

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural
Research Service, Lincoln, Nebraska

1995

Development of Maize Weevils (Coleoptera: Curculionidae) on Corn Lines Resistant to an Aflatoxin-Producing Fungus

James E. Throne

USDA-ARS, Manhattan, KS, james.throne@ars.usda.gov

James E. Baker

USDA-ARS

Gene E. Scott

USDA-ARS

Follow this and additional works at: <https://digitalcommons.unl.edu/usdaarsfacpub>

Throne, James E.; Baker, James E.; and Scott, Gene E., "Development of Maize Weevils (Coleoptera: Curculionidae) on Corn Lines Resistant to an Aflatoxin-Producing Fungus" (1995). *Publications from USDA-ARS / UNL Faculty*. 1996.

<https://digitalcommons.unl.edu/usdaarsfacpub/1996>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Development of Maize Weevils (Coleoptera: Curculionidae) on Corn Lines Resistant to an Aflatoxin-Producing Fungus

JAMES E. THRONE,¹ JAMES E. BAKER,¹ AND GENE E. SCOTT²

Stored-Product Insects Research and Development Laboratory, USDA-ARS, 3401 Edwin Street, Savannah, GA 31405

Environ. Entomol. 24(4): 944-949 (1995)

ABSTRACT Susceptibility to the maize weevil, *Sitophilus zeamais* Motschulsky, of corn lines bred for their resistance to the aflatoxin-producing fungus *Aspergillus flavus* Link ex Fries was determined in the laboratory. Duration of maize weevil development, number of progeny produced, and an index of susceptibility varied with corn line; number of eggs laid and survivorship from egg to adult emergence did not vary with corn line. Soluble carbohydrate, kernel hardness, lipid, soluble nitrogen, free and bound phenolic acids, Kjeldahl protein, and protein/carbohydrate ratio varied with corn line; equilibrium moisture content did not vary with corn line. The only relationship between biological parameters of maize weevils and chemical and physical properties of the stored corn that was predictable was that maize weevil development time increased as lipid content of the corn increased. Susceptibility to *A. flavus* infection in the field was not correlated with susceptibility to maize weevil population growth or with chemical or physical properties of the stored corn. Predicted weevil population growth over a 180-d period varied 50-fold among the corn lines. Some of the corn lines that are less susceptible to infection by *A. flavus* in the field were also less susceptible to maize weevils (particularly Mp420 and SC54).

KEY WORDS *Sitophilus zeamais*, aflatoxin, host plant resistance

INFECTION OF CORN, *Zea mays* L., kernels by aflatoxin-producing fungi is a chronic problem in the southeastern United States. Development of corn varieties resistant to aflatoxin-producing fungi is 1 method for reducing aflatoxin contamination of corn (Scott and Zummo 1988). However, most studies have concentrated only on resistance of preharvest corn to infection by aflatoxin-producing fungi, particularly *Aspergillus flavus* Link ex Fries. Dowd (1994) showed that an *A. flavus* resistant corn line (Mp313e) was cross resistant to insect vectors, *Carpophilus hemipterus* (L.) and *C. free-mani* Dobson (Coleoptera: Nitidulidae), of *A. flavus* in preharvest corn. Damage to stored corn by insects may increase *A. flavus* infection by providing a path for entry into the kernel and by increasing moisture content of the kernel (Sinha and Sinha 1992). Therefore, development of corn lines resistant both to infection by aflatoxin-producing fungi and storage insects is desirable for reducing aflatoxin contamination of stored corn.

Resistance of corn to insects and fungi may be associated with physical and chemical characteris-

tics of the corn. Resistance to the maize weevil, *Sitophilus zeamais* Motschulsky, an insect pest of stored corn has been associated with increased ferulic acid content of corn (Classen et al. 1990). Resistance to *Fusarium graminearum* Schwabe, a mycotoxin-producing fungus, has also been associated with ferulic acid content of corn (Assabgui et al. 1993).

The objective of our study was to determine the susceptibility to maize weevils of 8 inbred corn lines relatively resistant to infection by *A. flavus* (Scott and Zummo 1988). Maize weevils were chosen for the study because they are a major pest of stored corn throughout the corn-growing regions of the world (Throne 1986). We also determined whether infection of corn by *A. flavus* in the field and biological parameters of maize weevils on stored corn are correlated with several chemical and physical properties of the stored corn.

Materials and Methods

Corn Lines. The lines used in this study differed in their susceptibility to kernel infection by *A. flavus* in the field (Scott and Zummo 1988). Reported kernel infection levels were 5.6% for Mp313E, 6.0% for SC54, 6.1% for Mp420, 6.4% for Mo18W, 7.8% for SC76, 8.7% for TX601, 11.0% for Mp317, 12.7% for SC229, 16.8% for Mp68:616, and 30.1% for SC212M. 'Pioneer 3320',

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

¹U.S. Grain Marketing Research Laboratory, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502.

²Corn Host Plant Resistance Research Unit, USDA-ARS, P.O.B. 5248, Mississippi State, MS 39762.

Table 1. Biological parameters, mean \pm SD, of maize weevils reared on corn lines differing in their susceptibility to *A. flavus* infection

Corn line	No. eggs laid ^a	Duration of development, d ^b	Progeny produced ^c	Survivorship ^d	Susceptibility index ^e
Mo18W (R)	53.0 \pm 3.61	33.1 \pm 1.11ab	42.7 \pm 5.51ab	0.81 \pm 0.11	0.113 \pm 0.0066bcd
Mp313E (R)	65.7 \pm 7.02	31.2 \pm 0.18c	51.7 \pm 5.86a	0.79 \pm 0.06	0.126 \pm 0.0040a
Mp317 (R)	52.7 \pm 10.21	31.6 \pm 0.93bc	37.3 \pm 7.23bc	0.71 \pm 0.03	0.114 \pm 0.0085bcd
Mp420 (R)	46.7 \pm 3.21	33.5 \pm 0.75a	34.7 \pm 5.51bc	0.74 \pm 0.10	0.106 \pm 0.0050d
SC54 (R)	56.0 \pm 5.66 ^f	34.0 \pm 1.71a	37.0 \pm 4.24bc ^f	0.64 \pm 0.04	0.109 \pm 0.0062cd ^f
SC76 (R)	48.0 \pm 8.00	30.8 \pm 1.62cd	36.0 \pm 2.65bc	0.76 \pm 0.10	0.117 \pm 0.0082abc
SC229 (R)	55.3 \pm 4.93	31.6 \pm 0.40bc	40.7 \pm 0.58bc	0.74 \pm 0.07	0.117 \pm 0.0019abc
TX601 (R)	51.0 \pm 2.83 ^f	31.9 \pm 0.97bc	38.0 \pm 4.24bc ^f	0.69 \pm 0.13	0.116 \pm 0.0008abcd ^f
Mp68:616 (S)	55.0 \pm 12.73 ^f	29.4 \pm 0.63d	40.0 \pm 9.90bc ^f	0.79 \pm 0.10	0.124 \pm 0.0068ab ^f
SC212M (S)	44.7 \pm 9.61	32.9 \pm 0.54ab	31.7 \pm 4.73c	0.72 \pm 0.05	0.105 \pm 0.0027d
Pioneer 3320 (ND)	45.5 \pm 6.36 ^f	30.6 \pm 0.05cd	32.0 \pm 0.00bc ^f	0.70 \pm 0.07	0.113 \pm 0.0002bcd ^f
MSD ^g	— ^a	1.60	10.9	— ^d	0.011

Letters after the names of the corn lines indicate whether the lines are relatively resistant (R) or susceptible (S) to *A. flavus* infection, or whether susceptibility has not been determined (ND). Means within a column followed by the same letter are not significantly different ($\alpha = 0.05$; Waller-Duncan *k*-ratio test, $k = 100$ (SAS Institute 1988)).

^a Number of eggs laid did not vary with corn line ($F = 1.93$; $df = 10, 18$; $P = 0.11$).

^b Duration of development varied with corn line ($F = 6.43$; $df = 10, 22$; $P < 0.01$).

^c Number of progeny produced varied with corn line ($F = 3.27$; $df = 10, 18$; $P = 0.01$).

^d Survivorship from egg to adult emergence did not vary with corn line ($F = 1.01$; $df = 10, 22$; $P = 0.47$).

^e The Dobie index of susceptibility varied with corn line ($F = 4.00$; $df = 10, 18$; $P = 0.01$).

^f This mean is based on 2 observations. Other means are based on 3 observations.

^g A confidence interval can be calculated to determine by how much any 2 means differ. Calculate the confidence interval as $[\text{mean}_1 - \text{mean}_2] \pm \text{MSD}$, where MSD (shown at the bottom of each column) is the minimum significant difference calculated using the Waller-Duncan *k*-ratio test. For example, for duration of development on SC54 and Mp68:616, the confidence interval is $[34.0 - 29.4] \pm 1.60$, or 3.0 to 6.2. Thus, the true difference between the 2 means is between 3.0 and 6.2 d.

a commercial dent corn hybrid commonly grown in the southeastern United States, was also included; however, we do not know its susceptibility to *A. flavus* infection.

Insect Development. Weevils were from a culture maintained at $25 \pm 1^\circ\text{C}$, 65–70% RH, and a photoperiod of 12:12 (L:D) h. The culture was founded with weevils collected from grain storage areas in southern South Carolina in 1985, and weevils from the field were added to the culture monthly. Weevil generations were separated so that weevils only recently removed from the field were used for experiments. Voucher specimens were placed in the Florida State Collection of Arthropods (numbers: JT1–JT6).

The corn was X-rayed (Throne 1994) to ensure that no insects were present. Lots of 30.0 ± 0.1 g of each corn line were placed individually in each of three 40-dram vials. A 35-mm-diameter hole in the bottom of the vial and in the lid were covered with 64- μ -mesh nylon screen for ventilation. These vials were placed randomly on a perforated false floor in 3 covered plastic boxes (40 by 27.5 by 16 cm high; 1 replication of each corn line in each box). The boxes each contained a saturated sodium chloride (NaCl) solution below the false floor to maintain relative humidity at 75% (Greenspan 1977). An additional cage containing a 300-g sample of Pioneer 3320 corn was placed in each box and the moisture content of those 300-g samples was measured weekly with a moisture tester (Mottoco model 919 automatic grain moisture tester, Dickey-John, Auburn, IL). The boxes were placed in an environmental chamber maintained at $30 \pm$

1°C and a photoperiod of 12:12 (L:D) h, and the corn was equilibrated for 6 wk to these conditions before the insect bioassays were conducted.

After the 6-wk equilibration period, 5 females (3- to 4-wk-old F_3) were placed in each cage. Sex was determined by snout characteristics (Tolpo and Morrison 1965) after immobilization of weevils by chilling. Females were sieved from the cages after 72 h using a U.S. standard no. 6 sieve (3.36-mm openings), and species and sex were confirmed based on examination of genitalia (Halstead 1962) because of the external morphological similarity of the maize weevil to the rice weevil, *S. oryzae* (L.). Starting 2 wk after females were removed, emerging adult progeny were sieved from the corn every day. After all adults had emerged, each lot of corn was X-rayed (Throne 1994) to determine the presence of any weevil stage. This allowed us to determine number of eggs laid and rate of survival from egg to adult emergence.

An index (Dobie 1974) was used to compare the susceptibility to maize weevils of the corn lines. This index is calculated as $(\log_e Y)/T$, where Y = number of progeny produced on a given corn line and T = duration of development on that corn line. This index incorporates number of eggs laid, survival to the adult stage, and development time because number of progeny produced is based on the number of eggs laid and survival to the adult stage. Larger index values indicate greater susceptibility to maize weevils. Dobie (1974) suggested that this index approaches the intrinsic rate of increase, r_m . We used the susceptibility index as an

Table 2. Predicted number of adult maize weevils when 1 pair is reared for up to 180 d on corn lines differing in their susceptibility to *A. flavus* infection

Corn line	Day		
	60	120 ^a	180 ^a
SC212M (S)	1,070	0.57	310
Mp420 (R)	1,122	0.63	350
SC54 (R)	1,401	0.98	690
Pioneer 3320 (ND)	1,771	1.57	1,390
Mo18W (R)	1,792	1.61	1,440
Mp317 (R)	1,892	1.79	1,690
TX601 (R)	2,057	2.12	2,180
SC76 (R)	2,198	2.42	2,650
SC229 (R)	2,278	2.60	2,960
Mp68:616 (S)	3,325	5.53	9,190
Mp313E (R)	3,933	7.73	15,210

Letters after the names of the corn lines indicate whether the lines are relatively resistant (R) or susceptible (S) to *A. flavus* infection, or whether susceptibility has not been determined (ND).

^a Predicted number of maize weevils after 120 and 180 days, in millions.

estimate of r_m to calculate population growth over a 6-mo storage period as:

$$N_t = N_{t-1}e^{r_m t},$$

where N_t = number of weevils at time t .

Chemical and Physical Analyses. Relative size of kernels was determined by counting the number of kernels in each of three 10-g samples of each corn line. Lots of 15.0 ± 0.1 g of each corn line were equilibrated at 30°C over an NaCl solution for use in determining kernel hardness and equilibrium moisture content. Relative kernel hardness was determined by grinding 3 replicates (3.0 ± 0.1 g each) of each corn line in a 50-ml cup of a blender for 20 s, and determining the ratio of the portion of the corn held over a U.S. standard no. 35 sieve (0.5-mm openings) to the portion passing through the sieve (Dobie 1974). Equilibrium moisture content was determined using the air-oven method (method 44-15A, AACC 1969) and was assumed to be indicative of equilibrium moisture content in the insect bioassays.

Lots of 30.0 ± 0.1 g of each corn line were ground over a U.S. standard no. 40 sieve (0.425-mm openings) in a laboratory mill and analyzed for total Kjeldahl nitrogen, soluble nitrogen, soluble carbohydrate, total lipid, soluble free phenolics, and bound phenolics. Total Kjeldahl nitrogen determined by the method of Lang (1958) was based on analysis of 6 replicate 10-mg samples of each corn line, and expressed as percentage of protein ($N \times 6.25$) based on sample dry weight.

Soluble nitrogen was determined by extracting 100 mg samples of each corn line 5 times with 1 ml 0.15 M NaCl followed by brief centrifugation, combining supernatants, and analyzing 1-ml aliquots by the method of Lang (1958). Results are based on 4 replicates of each corn line and expressed as a percentage of dry weight.

Table 3. Physical properties, mean \pm SD, of corn lines differing in their susceptibility to *A. flavus* infection

Corn line	Kernel hardness ^a	% Moisture content ^b	No. kernels ^c
Mo18W (R)	2.43 \pm 0.26d	13.9 \pm 0.10	47.0 \pm 1.73e
Mp313E (R)	2.03 \pm 0.15f	13.9 \pm 0.31	30.0 \pm 0.00g
Mp317 (R)	2.15 \pm 0.08ef	14.2 \pm 0.01	40.3 \pm 0.58f
Mp420 (R)	2.89 \pm 0.15b	13.5 \pm 0.28	39.3 \pm 0.58f
SC54 (R)	3.38 \pm 0.20a	13.6 \pm 0.24	49.7 \pm 1.15d
SC76 (R)	2.72 \pm 0.04bc	13.8 \pm 0.34	57.0 \pm 2.00b
SC229 (R)	3.37 \pm 0.19a	13.8 \pm 0.20	52.3 \pm 1.53c
TX601 (R)	2.37 \pm 0.25de	13.8 \pm 0.65	52.0 \pm 1.73c
Mp68:616 (S)	2.87 \pm 0.22b	13.6 \pm 0.34	64.3 \pm 1.53a
SC212M (S)	2.48 \pm 0.12cd	13.7 \pm 0.38	51.7 \pm 0.58cd
Pioneer 3320 (ND)	1.77 \pm 0.01g	14.8 \pm 0.15	31.3 \pm 2.52g
MSD ^d	0.26	— ^b	2.2

Letters after the names of the corn lines indicate whether the lines are relatively resistant (R) or susceptible (S) to *A. flavus* infection, or whether susceptibility has not been determined (ND). Means within a column followed by the same letter are not significantly different, $k = 100$ ($\alpha = 0.05$; Waller-Duncan k -ratio test [SAS Institute 1988]).

^a Kernel hardness varied with corn line ($F = 27.70$; $df = 10, 22$; $P < 0.01$; $n = 3$).

^b Equilibrium moisture content did not vary with corn line ($F = 2.69$; $df = 10, 11$; $P = 0.06$; $n = 2$).

^c Number of kernels per 10-g sample varied with corn line ($F = 158.41$; $df = 10, 22$; $P < 0.01$; $n = 3$).

^d A confidence interval can be calculated to determine by how much any 2 means differ. Calculate the confidence interval as $[\text{mean}_1 - \text{mean}_2] \pm \text{MSD}$, where MSD (shown at the bottom of each column) is the minimum significant difference calculated using the Waller-Duncan k ratio test. For example, for SC54 and Pioneer 3320, the confidence interval for hardness is $[3.38 - 1.77] \pm 0.26$, or 1.35–1.87. Thus, the true difference between the 2 hardness ratios is between 1.35 and 1.87.

Soluble carbohydrate was determined by extracting 50-mg samples of each corn line 4 times with 5 ml H₂O for 5 min at 80°C. Supernatants were combined, brought to 50 ml volume, and 200- μ l aliquots assayed for carbohydrate (as glucose) with the method of Dubois et al. (1956). Results are based on 5 replicates per corn line and expressed as a percentage of dry weight.

Lipid content was estimated by extracting 5-g samples of ground corn with 180 ml n-hexane for 6 h in a Soxhlet extractor. Solvent was removed in tared 100-ml flasks on a rotoevaporator, and extract weight determined. Results are based on extraction of 3 replicates of each corn line and expressed as a percentage of sample dry weight.

Free phenolic acids were extracted from 1-g samples of the defatted ground corn (after lipid extraction) by the method of Krygier et al. (1982) and analyzed with the procedures of Swain and Hillis (1959) and Singleton and Rossi (1965). Results are based on 3 replicate samples of each corn line and expressed as chlorogenic acid equivalents.

Following removal of free phenolic acids, bound phenolics in the corn samples were released by alkaline hydrolysis and isolated with the procedures of Krygier et al. (1982) and Sosulski et al. (1982). Bound phenolic acids were analyzed and expressed as chlorogenic acid equivalents as above.

Table 4. Chemical properties, mean \pm SD, of corn lines differing in their susceptibility to *A. flavus* infection

Corn line	% soluble carbohydrate ^a	% lipid ^b	% soluble nitrogen ^c	% bound phenolics ^d	% free phenolics ^e	% protein ^f
Mo18W (R)	10.7 \pm 1.85ef	4.97 \pm 0.42a	0.34 \pm 0.039de	0.260 \pm 0.021b	0.143 \pm 0.021de	12.8 \pm 0.38d
Mp313E (R)	14.0 \pm 1.30ab	4.77 \pm 0.32abc	0.38 \pm 0.039bc	0.215 \pm 0.033bcde	0.190 \pm 0.010ab	11.6 \pm 0.41e
Mp317 (R)	11.7 \pm 1.64cde	3.97 \pm 0.32d	0.33 \pm 0.018e	0.209 \pm 0.045bcde	0.147 \pm 0.021de	11.7 \pm 0.50e
Mp420 (R)	12.8 \pm 0.72bc	4.90 \pm 0.44ab	0.36 \pm 0.022cde	0.354 \pm 0.035a	0.207 \pm 0.012a	14.3 \pm 0.89c
SC54 (R)	9.8 \pm 0.18f	5.07 \pm 0.51a	0.47 \pm 0.017a	0.247 \pm 0.001bc	0.187 \pm 0.021ab	17.2 \pm 0.74a
SC76 (R)	11.2 \pm 1.50de	4.47 \pm 0.35abcd	0.37 \pm 0.015cd	0.205 \pm 0.017bcde	0.150 \pm 0.010cd	13.0 \pm 1.03d
SC229 (R)	9.6 \pm 0.89f	4.67 \pm 0.31abcd	0.35 \pm 0.015de	0.238 \pm 0.013bcd	0.100 \pm 0.017f	14.3 \pm 0.61c
TX601 (R)	12.3 \pm 1.21cd	4.23 \pm 0.31bcd	0.47 \pm 0.013a	0.191 \pm 0.027de	0.123 \pm 0.006ef	15.5 \pm 0.77b
Mp68:616 (S)	12.4 \pm 0.48cd	4.07 \pm 0.15cd	0.41 \pm 0.036b	0.259 \pm 0.003b	0.173 \pm 0.006bc	12.7 \pm 0.58d
SC212M (S)	12.2 \pm 1.28cd	4.80 \pm 0.53ab	0.30 \pm 0.019f	0.196 \pm 0.035cde	0.140 \pm 0.026de	12.6 \pm 0.52d
Pioneer 3320 (ND)	14.8 \pm 0.86a	4.43 \pm 0.33abcd	0.36 \pm 0.015cde	0.162 \pm 0.026e	0.138 \pm 0.010de	8.5 \pm 0.44f
MSD ^g	1.3	0.72	0.03	0.045	0.025	0.6

Letters after the names of the corn lines indicate whether the lines are relatively resistant (R) or susceptible (S) to *A. flavus* infection, or whether susceptibility has not been determined (ND). Means within a column followed by the same letter are not significantly different ($\alpha = 0.05$; Waller-Duncan k ratio test, $k = 100$ [SAS Institute 1988]).

^a Percentage soluble carbohydrate varied with corn line ($F = 12.41$; $df = 10, 49$; $P < 0.01$; $n = 5$, except $n = 10$ for Pioneer 3320).

^b Percentage lipid varied with corn line ($F = 2.96$; $df = 10, 23$; $P = 0.02$; $n = 3$, except $n = 4$ for Pioneer 3320).

^c Percentage soluble nitrogen varied with corn line ($F = 20.53$; $df = 10, 37$; $P < 0.01$; $n = 4$, except $n = 8$ for Pioneer 3320).

^d Percentage bound phenolics varied with corn line ($F = 8.54$; $df = 10, 13$; $P < 0.01$; $n = 2$, except $n = 4$ for Pioneer 3320).

^e Percentage free phenolics varied with corn line ($F = 12.29$; $df = 10, 23$; $P < 0.01$; $n = 3$, except $n = 4$ for Pioneer 3320).

^f Percentage protein varied with corn line ($F = 102.27$; $df = 10, 61$; $P < 0.01$; $n = 6$, except $n = 12$ for Pioneer 3320).

^g A confidence interval can be calculated to determine by how much any two means differ. Calculate the confidence interval as $[\text{mean}_1 - \text{mean}_2] \pm \text{MSD}$, where MSD (shown at the bottom of each column) is the minimum significant difference calculated using the Waller-Duncan k ratio test. For example, for Pioneer 3320 and SC229, the confidence interval for percentage of soluble carbohydrates is $[14.8 - 9.6] \pm 1.33$, or $3.87-6.53$. Thus, the true difference between the 2 means is between 3.87 and 6.53.

Results are based on 2 replicate samples of each cultivar.

Statistical Analysis. The general linear models (GLM) procedure of SAS (SAS Institute 1987) was used to determine whether mean number of eggs laid by weevils; development time; number of progeny produced; Dobie index of susceptibility; survivorship from egg to adult emergence; kernel hardness; number of kernels per 10 g sample; or percentage protein, soluble nitrogen, soluble carbohydrate, lipid, free or bound phenolic acids, or equilibrium moisture content differed with corn line. The protein/carbohydrate ratio of the corn lines was included as an independent variable because it has been shown to be correlated with insect growth (Applebaum 1972). Variances for duration of development, Dobie index of susceptibility, soluble carbohydrate, kernel hardness, and bound phenolics were nonhomogeneous. However, equalizing variances by transformation changed F values and probability levels very little; hence, untransformed data are reported in the tables.

The correlation procedure (CORR) of SAS (SAS Institute 1988) was used to determine whether any of the dependent variables (biological parameters of the weevils) were correlated with any of the independent variables (chemical and physical properties of the corn). The stepwise regression procedure (REG) of SAS (SAS Institute 1987) was used to determine regression coefficients for the relationships between dependent and independent variables, including models with multiple predictor variables. We used the same analyses to identify relationships between preharvest aflatoxin infection levels (Scott and Zummo 1988) and the chem-

ical and physical properties of the dried corn and insect biology on the dried corn.

Results

Insect Development. Duration of maize weevil development, number of progeny produced, and the Dobie index of susceptibility differed with corn line (Table 1). However, differences in number of eggs laid and survivorship from egg-to-adult emergence were not detected among the corn lines. Decreased weevil development time indicates increased susceptibility of a corn line to this insect. The corn line on which weevil development was shortest, Mp68:616, is also highly susceptible to *A. flavus* infection. Maize weevil development time on corn lines SC54, Mp420, and Mo18W, which are relatively resistant to *A. flavus* infection, took 4-5 d longer than on Mp68:616.

More progeny were produced on Mp313E and Mo18W, which are relatively resistant to *A. flavus* infection. Fewest progeny were produced on SC212M, which is relatively susceptible to *A. flavus* infection. The Dobie index of susceptibility indicated that some of the corn lines that are less susceptible to *A. flavus* infection could be selected to limit weevil population growth (particularly Mp420 and SC54). There was nearly a 50-fold difference in predicted numbers of weevils present after 180 d on the various corn lines (Table 2).

Chemical Analyses. All of the chemical and physical properties varied with corn line, except equilibrium moisture content (Tables 3 and 4). Duration of maize weevil development increased with lipid content of the corn lines (Table 5), al-

Table 5. Correlations [r and $P(r > 0)$] among biological parameters of maize weevils reared on corn lines differing in their susceptibility to *A. flavus* infection and chemical and physical properties of the corn lines

Physical or chemical property	No. eggs laid	Duration of development	Progeny produced	Survivorship	Susceptibility index
Kernel hardness	-0.01 (0.95)	0.28 (0.118)	-0.11 (0.58)	-0.10 (0.57)	-0.16 (0.39)
% moisture content	-0.07 (0.72)	-0.29 (0.100)	-0.09 (0.66)	-0.08 (0.66)	0.11 (0.58)
No. kernels/10 g	-0.19 (0.34)	-0.14 (0.428)	-0.23 (0.23)	0.03 (0.87)	-0.04 (0.85)
% soluble carbohydrate	-0.01 (0.94)	-0.34 (0.050)	0.04 (0.83)	0.09 (0.63)	0.14 (0.48)
% lipid	0.04 (0.84)	0.64 (0.000)*	0.10 (0.61)	-0.01 (0.95)	-0.31 (0.11)
% soluble nitrogen	0.24 (0.21)	0.00 (0.997)	0.16 (0.41)	-0.22 (0.21)	0.25 (0.20)
% bound phenolics	-0.02 (0.94)	0.36 (0.040)	0.03 (0.87)	0.14 (0.42)	-0.23 (0.22)
% free phenolics	0.14 (0.46)	0.19 (0.293)	0.14 (0.46)	0.05 (0.79)	-0.02 (0.90)
% protein	0.06 (0.77)	0.48 (0.004)	-0.02 (0.91)	-0.22 (0.23)	-0.21 (0.28)
% protein/% carbohydrate	0.18 (0.60)	0.54 (0.088)	0.02 (0.96)	-0.38 (0.25)	-0.23 (0.50)

Numbers of observations are 33, 29, 33, 29, and 33 for number of eggs laid, duration of development, number of progeny produced, survivorship, and susceptibility index, respectively.

Asterisk indicates significance at the 0.05 table-wise error level [α /number of comparisons = 0.001 (Rice 1989)]. Thus, a P must be <0.001 to be significant at the 0.05 table-wise error level.

though the linear equation describing the relationship ($a = 21.05 \pm 2.645$, $b = 2.354 \pm 0.5713$) explained only 38% of the variation among observations. Stepwise regression analyses indicated no significant ($P < 0.05$) predictors for other dependent variables. Levels of *A. flavus* infection in the field were not correlated with insect biology or chemical and physical properties of the corn lines ($P \geq 0.17$ for all correlations).

Discussion

Among the corn lines tested that were less susceptible to *A. flavus* infection, some were also less susceptible to maize weevils. Weevil development time varied by 5 d among corn lines, and number of progeny produced could be halved based on the corn line selected. Weevils reared on some of the lines less susceptible to *A. flavus* infection had both relatively low progeny production and longer developmental time. Long-term maize weevil population growth was ≈ 25 - to 50-fold greater on Mp313E than on SC54 or Mp420. Further development of corn lines that are less susceptible to both *A. flavus* and to maize weevils would be desirable. Development of corn lines that are susceptible to *A. flavus* infection and to infestation by maize weevils, such as Mp68:616, should be avoided. We have shown in this study, and in a previous study (Throne 1989), that use of corn lines that limit insect growth can severely limit insect population levels in stored corn during a normal storage period which results in less damage to the corn and may reduce the cost of insect control.

Maize weevil fecundity, progeny production, and Dobie index of susceptibility previously were shown to be correlated with protein and ferulic acid (a phenolic acid) content of corn (Classen et al. 1990). Protein content was the best predictor of progeny production and fecundity, and ferulic acid content was the best predictor of Dobie index. Our results differed in that only development time could be predicted based on chemical and physical

properties of the corn, and lipid content was the best predictor. We did not measure ferulic acid content of the corn lines, but ferulic acid accounts for 65–85% of total phenolics in corn (Classen et al. 1990, Sosulski et al. 1982). Bound phenolics constitute $\approx 70\%$ of total phenolics in corn (Sosulski et al. 1982), therefore our measurements of bound phenolic acids may be indicative of ferulic acid content. Kernel hardness has previously been shown to be correlated with susceptibility to maize weevil infestation (Dobie 1974); we did not find this to be the case in our study.

Infection of corn in the field by *F. graminearum* was shown to be negatively correlated with ferulic acid content of corn and positively correlated with *p*-coumaric acid content, both of which are phenolic acids (Assabgui et al. 1993). We did not find any correlation between *A. flavus* infection of corn and any of the chemical or physical properties we measured in the stored corn. These properties may differ from those of preharvest corn. It is possible that a chemical or physical factor may be found in preharvest corn that can be used to predict the resistance of corn to *A. flavus* infection.

Acknowledgments

We thank Julia A. Barron, Patricia L. Lang, and Stanley M. Woo (USDA-ARS, Savannah, GA) for technical assistance; Victor Chew (USDA-ARS, Gainesville, FL) for advice on the statistical analyses; and Patrick F. Dowd (USDA-ARS, Peoria, IL) and Robert L. Meagher, Jr. (University of Minnesota) for reviewing an earlier version of the manuscript.

References Cited

- American Association of Cereal Chemists. 1969. Approved methods of the American Association of Cereal Chemists. American Association of Cereal Chemists, St. Paul, MN.
- Applebaum, S. W. 1972. The carbohydrate nutrition of *Tribolium*, pp. 288–297. In J. G. Rodriguez [ed.], Insect and mite nutrition, significance and implica-

- tions in ecology and pest management. North-Holland, Amsterdam.
- Assabgui, R. A., L. M. Reid, R. I. Hamilton, and J. T. Arnason. 1993.** Correlation of kernel (E)-ferulic acid content of maize with resistance to *Fusarium graminearum*. *Phytopathology* 83: 949-953.
- Classen, D., J. T. Arnason, J. A. Serratos, J.D.H. Lambert, C. Nozzolillo, and B.J.R. Philogène. 1990.** Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil, in CIMMYT's collections. *J. Chem. Ecol.* 16: 301-315.
- Dobie, P. 1974.** The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera, Curculionidae). *J. Stored Prod. Res.* 10: 183-197.
- Dowd, P. F. 1994.** Examination of an *Aspergillus flavus* resistant inbred of maize for cross-resistance to sap beetle vectors. *Entomol. Exp. Appl.* 71: 177-180.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956.** Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356.
- Greenspan, L. 1977.** Humidity fixed points of binary saturated aqueous solutions. *J. Res. Natl. Bur. Stand.* A81: 89-96.
- Halstead, D.G.H. 1962.** The rice weevils, *Sitophilus oryzae* (L.) and *Sitophilus zeamais* Mots.; identification and synonymy. *Trop. Stored Prod. Inf.* 5: 177-179.
- Krygier, K., F. Sosulski, and L. Hogge. 1982.** Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *J. Agric. Food Chem.* 30: 330-334.
- Lang, C. A. 1958.** Simple microdetermination of Kjeldahl nitrogen in biological materials. *Anal. Chem.* 30: 1692-1694.
- Rice, W. R. 1989.** Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- SAS Institute. 1987.** SAS/STAT guide for personal computers, version 6 ed. SAS Institute, Cary, NC.
- 1988.** SAS procedures guide, version 6.03 ed. SAS Institute, Cary, NC.
- Scott, G. E. and N. Zummo. 1988.** Sources of resistance in maize to kernel infection by *Aspergillus flavus* in the field. *Crop Sci.* 28: 504-507.
- Singleton, V. L. and J. A. Rossi. 1965.** Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16: 144-158.
- Sinha, K. K. and A. K. Sinha. 1992.** Impact of stored grain pests on seed deterioration and aflatoxin contamination in maize. *J. Stored Prod. Res.* 28: 211-219.
- Sosulski, F., K. Krygier, and L. Hogge. 1982.** Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* 30: 337-340.
- Swain, T. and W. E. Hillis. 1959.** The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* 10: 63-68.
- Throne, J. E. 1986.** A bibliography of maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). U.S. Dep. Agric., Agric. Res. Serv. 58.
- 1989.** Effects of noncatastrophic control technologies that alter life history parameters on insect population growth: a simulation study. *Environ. Entomol.* 18: 1050-1055.
- 1994.** Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. *Environ. Entomol.* 23: 1459-1471.
- Tolpo, N. C. and E. O. Morrison. 1965.** Sex determination by snout characteristics of *Sitophilus zeamais* Motschulsky. *Tex. J. Sci.* 17: 122-124.

Received for publication 20 October 1994; accepted 17 April 1995.