Stadia, Larval-Pupal Weight, and Width of Head Capsules of Corn Earworm (Lepidoptera: Noctuidae) After Feeding on Varying Resistance Levels of Maize Silks[1]

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ABSTRACT Weight of larvae, developmental time to pupation, weight of pupae, length of stadia, and width of head capsule were determined for larvae of the corn earworm, *Helicoverpa zea* (Boddie), that were fed on meridic diets with silks of 'Stowell's Evergreen', a susceptible genotype, or low, intermediate, and high levels of 'Zapalote Chico', a corn, *Zea mays* L., cultivar with antibiotic resistance. As the level of resistance increased, the weight of larvae at 9 days and weight of pupae significantly decreased, and developmental time to pupation significantly increased. Also, as the concentration of resistant material increased in the diet, stadial length also significantly increased. The intermediate and high levels of resistance also resulted in a significant reduction in the width of the head capsule of larvae. Thus, two new characteristics of resistance in maize silks to the corn earworm were identified: increased stadia and decreased width of head capsule.

KEY WORDS Insecta, *Helicoverpa zea*, antibiosis, corn.

Resistance in crop cultivars to insect pests has been reported to be the "most effective and ideal method of combating insects" (Luginbill 1969). The use of a resistant cultivar for pest management is biologically, ecologically, economically, and socially feasible (Teetes 1985). Headley (1979) suggested that "resistant varieties" of crop plants would have an increasingly important role in the control of insect pests until 1992, and that beyond that period of time the demand for resistant cultivars should increase, with greater emphasis on ground water quality, and decreasing emphasis on pesticides for the control of insect pests.

The effects of the resistance in plants to insects as a primary method of control have been specific, cumulative, and persistent (Dahms 1972). Resistance in plants to insect pests may be expressed as one or a combination of the three mechanisms, nonpreference, antibiosis, or tolerance (Painter 1951). All three mechanisms of resistance have been defined in corn cultivars that show resistance to the corn earworm, *Helicoverpa zea* (Boddie) (Wiseman *et al.* 1972, 1977, and 1983). Wiseman and Isenhour (1990) showed that the resistance to *H. zea* larvae by 'Zapalote Chico' was specific for several biological characters (lower larval weights, longer developmental time, lower pupal weights, and reduced fecundity) and that the effects of the resistance were cumulative and persistent over four generations.

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Here we report the effects of larval feeding on diets with various levels of resistance on the rate of corn earworm instar development as well as development within instars and width of instar head capsule.

Materials and Methods

The corn earworm larvae used in this study were obtained from a colony maintained at the Insect Biology and Population Management Research Laboratory, Tifton, GA (Perkins *et al.* 1973).

The maize silks were produced from two genotypes, a susceptible genotype, 'Stowell's Evergreen' (SEG), and a resistant genotype, 'Zapalote Chico 2451# (PC3)' (ZC) (Wiseman and Wilson 1987). Both genotypes were grown in the field using accepted agronomic practices. Open pollinated silks of each cultivar were harvested after the silks had emerged for 2 days. Silks were excised at the ear tip, removed from the husk channel, bulked by cultivar, and oven-dried at 41°C for 10 days. The dried silks were then finely ground (1 mm screen) using a Cyclotec® 1093 sample mill (Atlanta, GA).

Pinto bean diet (Perkins *et al* 1973) was obtained in bulk from the rearing section of the Insect Biology and Population Management Research Laboratory. For each treatment, the standard pinto-bean diet was diluted by 3:1 with distilled water. Levels of resistance were obtained by incorporating different concentrations of ZC silks in the diet, or SEG silks for the susceptible check. Treatments were 1) susceptible check = SEG at 25 mg of dry silk/ml diet; 2) low level of resistance = ZC at 25 mg of dry silk/ml diet; 3) intermediate level of resistance = ZC at 50 mg of dry silk/ml of diet; 4) high level of resistance at 75 mg of dry silk/ml of diet; and 5) a check diet of dilute pinto bean. The diet mixtures were dispensed into 30 ml plastic diet cups (36) at about 10 ml/cup and allowed to cool and solidify at room temperature. One corn earworm neonate was placed on top of the diet and the cup was capped. The test was repeated three times; the first test was maintained at 23.9 ± 2°C, 75 ± 5% relative humidity and a photoperiod of 14:10 (L:D), and the second and third tests were maintained at 26.7 ± 2°C and 80 ± 5% RH and a photoperiod of 14:10 (L:D). All treatments of each test were arranged as a randomized complete block design (all treatments blocked within each replication) with 36 replications and 1 cup/replicate. Data from the first test included the weight of larvae at 9 days, development time to pupation, weight of pupae, and stadia. Data from tests 2 and 3 included stadia and width of head capsule for each instar, using an ocular micrometer. Larvae were observed twice daily for cast head capsules which were indicative of a change in instar.

Statistical analyses were applied to the data from each measured parameter (SAS Institute, 1985) and significantly different means were separated by Waller-Duncan k-ratio t-test at k-ratio = 100 and $P < 0.05$ (Waller and Duncan, 1969).

Results and Discussion

The effects that various low, intermediate, and high concentrations of resistant corn silks had on the growth and development of corn earworm larvae are illustrated in Figure 1. Initially, even larvae fed diet with susceptible silks showed a significant reduction in weight at 9 days compared with larvae fed the bean diet check. However, there were no significant differences between these two treatments in

weight of pupae (Fig. 1). Conversely, the diet containing 25 mg/ml of ZC silks (low level of resistance) resulted in more than a 50% decrease in weight of larvae at 9 days compared with larvae that fed on the susceptible and bean diet check. The larvae on the diet containing 50 mg/ml of ZC silks (intermediate level of resistance) were much smaller than those fed on the diet with a low level resistance, yet the significantly longer developmental time for the larvae on the former diet apparently allowed time to consume more diet and attain a pupal weight comparable with those from the low level of resistance. Larvae that were fed on the diets with intermediate (50 mg/ml) and high levels of resistance (75 mg of ZC silk/ml diet) showed an even greater reduction of larval weight compared with those that were fed the other diets. Lastly, the weight of pupae showed an inverse relationship with the amount of resistant silks added to the diet (Fig. 1).

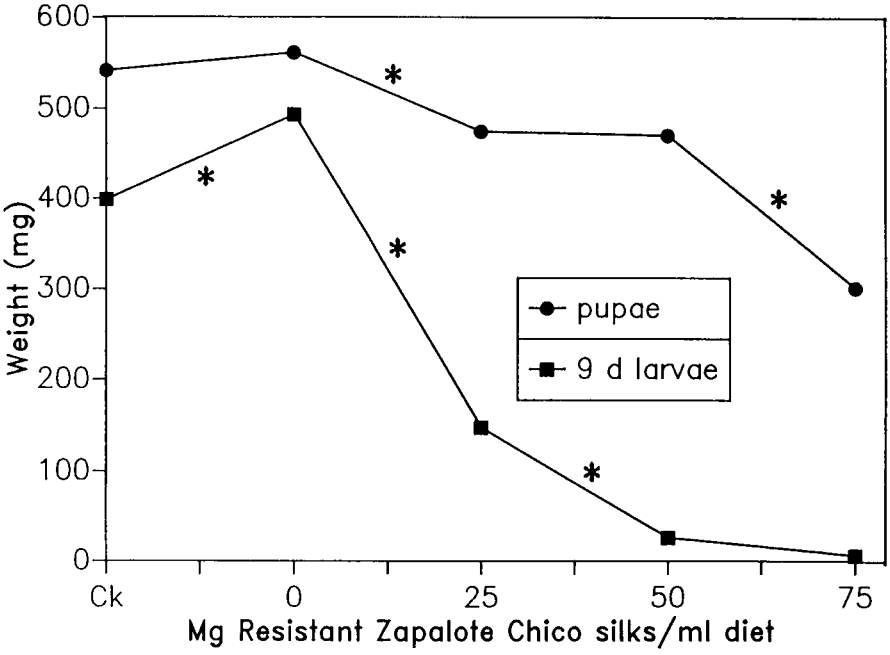

Fig. 1. Mean weight of larvae at 9 days and weights of pupae of corn earworm that were fed diets with various levels of resistant silks of maize. Ck = 'Stowell's Evergreen'. * = Means significantly different ($P < 0.05$ and k-ratio = 100; Waller and Duncan 1969).

The cumulative developmental times for the various instars, prepupae, and pupae for the corn earworm larvae that were fed the diets with various levels of resistance are shown in Table 1. Combined analyses of the data for each instar for Tests 1-3 revealed a significant test by instar interaction, indicating that the magnitude of instar length was not the same for each test. Thus, the data are presented accordingly (Table 1). In greater than 80% of the cases, larvae fed the diet with a low level of resistance had a significantly longer development time to pupation than those larvae that were fed on the susceptible diets. In every case, larvae fed the intermediate and high level of resistance were significantly slower in developing to pupation compared with those that were fed the low level of resistance. This prolonged development was also true for the stadial length of each instar for larvae that were fed the intermediate and high levels of resistance. In addition, more than 40% and 90% of the larvae fed the intermediate and high level of resistance, respectively, developed to the sixth larval instar (Test 1). However, for Tests 2 and 3, only those larvae (50%) fed the high level of resistance developed into the sixth instar. This may be due to the lower temperature and humidity in Test 1 than in Tests 2 and 3.

Wiseman and Isenhour (1990) demonstrated in a laboratory study that low levels of resistant maize silks in diets reduced corn earworm larval growth and extended the life cycle by 3 days. An intermediate level of antibiotic resistant silks reduced larval growth, extended developmental time by about 8 days per generation, and subsequently reduced fecundity by approximately 30%. The high concentration of antibiotic silks reduced corn earworm growth, extended the life cycle by about 20 days, and reduced fecundity by about 65% per generation. They predicted that the intermediate levels of antibiotic resistance could eliminate about two generations of corn earworms per year, whereas the high level of antibiotic resistance could reduce the number of corn earworm generations by 40-50% per year.

Again, combined analyses of the data for each instar head capsule width revealed a significant test by instar interaction. Thus, the data for Tests 2 and 3 are presented separately. Yet, there were no differences in head capsule widths of the second instars that were fed the various diets (Table 2). However, differences were detected in Test 3, but not in Test 2 for the third instar larvae fed the high level of resistance. The third instar fed the higher concentration of silk/diet had a significantly smaller head capsule width than larvae fed the low level of resistance, and larvae fed all levels of resistance had smaller capsule widths than larvae fed the susceptible diets. The fourth and fifth instars fed the intermediate and high levels of resistance had significantly smaller head capsule width than larvae fed the susceptible or bean diet check. Only larvae that were fed the intermediate and high levels of resistance (Test 2) or high level of resistance (Test 3) developed a sixth instar.

Dyar (1890) reported that widths of lepidopteran head capsules follow a regular geometrical progression unless the larva behaved abnormally. However, Ghent (1956) reported that, in some cases, head capsule width follows a linear progression. Corn earworm larvae fed the intermediate and high levels of resistance developed slower than normal compared with larvae fed the susceptible or bean diet check. Therefore, the differences in head capsule widths recorded from larvae fed diets with higher concentrations of resistant silks compared with larvae fed the susceptible diets follow a progression similar to that reported by Gunasena et al. (1989).

Table 1. Cumulative developmental time for corn earworm larvae that were fed diets with various levels of resistant silks of maize.

Treatments	$\bar{X}$ cumulative developmental time in days to stage:*						
	Instar						
	2nd	3rd	4th	5th	6th	Prepupae	Pupae
Test I							
Bean diet check	2.9 a	4.6 a	6.3 a	8.5 a	-	11.6 a	14.4 a
Susceptible check	2.9 a	4.7 a	6.3 a	8.7 a	-	11.8 a	14.9 a
Low resistance	3.2 b	5.3 b	7.9 b	10.9 b	-	14.7 b	17.3 b
Intermediate resistance	3.8 c	8.0 c	9.0 c	12.7 c	15.6	18.1 c	21.1 c
High resistance	4.8 d	9.0 d	12.5 d	16.6 d	19.5	26.3 d	30.4 d
Mean	3.5	6.3	8.4	11.4	-	16.1	19.0
Mean SE	0.25	0.48	0.67	0.91	-	2.86	3.39
Test 2							
Bean diet check	2.0 a	4.0 a	5.3 a	7.3 a	-	10.2 a	12.1 a
Susceptible check	2.0 a	4.0 a	5.9 b	7.9 b	-	10.8 a	13.1 b
Low resistance	2.2 b	4.1 b	6.1 c	8.3 c	-	12.1 b	14.1 c
Intermediate resistance	3.0 c	5.0 c	7.0 d	9.8 d	-	14.6 c	17.8 d
High resistance	3.3 d	5.3 d	7.5 e	10.9 e	15.6	21.0 d	24.7 e
Mean	2.5	4.5	6.3	8.8	-	13.7	16.4
Mean SE	0.08	0.05	0.14	0.36	-	3.87	3.71
Test 3							
Bean diet check	2.2 a	4.0 a	5.4 a	7.7 a	-	10.7 a	13.0 a
Susceptible check	2.1 a	4.1 a	5.9 b	8.0 a	-	11.5 b	13.8 a
Low resistance	2.0 a	4.2 a	6.4 c	9.1 b	-	13.1 c	15.7 b
Intermediate resistance	2.6 b	5.1 b	8.1 d	11.1 c	-	16.2 d	18.8 c
High resistance	3.1 c	5.8 c	8.9 e	13.8 d	21.4	29.9 e	33.7 d
Mean	2.4	4.6	6.9	9.9	-	16.3	19.0
Mean SE	0.19	0.32	1.28	1.99	-	3.25	5.02

* Means within a column for each test followed by the same letter are not significantly different ($P < 0.05$ and k-ratio = 100; Waller-Duncan 1969). Susceptible = 25 mg/ml diet of Stowell's Evergreen silk. Low, intermediate, and high resistance = 25, 50, and 75 mg, respectively, of Zapalote Chico silks/ml diet.

Gunasena et al. (1989) found that head capsule widths of larvae that produced a sixth instar were smaller than those that produced only five normal instars.

In summary, the increased length of time required for each instar, increased number of instars, and decreased head capsule widths in response to ingestion of resistant plant material are new characteristics which may be utilized to describe the adverse effects of the resistant plant on the development of the insect pest. Stadia of corn earworm larvae were significantly extended in >80% of the instances for larvae fed the low level of resistance. The stadia for the instars, prepupal, and pupal stages were significantly extended in every case for larvae fed the intermediate and high levels of resistance. Head capsule widths were also significantly smaller for later instars developing on the intermediate and high levels of resistance. Furthermore, larvae fed the high level of resistance consistently developed an extra, sixth instar.

Table 2. Widths of head capsules of corn earworm larvae that were fed diets with various levels of resistant silks of maize.

Treatments	$\bar{X}$ width (mm) of head capsule*				
	Instar				
	2nd	3rd	4th	5th	6th
Test 2					
Bean diet check	0.36 a	0.57 a	1.09 a	1.99 a	-
Susceptible check	0.33 a	0.56 a	1.09 a	2.00 a	-
Low resistance	0.34 a	0.57 a	1.08 a	2.00 a	-
Intermediate resistance	0.33 a	0.56 a	1.03 b	1.97 a	2.20 a
High resistance	0.34 a	0.56 a	1.01 b	1.80 b	2.20 a
Mean	0.34	0.56	1.06	1.96	-
Mean SE	-	-	0.03	0.22	-
Test 3					
Bean diet check	0.30 a	0.62 a	1.09 a	2.13 a	-
Susceptible check	0.28 a	0.55 b	1.08 ab	2.13 a	-
Low resistance	0.30 a	0.51 c	1.05 abc	2.06 b	-
Intermediate resistance	0.31 a	0.49 cd	1.03 bc	1.98 c	-
High resistance	0.31 a	0.48 d	1.00 c	1.87 d	2.36
Mean	0.30	0.53	1.05	2.03	-
Mean SE	0.02	0.03	0.06	0.14	-

* Means within a column for each test followed by the same letter are not significantly different ($P < 0.05$ and k-ratio = 100; Waller-Duncan 1969). Susceptible = 25 mg/ml of Stowell's Evergreen silk. Low, intermediate, and high resistance = 25, 50, and 75 mg, respectively, of Zapalote Chico silks/ml diet.

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