANCE SILK-DIETS OVER FIVE GENERATIONS.

Treatment[a]	9-d wt. (mg) larvae	Day to pupation	Wt. (mg) of pupae	Day to adult eclosion
Generation 1				
Lab C	—	—	—	—
Susceptible	537 a	11.7 a	259 a	18.1 a
Low-resistance	346 b	12.9 b	246 ab	19.6 b
Intermediate resistance	215 c	14.1 c	239 ab	21.0 c
High-resistance	17 d	21.9 d	234 b	28.8 d
SEM	21.1	0.23	6.38	0.37
Generation 2				
Lab C	302 a	13.3 a	259 a	21.7 a
Susceptible	279 a	13.4 a	258 a	21.3 a
Low-resistance	142 b	15.0 b	228 b	24.2 b
Intermediate resistance	50 c	17.4 c	238 b	25.7 c
High-resistance	24 c	20.0 d	242 b	27.9 d
SEM	13.2	0.18	5.63	0.29
Generation 3				
Lab C	380 a	12.7 a	283 a	20.5 a
Susceptible	299 b	12.8 a	250 c	20.5 a
Low-resistance	160 c	16.3 b	271 ab	23.4 b
Intermediate resistance	78 d	16.2 b	223 d	24.8 c
High-resistance	28 e	19.5 c	258 bc	26.9 d
SEM	13.1	0.14	5.56	0.18
Generation 4				
Lab C	251 a	13.5 a	282 a	21.8 a
Susceptible	253 a	13.1 a	250 b	21.4 a
Low-resistance	137 b	15.9 b	260 b	24.0 b
Intermediate resistance	35 c	18.4 c	210 c	26.3 c
High-resistance	6 d	28.3 d	162 d	36.1 d
SEM	10.2	0.34	6.88	0.32
Generation 5				
Lab C	539 a	12.4 a	285 a	20.7 a

Means within a column for each generation not followed by the same letter are significantly different (*k* ratio = 100, $P \leq 0.05$ [Waller & Duncan 1969]). Lab C is laboratory control larvae from the laboratory culture maintained on pinto bean diet; susceptible, diet of continuous pinto bean; low-resistance is diet of 12.5 mg dried resistant silks/ ml dilute pinto bean diet; intermediate-resistance is a diet of 25 mg dried resistant silks in dilute pinto bean diet; high resistance is a diet of 50 mg dried resistant silks in dilute pinto bean diet. SEM = standard error of the mean was based on pooled error mean square in the analysis.

TABLE 1. (CONTINUED) MEAN GROWTH AND DEVELOPMENTAL TIME OF FALL ARMYWORM AFTER HAVING FED ON SUSCEPTIBLE, LOW-RESISTANCE, INTERMEDIATE AND HIGH-RESISTANCE SILK-DIETS OVER FIVE GENERATIONS.

Treatment[a]	9-d wt. (mg) larvae	Day to pupation	Wt. (mg) of pupae	Day to adult eclosion
Generation 5				
Susceptible	292 b	13.2 b	227 b	21.1 a
Low-resistance	108 c	16.1 c	275 a	23.5 b
Intermediate resistance	46 d	17.4 d	212 c	25.9 c
High-resistance	10 e	24.4 e	183 d	31.8 d
SEM	12.7	0.26	5.38	0.30

Means within a column for each generation not followed by the same letter are significantly different (*k* ratio = 100, $P \leq 0.05$ [Waller & Duncan 1969]). Lab C is laboratory control larvae from the laboratory culture maintained on pinto bean diet; susceptible, diet of continuous pinto bean; low-resistance is diet of 12.5 mg dried resistant silks/ ml dilute pinto bean diet; intermediate-resistance is a diet of 25 mg dried resistant silks in dilute pinto bean diet; high resistance is a diet of 50 mg dried resistant silks in dilute pinto bean diet. SEM = standard error of the mean was based on pooled error mean square in the analysis.

For generation two, the weight of larvae at 9-d that fed on the intermediate and high resistant silk-diet were not significantly ($P \leq 0.05$) different (df = 14,4; F = 94.5) (Table 1), though the larvae fed on the intermediate resistant silk-diet were more than twice the weight of those fed on the high resistant silk-diet. The development time of larvae to pupation among the various resistance diet levels was significantly (P ≤ 0.05; F = 260.1) longer than for larvae that were fed on the two check diets. Development time for larvae that were fed on the high resistant silk-diet were only 2.5 d longer than for larvae that were fed on the intermediate silk-diet. As before, it appeared that the longer the larvae fed on a diet before pupation, compensation of weight occurred (P ≤ 0.05; F = 5.58). Again, the development time to adult eclosion was longer (P ≤ 0.05; F = 87.8) as the level of resistance in the silk-diets was increased.

Nine d weight and development time of larvae fed the various resistant silk-diets during generation three were significantly ($P \leq 0.05$) (P ≤ 0.05; F = 129.0) less than those that were fed the susceptible checks (Table 1). Larvae that were fed the high resistant silk-diet weighed significantly less than those fed the intermediate silk-diet. Weight of pupae for larvae fed the various diets was similar to those in generations one and two (P ≤ 0.05; F = 16.7). Development time to adult eclosion was clearly separable (P ≤ 0.05; F = 238.0) among the various levels of resistant silk-diets. Larvae that were fed on the high resistant silk-diet required the longest time before eclosion.

For generations four and five clearer separations occurred among the various treatment levels for 9-d weight of larvae, development time of larvae, weight of pupae and days to adult eclosion (Table 1). The weight of larvae at 9-d that were fed the high resistant silk-diets weighed significantly ($P \leq 0.05$) (df = 14, 4) (F = 130.8, 298.5) less than larvae fed the intermediate silk-diets for both generation four and five. Weight of pupae were also significantly less for larvae feeding on the high resistant silk-diet for both generations (P ≤ 0.05; F = 45.0, 62.8). Likewise, days to adult eclosion was similar for generation four and five in that larvae feeding on the high resistant silk-diet (P ≤ 0.05; F = 300.8, 176.8) required the longest to emerge as adults.

From the data reported here on fall armyworms maintained on various levels of resistant silk-diets, it appears that the larvae were unable to adjust to the resistant

TABLE 2. ESTIMATED MEAN EGG PRODUCTION OF FALL ARMYWORM ADULT FEMALES REARED FROM LARVAE ON VARIOUS LEVELS OF RESISTANT SILK-DIETS.

	Generation				
Treatment[1]	1	2	3	4	5
Lab C	—	961 a	1082 a	1081 a	1004 a
Susceptible	960 a	952 a	911 c	912 b	788 b
Low-resistance	886 ab	793 b	1021 ab	965 b	1040 a
Intermediate resistance	853 ab	848 b	764 d	698 c	709 c
High-resistance	823 b	867 b	951 bc	441 d	592 d
SEM	35	30	30	36	29

[1]Fecundity per female was based on the regression equation of Leuck & Perkins (1972) where $\hat{Y} = 5.33X - 423.23$ and X = average weight of pupae/replicate/treatment. Means within a column for each generation not followed by the same letter are significantly different (*k* ratio = 100, $P \leq 0.05$ [Waller & Duncan 1969]). Lab C is laboratory control larvae from the laboratory culture maintained on pinto bean diet; susceptible, diet of continuous pinto bean; low-resistance is diet of 12.5 mg dried resistant silks/ ml dilute pinto bean diet; intermediate-resistance is a diet of 25 mg dried resistant silks/ml dilute pinto bean diet; high resistance is a diet of 50 mg dried resistant silks/ml dilute pinto bean diet. SEM = standard error of the mean was based on pooled error mean square in the analysis.

silks even after five generations. Differences were detected between the two checks (generation two-five) among the various parameters measured in only six of sixteen comparisons. Three of these instances were for weight of pupae where compensation appeared when larvae fed longer on the various resistant diets. However, only one difference between the two checks occurred for development time of larvae and that was in generation five. The mean separations for the various parameters measured became more distinct for larvae that were fed the resistant silk- diets as the number of generations increased.

The estimated fecundity of fall armyworm fed the various silk-diets varied among treatment levels (Table 2) ($P \leq 0.05$). It appeared that the intermediate and high resistant silk-diets that were fed to larvae reduced the fecundity of females emerging from these diets, especially in generations four and five (F = 16.7, 62.8). Though the estimated fecundity was not greatly reduced, there certainly was some impact on fecundity to the higher levels of resistance as compared to earlier generations.

In summary, in each generation, weight of larvae was significantly lower and development time was significantly longer for larvae that were fed the high-resistant diets than for larvae fed the intermediate silk-diet and especially for larvae fed the other treatment diets. Estimated fecundity was reduced, especially for generation four and five for larvae that were fed the high resistant silk-diet. Though the estimated fecundity was not greatly reduced in all generations, there were no apparent adjustments to the stress of resistance occurring in any of the five parameters measured after five generations. Thus, fall armyworm fed on various resistant levels of silk-diets did not appear to adjust to the stress of the various levels of resistance after five generations.

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