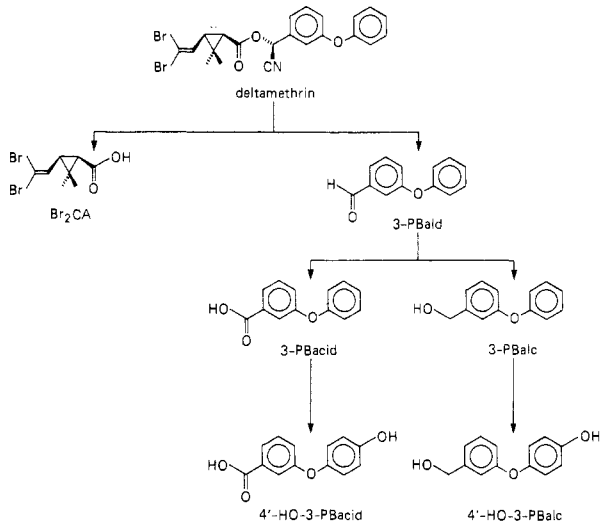

Figure 2. Metabolic pathways of deltamethrin in enzyme preparations from liver of cow and chicken.

deltamethrin contained unchanged insecticide, Br_2CA, and other unidentified products. However, the amounts of unchanged deltamethrin and Br_2CA were higher in chicken liver systems than those from cow liver. This may be, in part, due to the higher fat content of the chicken liver.

The data presented above provide evidence for the metabolic pathway of deltamethrin in in vitro incubation with various enzymatic fractions of a cow and chicken liver homogenates. The preferred metabolic pathway for deltamethrin is the cleavage of the ester bond to yield to Br_2CA and 3-PBald (after fast elimination of HCN from 3-PBald cyanohydrin). 3-PBald can undergo both oxidation and reduction to afford 3-PBacid and 3-PBalc, respectively. In the present study 3-PBacid and 3-PBalc were produced in almost equal quantities, suggesting efficient oxidase and reductase in liver systems. Shono et al. (1979) also found sufficient quantities of 3-PBacid and 3-PBalc when they incubated deltamethrin with mouse liver microsomes (oxidase or oxidase plus esterase). However, the amount of 3-PBacid produced was always high (4.7–6.5 times that of 3-PBalc).

Although all the polar metabolites were not identified in the present study, the amounts of these metabolites were very small. Thus, it appears that hydroxylation of the ring(s) and the methyl group(s) is not an efficient metabolic pathway. Based on the metabolites identified, a metabolic pathway for deltamethrin in the liver homogenates of cow and chicken is proposed in Figure 2.

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Registry No. Deltamethrin, 52918-63-5; Br_2CA, 53179-78-5; 3-PBald, 39515-51-0; 3-PBalc, 13826-35-2; 3-PBacid, 3739-38-6; 4'-HO-3-PBalc, 63987-19-9; 4'-HO-3-PBacid, 35065-12-4.

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Possible Factors of Leaf-Feeding Resistance in Corn to the Southwestern Corn Borer

Paul A. Hedin,* Frank M. Davis, William P. Williams, and Marvin L. Salin

This report describes results of studies to chemically describe differences between leaf feeding resistant and susceptible corn genotypes to the southwestern corn borer, *Dietraea grandiosella* (Dyar), a major pest of corn (*Zea mays* L.) in parts of the southwestern and southeastern United States. The compound 6-methoxybenzoxazolinone was present in very low concentrations (20 $\mu g/g$) in the corn lines tested and was relatively nontoxic for this insect. Crude fiber and residue content, the latter obtained by 70% aqueous methanol extraction of inner whorl tissue, were consistently higher in resistant lines and were negatively correlated with insect weight ($r = -0.84^{**}$, 15 df) and with insect damage ($r = -0.57^{**}$, 20 df). The hemicellulose content of the fiber from resistant whorls (24.0%) was higher than in susceptible whorls (17.6%), while the cellulose content was unchanged. The susceptible lines were at least 25% higher in several constituents, notably crude protein, lipid, total sugars, ash, and polyphenol oxidase activity. These factors explain at least in part the bases of resistance and susceptibility in the cultivars studied.

The southwestern corn borer [*Diatraea grandiosella* (Dyar)] (SWCB) is a native of Mexico and was first reported in New Mexico in 1913. Since that time, the borer has spread northward and eastward (Henderson et al., 1966). The northern limits (Kansas) are restricted by its inability to survive winter temperatures (Chippendale, 1974). Presently, the SWCB infests corn eastward to the Alabama–Georgia border.

The damage to corn plants is caused by larvae. Normally, corn plants are in the whorl stage of growth when moths that have emerged from the overwintering generation lay their egges on the corn leaves. After the eggs hatch, the young larvae move into the whorl where they

Boll Weevil Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Mississippi State, Mississippi 39762 (P.A.H.), Crop Science Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Mississippi State, Mississippi 39762 (F.M.D. and W.P.W.), and Department of Biochemistry, Mississippi State University, Mississippi State, Mississippi 39762 (M.L.S.).

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feed for 9–10 days before they move to the outside of the stalk and start tunneling into the stalk (Davis et al., 1972). Feeding in the whorl results in extensive leaf damage that is similar to the type caused by the European corn borer, *Ostrinia nubilalis* (Hubner) (ECB).

Losses due to the SWCB occur both directly and indirectly. Reportedly, up to 20% yield loss can occur from first-brood damage and 9% yield loss from second-brood damage (Scott and Davis, 1974). Although direct effects of feeding of the larvae are very significant, the indirect losses due to increased lodging, a result of stalk girdling by overwintering larvae, cause the most obvious and greatest damage. Corn fields vulnerable to the late summer generation larvae may have as much as 50–75% stalk lodging.

Several studies on the chemical and physical mechanisms of resistance in maize/corn to insects have been carried out on the ECB. The role of 6-methoxybenzoxazolinone (6-MBOA) in inbred resistance of maize to first-brood ECB larvae was elucidated by a number of studies in the 1950s (Klun and Brindley, 1966). Actually 6-MBOA is not an in vivo constituent of corn tissue but a degradation product from 4-*O*-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, which upon injury or crushing is hydrolyzed enzymatically to the corresponding aglycon, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). DIMBOA is quite unstable, being degraded at elevated temperatures (above 25 °C) to 6-MBOA. It is hypothesized that in the plant DIMBOA is the primary factor of resistance; however, only the degradation product 6-MBOA has been fed in diets and shown to decrease the larval growth rate of this insect (Klun and Brindley, 1966).

Recently, we synthesized 6-MBOA and also 5- and 7-MBOA to determine their effect on the growth rate of the SWCB, the ECB, and the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (FAW). In larval feeding tests, the three compounds were essentially equal in their activity but 3× more growth depressing to the ECB than the FAW or SWCB (Nicollier et al., 1982). The toxicity of DIMBOA is difficult to evaluate because it is rapidly converted to 6-MBOA after its incorporation in diet, even when precautions are taken to minimize heat during formulation. In one test, DIMBOA was 6× more toxic to the ECB than 6-MBOA, but only equally toxic in a second test. In both instances, chemical tests showed that DIMBOA had been at least partially converted to 6-MBOA (Hedin et al., 1983).

Guthrie et al. (1971) had observed that an inbred line with a relatively low DIMBOA concentration had high resistance to second-generation ECB. Sullivan et al. (1974) also found ECB resistance in several exotic maize varieties low in DIMBOA; hence, DIMBOA is not the only factor of resistance. A nonpreference mechanism of leaf feeding resistance related to DIMBOA was proposed for the ECB (Robinson et al., 1978). These various studies suggest that there are different mechanisms of resistance in corn to these insects and that DIMBOA is not the primary resistance factor for the SWCB or the FAW.

This work describes results of studies to chemically describe differences between leaf feeding resistant and susceptible corn genotypes to the southwestern corn borer. For experimental purposes, it was hypothesized that the resistant genotypes contained a growth depressant that was absent or less prevalent in the susceptible genotypes.

MATERIALS AND METHODS

Corn Lines and Harvesting, Entomological, and Statistical Procedures. Corn lines used in these studies were developed by the Corn Host Plant Resistance Unit at Mississippi State, MS, and were selected for desirable resistance properties to the SWCB. Agronomic practices were normal for this area. Genotypes were sampled from blocks of genotype or entire rows were sampled from randomized complete blocks. Whorls were excised in the mid-whorl stage on site and transported in an ice chest to the laboratory.

Entomological and statistical procedures were carried out as described by Davis and Williams (1980). Analyses and bioassays were carried out on field-grown tissues unless otherwise specified. Insect growth evaluations were carried out as randomized complete block designs with three to four replications. Larvae were obtained from 10 plants from each replicate on the 14th day after field infestation and weighed. Plant ratings were also from randomized complete blocks and recorded on the 14th day. Ratings and insect weighings were subjected to ANOV and to Duncan's multiple range test.

Analysis and Synthesis of 6-MBOA. The analysis was performed by the procedure of Venis and Watson (1978) in which inner whorl tissue [AB24E × Mp305 (S) and Mp701 × Mp496 (R); S = susceptible and R = resistant] was homogenized in water and allowed to stand at room temperature for 1 h so that the DIMBOA-glucoside was hydrolyzed by in situ enzymes to DIMBOA. It was then heated at 100 °C for 1 h to convert the DIMBOA to 6-MBOA. The 6-MBOA was extracted with CH_2Cl_2, and an appropriate aliquot was analyzed by GLC on a 1% OV-225 Gas-Chrom Q glass column 150 cm × 0.32 cm at 200 °C). The 6-MBOA was synthesized to serve as an analytical standard by the procedures of Matsumoto and Okazawa (1974) and Nicollier et al. (1982).

SWCB Larval Feeding Studies with Corn Whorl Tissue Fractions and Compounds. Newly hatched SWCB larvae were placed on a custom Bio-Mix No. 1107 diet prepared by Bioserv, Inc., Frenchtown, NJ, for 5 days after which they were weighed. The diet was based on wheat germ, casein, sucrose, vitamins, salts, agar, and antimicrobial agents. The candidate fractions or compounds were either added directly as an aqueous solution or, if required, added in a nonaqueous solvent to cellulose powder and rotoevaporated to remove the solvent. Four replications of six insects each were employed (Nicollier et al., 1982).

Test A. Whorl powder (10 g), obtained by grinding freeze-dried whorl tissue, was extracted by Soxhlet with cyclohexane/ethyl acetate/acetic acid, 500/500/1 (CHEA). The yield was typically 0.3 g (3%). The powder was subsequently extracted at boiling reflux with methanol/water, 7/3 (MW). The yield was typically 3 g (30%). For incorporation in the diet, the CHEA extract was dissolved in hexane and rotoevaporated to dryness with 2 g of cellulose powder so that the extract was evenly deposited. The cellulose was then added during routine diet preparation. The 10-g quantity of dehydrated whorl powder was used because it was comparable to 50 g of fresh tissue, and the diet was conveniently divided into 50-g units to yield sufficient quantities for 20 2.5-g vials. One-third strength extracts were also evaluated.

Test B. For further evaluation of the methanol/water, 7/3, extract (a larger preparation from 100 g of powder) the solvent was removed by evaporation, and the extract was then redissolved in water and extracted with chloroform. The yield of the chloroform phase was typically 10% of that of the MW phase. The chloroform phase (1.3 g) was chromatographed on a 2 × 30 cm Biosil A column and eluted with chloroform and later acetone and methanol to give four distinct fractions yielding a total of 0.95 g of solids. The methanol/water, 7/3, extract (MW) (12.1 g)

was partition chromatographed on a 2 × 30 cm Sephadex G-25 column with water, 70% aqueous methanol, and methanol. Nine fractions with a total weight of 10.03 g were obtained. For this test, 30, 50, 70, 100, or 150 mg of each fraction (multiple levels where adequate material was available) was incorporated in the laboratory diet by procedures as described in the previous section and evaluated for SWCB larval growth inhibition.

Laboratory Feeding Studies with Dietary Additives. Ground corn cobs (Anderson Cob Co., Maumee, OH), ground corn whorl residue (see test A), and α-cellulose powder (Sigma Chemical Co., St Louis, MO) were evaluated at 2 and 6% for effect on larval growth rate of the diet according to the SWCB larval dietary procedures with four replications of six insects each as described in a previous section.

Assorted Chemical, Physical, and Biological Analyses of Whorl Tissues. Proximate analyses were obtained by standard AOAC procedures. Fatty acids were analyzed after methylation by GLC on a 0.2 × 1820 cm column packed with 10% Silar-10 C on 100–120-mesh Gas-Chrom Q operated at 180 °C in a N_2 flow of 20 mL/min. Analysis for acid detergent (extracted) fiber, lignin, and silica were obtained by standard AOAC procedures. Total sugars were determined by the colorimetric anthrone procedure. Cold-pressed corn serum was obtained by placing a plastic bag containing corn tissue between the plates of an industrial press at 10 000 lb. The juice was centrifuged and submitted for a medical series automated analysis of lactic acid, triglycerides, calcium, chloride, magnesium, cholesterol, glucose, lactic dehydrogenase, and glutamate–oxaloacetate transaminase. The enzymes hexokinase, phosphoglucomutase, UDP-glucose pyrophosphorylase, catalase, and polyphenol oxidase were determined by standard enzyme assay procedures. Intestinal microbial digestibility evaluation for in vitro dry matter digestibility was performed by Dr. W. H. Essig, Department of Animal Science, Mississippi State University. Leaf thickness was determined with a micrometer.

RESULTS AND DISCUSSION

Studies of 6-MBOA. Because 6-MBOA, DIMBOA, and DIMBOA glucoside had been reported to be ECB resistance factors (Klun and Brindley, 1966), analysis for 6-MBOA was performed in 1977 on susceptible (AB24E × Mp305) and resistant (Mp701 × Mp496) lines. While 6-MBOA could be detected, it was present at about 1% of that reported by Venis and Watson (1978) (19.1 ± 0.2 μg/g, fresh weight basis, in AB24E × Mp305; 21.2 ± 0.3 μg/g in Mp496 × Mp701). In 1981, analyses for 6-MBOA were conducted on four S × S, four R × R and two R × S lines. The mean 6-MBOA contents again were very low and not appreciably different (S × S 19.4 ± 0.4 μg/g, R × S 17.6 ± 0.2 μg/g, and R × R 24.5 ± 0.3 μg/g).

Evaluation of Corn Whorl Tissue Extracts and Fractions as SWCB Larval Growth Inhibitors. Freeze-dried corn whorl tissue was extracted and incorporated in diets for evaluation of larval growth inhibition as described under Materials and Methods. The levels were chosen to be comparable to those encountered by larvae in fresh leaf tissue as related in the materials and methods section. The results (Table I) show that the extracts from both SWCB-susceptible and -resistant whorl tissue caused only a limited inhibition of larval growth that was biologicaly not significant. The resistant CHEA fraction at 0.3 g decreased growth in this particular test to 69.8% of the control value. However, in 12 subsequent tests, insects fed the diet with CHEA extract at 0.3 g weighed an average of 77.8% of control insects. Growth retardation was limited and could be attributed at least in part to a small amount of 6-MBOA (15.2 μg/g, converted to a fresh weight basis), which was found by GLC to be present in this fraction; no further investigation was made.

Table I. Feeding of SWCB Larvae on Diets Supplemented with CHEA and MW[a] Extracts (Test A)

sample	field rating	extract	amount incorporated, % of diet	growth, % of control[b]
AB24E ×	S	CHEA	0.2	95.2
Mp305	S	CHEA	0.6	95.0
	S	MW	2.0	80.3
	S	MW	6.0	82.7
Mp701 ×	R	CHEA	0.2	87.7
Mp496	R	CHEA	0.6	69.8
	R	MW	2.0	91.5
	R	MW	6.0	84.5

[a] CHEA = chloroform/hexane/ethyl acetate, 500/500/1; MW = methanol/water, 7/3. [b] Control insects weighed 17.98 mg at 7 days; four replications of five insects per test.

Although the MW extracts did not appear very active, additional evaluation seemed prudent to be certain that no factor was overlooked. Only fraction 3 of the chloroform phase was active in that it depressed growth significantly ($P < 0.05$) to 68.2% of the control. This sample, likewise as in test A, was found by GLC to contain a small amount of 6-MBOA (20.8 μg/g) that evidently had not been completely extracted from the tissue in the CHEA Soxhlet procedure.

Effects of Dietary Additives on Larval Growth. The growth of larvae fed diets with 2 and 6% additives, respectively, expressed as percent of control were as follows: growth corn cobs, 94.8 and 95.9; whorl residue, 114.5 and 113.6; α-cellulose, 107.6 and 94.8. The testing of these additives was an effort to evaluate the effect of poorly digestible ingredients. Because they were of necessity ground, the added in situ dimension of toughness could not be assessed appropriately.

Chemical, Physical, and Biological Composition of Susceptible and Resistant Whorl Tissues. In 1977 and 1978, analyses were conducted mostly on one SWCB-susceptible (AB24E × Mp305) and one resistant (Mp701 × Mp496) genotype (Table II). Recognizing that a number of susceptible and resistant genotypes must be evaluated to identify true trends, several statistically significant differences appeared. The analyses for which the susceptible line was at least 25% lower (Table II) were crude fiber, acid detergent fiber, lactic acid, calcium, and glutamate–oxaloacetate transaminase. The susceptible line was at least 25% higher for crude protein, crude lipid, ash, stearic and oleic acids, and silica. There were also statistically significant differences between resistant and susceptible tissues for several other analyses.

Silica content was analyzed in plant material from a ten-genotype test of four resistant (450 ± 43 ppm), four susceptible (540 ± 35 ppm), and two intermediate (385 ± 26 ppm) genotypes harvested in 1981. However, it is difficult to comprehend silica as a factor of resistance at only 0.04–0.07%. The general trend of the other differences seemed to be that components associated with nutrition (protein, minerals, and lipids) were higher in the susceptible line, whereas fiber (noncaloric and nonassimilative) was higher in the resistant line.

In a subsequent analysis in 1979 of four susceptible and 11 lines known to possess varying degrees of resistance, the trends of the previous series of analyses were generally supported (see Figure 1 for identities). The average pro-

Table II. Chemical, Physical, and Biological Survey of SWCB-Susceptible and -Resistant Corn Inner Whorl Tissue; Analyses in Duplicate or Greater on Composited Samples

analysis	hybrid: AB24E × Mp305 (S)	hybrid: Mp701 × Mp496 (R)
crude protein, %	13.6 ± 0.2	10.1 ± 0.3
crude lipid, %	2.6 ± 0.1	1.6 ± 0.1
ash, %	6.6 ± 0.2	4.4 ± 0.1
nitrogen-free extract, %	59.5 ± 1.2	59.9 ± 1.5
crude fiber, %	17.7 ± 0.4	24.0 ± 0.5
fatty acids, %		
16:0	18.2 ± 0.2	20.8 ± 0.2
18:0	2.8 ± 0.1	1.7 ± 0.1
18:1	9.3 ± 0.3	7.3 ± 0.2
18:2	42.1 ± 0.4	42.5 ± 0.5
18:3	27.5 ± 0.2	27.5 ± 0.2
silica, ppm	720 ± 38	470 ± 29
acid detergent fiber, %	19.7 ± 0.4	26.5 ± 0.5
lignin, %	1.5 ± 0.2	1.6 ± 0.2
aqueous methanolic residue, %	65.0 ± 0.9	72.7 ± 1.1
total sugars, mg/g	113.8 ± 20.4	105.3 ± 16.7
lactic acid, mg/dL	0.6 ± 0.2	1.5 ± 0.4
triglycerides, mg/dL	24.0 ± 1.8	30.0 ± 2.5
glucose, mg/dL	78.0 ± 3.5	80.0 ± 2.6
calcium, mg/dL	4.8 ± 0.7	7.3 ± 1.2
chloride, mequiv/L	58.0 ± 2.5	62.0 ± 3.4
magnesium, mg/dL	6.5 ± 0.8	6.7 ± 0.4
cholesterol, mg/dL	8.0 ± 0.7	10.0 ± 1.2
lactic dehydrogenase, μmol/L	2.0 ± 0.4	2.0 ± 0.6
glutamate-oxaloacetate transaminase, μmol/L	239 ± 28	368 ± 45
leaf thickness, mm	2.3 ± 0.2	2.5 ± 0.2
in vitro matter digestibility, %	89.5 ± 0.8	90.5 ± 0.7

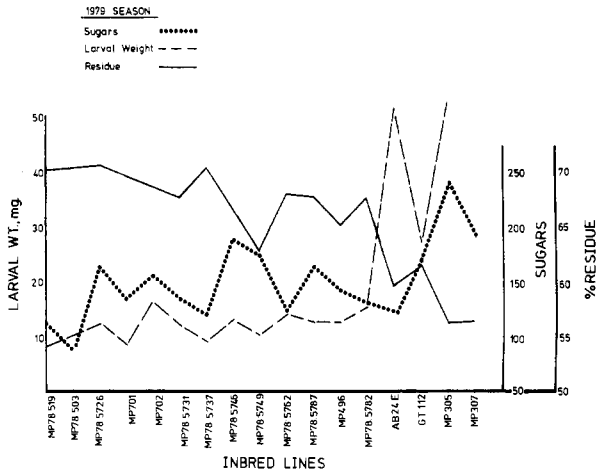

Figure 1. SWCB larval growth in 1979 on whorls of 11 resistant and 4 susceptible inbred lines in the field as related to whorl residue and sugar contents. Susceptible lines are to the right. Correlations: residue/larval weight, $r = -0.84^{**}$, 15 df; sugar/larval weight, $r = 0.45$, 15 df.

tein, fat, and ash contents were slightly higher in the susceptible lines (14.8 ± 1.3, 2.9 ± 0.2, and 5.8 ± 0.4 vs. 12.8 ± 1.2, 2.8 ± 0.3, and 5.5 ± 0.3, respectively), whereas the average crude fiber content was higher in the resistant lines (27.3 ± 1.0 vs. 25.5 ± 1.4). These lines were also analyzed for activity of five characteristic enzymes (see Materials and Methods) and the chlorophyll content. Each was as great or greater in the susceptible lines as in the resistant lines: hexokinase [μmol of NADPH min^{-1} (mg of protein)$^{-1}$], S, 0.06 ± 0.02, and R, 0.02 ± 0.01 phosphoglucomutase [μmol of NADPH min^{-1} (mg of protein)$^{-1}$], S, 1.7 ± 0.4, and R, 1.3 ± 0.3; UDP-glucose pyrophosphenylase [μmol of NADPH min^{-1} (mg of protein)$^{-1}$], S, 0.22 ± 0.06 and R, 0.23 ± 0.08; catalase [μmol of H_2O_2

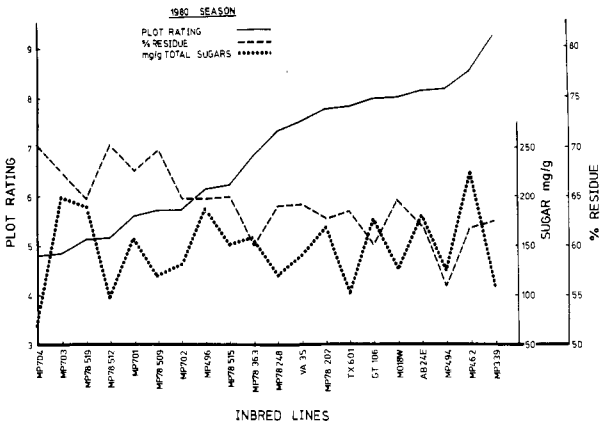

Figure 2. Field plot ratings in 1980 for SWCB larval feeding on 20 resistant and susceptible inbred lines as related to whorl residue and sugar contents. Susceptible lines are to the right. Correlations: residue/plot ratings, $r = -0.57^{**}$, 20 df; sugar/plot ratings, $r = 0.12$, 20 df.

min^{-1} (mg of protein)$^{-1}$], S, 0.52 ± 0.09, and R, 0.21 ± 0.06; polyphenol oxidase [μmol of O_2 min^{-1} (mg of protein)$^{-1}$], S, 1.38 ± 0.15, and R, 0.72 ± 0.18; chlorophyll [μg (mg of protein)$^{-1}$], S, 13.9 ± 0.5, and R, 11.3 ± 0.4.

Perhaps the most interesting of the enzymatic differences was that of polyphenol oxidase (PPO), which was 94% higher in the susceptible lines. Because there had been some feeding of larvae on the susceptible tissue brought directly from the field for analysis, it is possible that the increase in PPO was due to an induction of the enzyme activity caused by wounding by the insect. Such an induction has been found in sliced sweet potato (Hyodo and Uritani, 1967) and virus-infected soybean (Batra and Kuhn, 1975).

Figure 1 is a three-factor graph of weights of larvae that had fed for 14 days on whorl tissue in the field on 15 inbred lines, 11 of which were obtained from 3 known to possess varying degrees of resistance, obtained from 3 replications of 10 plants/replication, as related to percent tissue residue content (after extraction with 70% aqueous methanol) and mg/g total sugars. The resistant lines are to the left, and the susceptible lines are to the right in Figure 1. There is a significant ($P < 0.01$; i.e., **) negative correlation of tissue residue content and larval weight ($r = -0.84^{**}$, 15 df) but no correlation between sugars and larval weight ($r = 0.45$, 15 df).

In 1980, a second field test was conducted to determine the relationships between leaf feeding plot ratings and percent tissue residue content and total sugar content. Twenty inbred lines were plated in three replications by using a randomized complete block design. Twenty plants of each genotype in each replicate were rated for leaf feeding and 40 plants were used in the chemical analyses. Only 4 of these 20 lines had been included in the 1979 test and 9 were known to possess varying degrees of resistance. Figure 2 is a three-factor graph of leaf feeding plot ratings as related to percent residue and mg/g total sugars. The lines are arranged from left to right in Figure 2 in order of increasing susceptibility according to plot ratings. Figure 2 shows that there was a significant ($P < 0.01$) negative correlation between percent tissue residue and plot ratings ($r = -0.57^{**}$, 20 df) but sugars and leaf feeding plot ratings were uncorrelated ($r = 0.12$, 20 df).

In another 1980 field test (Table III), three susceptible and four resistant genotypes were evaluated in four replications by using a randomized complete block design both for leaf feeding ratings and larval biomass per plant and for whorl residue and sugar contents. All of these geno-

Table III. Leaf Feeding Ratings, Total Larval Biomass per Plant, and Whorl Residue and Sugar Contents for Three Susceptible and Four Resistant Genotypes, 1980

genotype	leaf feeding rating	larval biomass/plant, mg	residue, %[a]	sugars, mg/g[a]
Mp703	3.36 a[b]	44.14 a	67.4 ± 1.1	134.2 ± 8.2
Mp701	4.22 ab	66.40 ab	67.6 ± 1.0	157.6 ± 3.9
Mp702	5.02 ab	92.60 b	67.2 ± 1.3	139.4 ± 5.1
Mp496	5.86 bc	72.35 ab	66.8 ± 0.7	157.1 ± 4.7
Va35	7.36 cd	327.61 c	65.3 ± 0.9	148.2 ± 5.3
AB24E	7.81 d	584.94 e	62.0 ± 1.2	160.0 ± 5.6
SC229	8.29 d	371.67 d	61.7 ± 1.8	195.4 ± 8.7

[a] $X \pm S_X$; four or more replications. [b] 5% probability level (Duncan's multiple range test).

types were also included in the larger 1980 test that was summarized in Figure 2, but larval biomass per plant was additionally obtained in this test. Data were analyzed and means were compared by using Duncan's multiple range test. As expected, there were less leaf feeding and lower larval weight gains on the resistant genotypes. The percent residue was higher with the resistant genotypes as in previous tests. Thus, the negative correlation between residue content and larval feeding damage and weight gains continued to be manifested.

Davis et al. (1972) and Scott and Davis (1974) had reported that after SWCB egges have been laid on corn leaves, the larvae move into the whorl upon hatching, feed for 9 or 10 days, move to the outside of the stalk, and then tunnel into the stalk. If larvae reach the stalk, even of resistant lines, they then grow rapidly. Observations by Davis et al. (1972) have revealed that the yellow-green portion of the whorl is the favored larval feeding site. Proximate analyses of the outer leaves, inner whorl, and the stalk (Table IV) indicate that the protein, crude fiber, and ash contents of stalks are low, while the fat- and nitrogen-free extract (high in soluble sugars) are high compared to those of leaves and whorl. Though low, the protein content is evidently adequate in view of the rapid growth of larvae that feed on the stalk. By comparison, the protein, crude fiber, and ash contents of outer leaves are high, while the nitrogen-free extract is low compared to that of the inner whorl tissue. This composition could contribute to toughness and may account in part for the fact that young larvae move upon hatching from the outer leaves to the inner whorl and subsequently to the stalk, which is a high energy source. As in previous analyses (Table II and III), crude fiber in the inner whorl was significantly higher than that in resistant tissue. Protein, fat, and ash were significantly higher in all three tissues of the susceptible line.

Composition of the Residue Content. On the assumption that the residue (including crude fiber) in corn is a factor of resistance (Table II and III; Figures 1 and 2), a knowledge of composition could lead to an understanding of the mechanism(s) by which the residue contributes to resistance.

Extraction with hot 70% aqueous methanol was employed initially for several reasons. It solubilizes amino acids and some proteins, sugars, and other carbohydrates. It also solubilizes a number of so-called secondary plant constituents such as flavonoids and vitamins, some of which because of their solubility in polar solvents could be expected to possess some favorable or unfavorable biological activity. The extractables (30–40% of the total depending on the genotype) upon analysis yielded 32.5% crude protein and 25.7% carbohydrates (mostly sugars). The remainder consisted largely of salts and flavonoids. Proximate analysis of the residue (Table V) gave results not greatly different from that of the original whorl tissue although the protein content of susceptible tissue was higher than that of the resistant tissue. The crude fiber contents were nearly equal, but the preceding analyses had established that there was more fiber in the resistant tissue. Thus, proximate analyses of the residue did not provide results that could suggest an evident mechanism by which the residue could manifest resistance.

Another approach to the determination of the chemical composition of the residue was to analyze for the total sugar content of whorl tissue and thereby to infer the relative contents of the cellulose and hemicellulose. Whorl tissue was hydrolyzed and converted into the aldonitrile acetate derivatives and then analyzed by GLC according

Table IV. Proximate Analysis of Outer Leaves, Inner Whorls, and Stalks of a Susceptible (AB42E × Mp305) and a Resistant (Mp701 × Mp496) Line

component, %	outer leaves		inner whorl		stalk	
	S	R	S	R	S	R
crude protein	21.7 ± 0.3	18.6 ± 0.2	13.6 ± 0.2	10.1 ± 0.3	3.1 ± 0.3	2.2 ± 0.3
fat	2.4 ± 0.1	4.3 ± 0.2	2.6 ± 0.1	1.6 ± 0.1	6.8 ± 0.3	5.9 ± 0.2
crude fiber	26.9 ± 1.2	29.0 ± 1.0	17.7 ± 0.4	24.0 ± 0.5	18.7 ± 1.1	19.2 ± 0.8
nitrogen-free extract	36.7 ± 2.1	38.2 ± 1.3	59.9 ± 1.5	59.9 ± 1.5	69.9 ± 2.4	70.6 ± 1.9
ash	11.4 ± 0.6	9.9 ± 0.4	6.6 ± 0.2	4.4 ± 0.1	1.4 ± 0.2	2.1 ± 0.2

Table V. Proximate Analysis of the Residue of SWCB-Susceptible (AB24E × Mp305) and -Resistant (Mp701 × Mp496) Corn Inner Whorl Tissue

variety	crude protein, %	lipid, %	crude fiber, %	ash, %	nitrogen-free extract, %
AB24E × Mp305 (S)	13.4 ± 0.4	0.7 ± 0.2	31.0 ± 1.5	2.5 ± 0.2	53.4 ± 2.1
Mp701 × Mp496 (R)	9.8 ± 0.3	0.7 ± 0.2	32.4 ± 1.6	1.3 ± 0.2	55.8 ± 1.8

Table VI. Total Sugars in Corn Whorl, Cob, Husk, Stalk, and Leaf Tissues

tissue	arabinose, %	xylose, %	mannose, %	galactose, %	glucose, %
AB24E × Mp305 (S)	2.3 ± 0.3	13.9 ± 0.5	1.4 ± 0.3		40.0 ± 0.8
Mp701 × Mp496 (R)	3.1 ± 0.3	20.5 ± 0.5	0.4 ± 0.2		43.7 ± 1.3
cob[a]	4.6	31.2	0.1	0.1	41.7
husk[a]	6.8	29.6	0.3	0.6	45.1
stalk[a]	2.4	18.9	0.6	1.1	35.9
leaf[a]	3.0	18.7	0.3	0.5	31.0

[a] Krull and Inglett (1978); statistical calculations not available.

to the procedures of Chen and McGinnis (1981). The results (Table VI) show that there is significantly more xylose; 20.5% (and thus xylan; hence, hemicellulose) in resistant whorl tissue than in susceptible whorl tissue; 13.9%, while the glucose (hence cellulose) content was slightly higher in resistant tissue but probably not biologically important. The presence of the sugars arabinose and mannose is presumptive evidence of two other hemicelluloses, the arabans and the mannans. Thus, the total hemicellulose content was 17.6% for the susceptible line and 24.0% for the resistant line. The difference in the sum of the sugars from 100% can be attributed to proteins, salts, and other non-sugar constituents. The higher analysis for xylose in MpSWCB-1 × Mp496 suggests that xylans may be a source of resistance.

Comparable results for sugars in cob, husk, stalk, and leaf were determined by Krull and Inglett (1978) and are also given in Table VI. From this analysis, it can be deduced that cellulose, starch, and xylan polymers are the major sources of residue in corn leaf. Lignin (1.52–1.63%) and salts (analyzed as ash, 4.4–6.4%) evidently contribute only to a limited degree.

In summary, the fiber and residue contents are significantly higher in a number of resistant lines. The cellulose and hemicelluloses comprise an important portion of the fiber and can be expected to contribute to leaf toughness, indigestibility, and intractibility to metabolism by the insect. Thus, they evidently explain at least in part a basis of resistance in the cultivars studied.

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Roles of Tobacco Cellulose, Sugars, and Chlorogenic Acid as Precursors to Catechol in Cigarette Smoke

Steven G. Carmella,* Stephen S. Hecht, T. C. Tso, and Dietrich Hoffmann

Tobacco was extracted sequentially with hexane and methanol–H_2O, and the extracts were pyrolyzed at 650 °C in order to identify likely leaf precursors to the tobacco smoke cocarcinogen catechol. The results demonstrated that the methanol–H_2O extract and the extracted tobacco residue were good pyrolytic precursors to catechol. Subfractions of the methanol–H_2O extract were isolated by HPLC and pyrolyzed. Fructose, glucose, sucrose, and chlorogenic acid were thus identified as important pyrolytic precursors to catechol. Cellulose, a component of the extracted tobacco residue, was also found to be a good precursor to catechol in pyrolysis experiments. To determine the role of these substances as precursors to catechol under the conditions prevailing in a burning cigarette, either [^{14}C(U)]cellulose, [^{14}C(U)]fructose, or various levels of the unlabeled polyphenols chlorogenic acid or rutin were added to cigarettes and the mainstream smoke was analyzed for [^{14}C]catechol and catechol. On the basis of these experiments, we estimated the minimum contributions of these compounds to mainstream smoke catechol levels as follows: cellulose, 7–12%; total of fructose, glucose, and sucrose, 4%; chlorogenic acid, 13%; rutin, <1%. It is suggested that a significant portion of the remaining catechol in mainstream cigarette smoke is formed from pectin, starch, and hemicellulose.

The cocarcinogens of tobacco smoke are likely to be among the most important constituents responsible for its carcinogenic activities in experimental animals and man (Hoffmann et al., 1978; U.S. Department of Health and Human Services, 1982). These compounds, while not carcinogenic themselves, significantly enhance the tumorigenic activities of the polynuclear aromatic hydrocarbon carcinogens. In the absence of cocarcinogens and tumor promoters, the levels of polynuclear aromatic hydrocarbons present in tobacco smoke are not sufficient to induce tumors on mouse skin (Hoffmann et al., 1978). Catechol

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595, and Tobacco Laboratory, Beltsville Agricultural Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

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