Cuticular Lipids from Silks of Seven Corn Genotypes and Their Effect on Development of Corn Earworm Larvae [*Helicoverpa zea* (Boddie)]

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Cuticular lipids were extracted from the silks of seven corn genotypes, GT201wx, GT202wx, GT210wx, GT113, GT115, Antigua 2D-118, and Pioneer X304C. These lipids were identified by combined gas chromatography–mass spectrometry. There were compositional differences between genotypes, but the major components for each extract were *n*-alkanes, alkenes, aldehydes, fatty acids, and fatty alcohols. Alkenes comprised a much higher percentage (between 3% and 15%) of the surface lipids than they do in most plant cuticular lipids. Corn earworm larvae, *Helicoverpa zea*, were reared on meridic diet containing either chloroform-extracted or unextracted silks from the seven genotypes. Growth of larvae was monitored and shown to be significantly greater when larvae were reared on diet containing silks from which the cuticular lipids had been removed than when they were reared on diet containing unextracted silks.

The corn earworm, *Helicoverpa zea* (Boddie), is a serious economic pest of agricultural crops in the southeastern United States. The corn earworm develops on corn, *Zea mays* L., early in the growing season (Wiseman, 1985). The larvae feed on the whorl leaves, the emerging tassel, and the silks in the tips of the ears, where they are protected from many predators and parasitoids and where they are less susceptible to pesticide applications. The corn earworm then moves to other host plants where it often causes severe economic losses (Wiseman, 1985, 1987).

Some corn genotypes have been reported to be resistant to silk-feeding by the corn earworm (Wiseman et al., 1972, 1981, 1983, 1991). The isolation and identification of the chemical bases of corn earworm resistance in corn silks are important aspects in breeding and developing resistant corn plants. Resistance factors in corn silks that result in inhibition of corn earworm growth extend the life cycle of this insect and could, therefore, reduce the number of generations per year of this important pest (Wiseman and Isenhour, 1990).

There is evidence that plant cuticular lipids influence some herbivorous insects in the selection of host plants (Woodhead and Chapman, 1986; Chapman and Bernays, 1989; Espelie et al., 1991). Specific plant cuticular lipids can contribute to resistance to insect pests by affecting insect feeding behavior, such as palpation, biting, and movement (Bernays et al., 1976, 1985; Chapman, 1977; Eigenbrode et al., 1991). The addition of cuticular lipids to the diet of lepidopteran insects inhibits larval growth, and genotypic variation in these lipids may also play a role in host plant resistance (Quisenberry et al., 1988; Yang et al., 1991). In the present paper we characterize the cuticular lipids from the silks of seven corn genotypes, and we compare the growth of corn earworm larvae on these corn silks to the growth of larvae reared on silks from which the cuticular lipids have been removed.

MATERIALS AND METHODS

Corn Silks. Corn silks were collected from seven genotypes, GT201wx, GT202wx, GT210wx, GT113, GT115, Antigua 2D-118, and Pioneer X304C. The entries were grown in bulk plantings at Tifton, GA, in the summer of 1991, using agronomic practices common to the area. 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 genotype, air-dried at 41 °C, and then stored in the freezer (−10 °C) for further processing (Wiseman et al., 1991).

Extractions. Corn silks (approximately 10 g) from each genotype were immersed in 250 mL of redistilled chloroform for 1 min at room temperature to remove the cuticular lipids. A brief extraction period was used to remove primarily surface lipids (Espelie et al., 1980; Misra and Ghosh, 1991). The extracted silks were air-dried in a flow hood for 1 h to remove residual solvent. Chloroform extracts were concentrated to a volume of approximately 5 mL in a rotary evaporator at 40 °C and then stored at −20 °C for analysis.

Chemical Analysis. Aliquots (equivalent to the extract from 2 g of silks) were dried under a stream of N_2 and then derivatized with *N,O*-bis(trimethylsilyl)acetamide at 110 °C for 10 min. Excess derivatizing reagent was removed under N_2, and the derivatized extract was resuspended in 1 mL of hexane. Aliquots (0.1%) were analyzed by combined gas chromatography–mass spectrometry (Hewlett-Packard 5890A/5970). The capillary column (25-m cross-linked methyl silicone, 0.2-mm internal diameter, 0.33-μm film thickness) with helium as the carrier gas was held at 55 °C for 3 min after sample injection (splitless), and then the oven temperature was raised to 305 °C at a rate of 15 °C/min and held at this temperature for 20 min. The column was connected to a mass spectrometer, and mass spectra were recorded at 70 eV at 0.8-s intervals. Individual components were identified by their mass spectra, which were compared to those of standards, and were matched by computer search with the National Bureau of Standards Mass Spectral Library. Quantitation was based upon total ion chromatogram integrations which were corrected for response factors by utilizing a standard for each class of cuticular lipid component (Mattheis et al., 1991). Standards were tetradecanal, 1-hexadecanol, hexadecanoic acid, *cis*-9-tricosene, and *n*-pentacosane.

Bioassay. Extracted and unextracted silks were ground to a fine powder (1-mm screen) using a Cyclotec TC1093 (Fisher Scientific, Atlanta, GA) sample mill. The silk powders were mixed into a diluted pinto bean diet at a concentration of 50 mg of silks/mL of diluted diet (Wiseman and Isenhour, 1989). Diets were dispensed into 30 detached 7.5-mL pipet bulbs (Wiseman and Isenhour, 1991) at approximately 2 mL/bulb and then placed in 1-oz plastic diet cups for each treatment. The treatment diets were allowed to solidify for approximately 2 h. One corn earworm neonate was placed on top of the diet mixture within each

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Table I. Cuticular Lipid Composition (Percent) of Silks from Seven Corn Genotypes

RT[a]	component	corn genotype						
		GT201wx	GT202wx	GT210wx	GT113	GT115	2D-118[b]	X304C[c]
12.10	nonanoic acid	0.3	0.3	0.3	0.6	D[d]	0.6	0.6
13.04	decanoic acid	0.3	0.1	0.1	0.3	D	0.1	D
13.46	tridecanal	D	0.4	D	D	D	D	D
13.92	undecanoic acid	0.1	0.3	0.1	0.1	D	0.1	D
14.37	tetradecanal	0.3	0.4	0.3	0.2	0.2	0.3	0.2
14.76	dodecanoic acid	0.4	0.4	0.4	0.3	0.2	0.3	0.1
15.22	pentadecanal	0.6	2.3	1.4	0.8	0.7	1.1	0.6
15.55	tridecanoic acid	0.4	0.8	0.4	0.3	0.3	0.4	0.3
16.02	hexadecanal	0.3	0.6	0.5	0.3	0.3	0.5	0.3
16.31	tetradecanoic acid	1.3	0.9	1.4	1.1	1.1	1.1	0.7
16.79	heptadecanal	0.9	1.9	1.7	0.9	0.7	1.1	0.9
17.04	pentadecanoic acid	1.8	3.3	2.8	2.0	1.7	2.1	1.8
17.52	octadecanal	1.1	1.7	1.1	1.1	0.8	1.1	0.9
17.58	hexadecenoic acid	D	D	D	D	D	0.3	0.4
17.73	hexadecanoic acid	3.9	3.8	4.7	5.0	4.0	4.4	4.7
18.14	*n*-heneicosane	0.2	0.4	0.2	0.2	0.2	0.3	0.2
18.21	nonadecanal	0.8	0.9	0.9	0.6	0.5	0.6	0.5
18.38	heptadecanoic acid	1.4	1.0	1.6	1.3	0.6	1.1	1.0
18.83	octadecadienoic acid[e]	0.8	0.4	0.7	0.7	0.5	1.3	2.8
18.87	eicosanal[e]	4.2	5.3	3.9	3.9	1.5	6.3	5.3
18.89	octadecenoic acid[e]	1.3	0.3	0.7	0.6	0.3	1.0	1.7
19.01	octadecanoic acid	4.3	1.7	3.2	3.6	1.2	1.9	2.3
19.41	*n*-tricosane	0.6	0.8	0.6	0.7	0.8	2.2	2.0
19.50	heneicosanal	1.1	1.6	0.9	0.8	0.5	0.9	0.6
20.01	*n*-tetracosane	0.6	0.5	0.4	0.7	0.9	1.0	1.3
20.12	docosanal	6.6	10.7	5.4	5.0	3.2	6.1	4.7
20.21	eicosanoic acid	0.8	D	D	D	D	0.8	1.1
20.48	pentacosene	0.7	D	D		D	0.7	1.6
20.65	*n*-pentacosane	5.4	4.4	3.3	3.5	6.4	6.2	6.4
20.79	tricosanal	1.8	2.3	1.6	1.9	1.0	1.1	1.4
20.22	heneicosanoic acid						0.6	
21.18	hexacosene	0.2						
21.30	*n*-hexacosane	1.8	0.9	1.2	1.8	2.0	1.8	2.1
21.46	tetracosanal	2.8	5.7	4.3	2.5	2.4	2.9	2.5
21.53	docosanoic acid	1.4		D	1.6		0.7	1.3
21.76	2-methylhexacosane	D	0.3	D	D	D	D	D
21.88	heptacosene	3.6	1.4	2.0	1.6	2.0	3.7	5.2
22.08	*n*-heptacosane	13.1	13.2	15.1	14.6	19.9	14.1	12.2
22.41	tetracosanol	0.2	0.7	D	D		0.3	
22.70	octacosene	0.5		0.3	0.6	0.5	0.2	0.7
22.87	*n*-octacosane	3.8	2.0	4.2	5.0	5.2	3.4	2.7
23.11	hexacosanal	1.5	2.0	1.7	2.2	1.7	2.6	1.6
23.19	tetracosanoic acid							0.6
23.28	pentacosanol		0.2				D	
23.46	2-methyloctacosane	D	0.4	D	D	D	D	D
23.63	nonacosene	4.9	1.9	4.5	3.5	5.1	4.8	6.2
23.89	*n*-nonacosane	13.5	12.2	16.8	17.6	22.3	12.3	9.9
24.29	hexacosanol	0.5	1.6	D	0.5		0.6	D
24.93	*n*-triacontane	0.8	0.8	1.2	1.2	1.2	0.5	0.4
25.28	octacosanal	0.3	D	D	0.5	0.7	D	D
25.90	hentriacontene	0.8	D	0.9	0.7	1.6	0.7	1.4
26.24	*n*-hentriacontane	2.3	2.0	4.2	3.6	3.4	1.5	1.5

[a] Retention time (minutes). Components are listed only if they were identified by mass spectra. [b] Antigua 2D-118. [c] Pioneer X304C. [d] D, detectable but less than 0.1%. [e] Estimated by selected ion chromatography.

pipet bulb, and the cups were capped with paper lids. Corn earworm larvae were obtained from a colony maintained at the Insect Biology and Population Management Research Laboratory, Tifton, GA (Perkins et al., 1973). The experiment was maintained in incubators at 25 °C with a relative humidity of 80% and a 14:10 light/dark photoperiod. The experiment was arranged as a split plot randomized complete block design with 30 replications with 1 cup/replicate. Whole plots were normal or extracted-silk diets, and the split plots were corn genotypes. The weight of the larvae was recorded after 8 days. These data were subjected to an analysis of variance (SAS Institute, 1985), and means were separated by Waller–Duncan *k*-ratio *t* test (*k* ratio = 100; $P \leq 0.05$) (Waller and Duncan, 1969).

RESULTS

The cuticular lipids from the silks of seven corn genotypes, GT201wx, GT202wx, GT210wx, GT113, GT115, Antigua 2D-118, and Pioneer X304C, were identified by combined gas chromatography–mass spectrometry. The major components were primarily alkenes, *n*-alkanes, aldehydes, and free fatty acids (Table I). The compositions of the lipids for the seven genotypes were similar, but the cuticular lipid pattern for each genotype was distinct.

n-Alkanes were the major components of the cuticular lipids of the silks in all seven genotypes. The proportion of alkanes varied from 38% of the total surface lipids in GT202wx to 62% in GT115 (Table II). The alkanes *n*-heptacosane and *n*-nonacosane were the two dominant components of the surface lipids from each genotype. Alkenes, dominated by heptacosene and nonacosene, were present in the surface wax of the silks from all seven genotypes (Table II). The genotype Pioneer X304C had the highest proportion of alkenes (15.1%), while GT202wx had the lowest amount (3.3%).

Aldehydes were also major components of the cuticular

Table II. Composition (Percent) by Class of Cuticular Lipid Component of Silks from Seven Corn Genotypes

class of component	corn genotype						
	GT201wx	GT202wx	GT210wx	GT113	GT115	2D-118[a]	X304C[b]
n-alkanes (C_{21}–C_{31})	42.1	37.9	47.2	48.9	62.3	43.3	38.7
alkenes (C_{25}–C_{31})	10.7	3.3	7.7	6.4	9.2	10.1	15.1
primary alcohols (C_{24}–C_{26})	0.7	2.5	D[c]	0.5		0.9	D[c]
aldehydes (C_{13}–C_{28})	22.3	35.8	23.7	20.7	14.2	24.6	19.5
fatty acids (C_9–C_{24})	18.5	13.3	16.4	17.5	9.9	16.8	19.4

[a] Antigua 2D-118. [b] Pioneer X304C. [c] Detectable, but less than 0.1%.

Table III. Weight (Milligrams) of Corn Earworm Larvae after 8 Days on Meridic Diet Containing Unextracted Silks or Chloroform-Extracted Silks from Seven Corn Genotypes[a]

treatment	diet-check	corn genotype							mean
		GT201wx	GT202wx	GT210wx	GT113	GT115	2D-118[b]	X304C[c]	
unextracted silks	477.3b	546.7a	326.3c *	190.3e	305.2cd *	66.3f	273.8d *	103.2f	284.3 *
extracted silks	479.9b	571.2a	514.8b	198.9c	504.0b	55.0e	209.9c	124.6d	316.8

[a] Means within a row followed by the same letters were not significantly different ($P > 0.05$); means separated by an asterisk in the same column were significantly different ($P \leq 0.05$). [b] Antigua 2D-118. [c] Pioneer X304C.

lipids of the corn silks from each genotype. The aldehydes ranged in chain length from C_{13} to C_{28}, with docosanal and tetracosanal being the most prevalent (Table I). The proportion that aldehydes comprised of the total cuticular lipids varied from 36% (genotype GT202wx) to 14% (GT115) (Table II).

Free fatty acids were present in the surface lipids of the corn silks in a range from 9.9% (genotype GT115) to 19.4% (Pioneer X304C). Hexadecanoic and octadecanoic acids were the most prevalent components in this class of cuticular lipids (Table I). Free fatty alcohols comprised less than 1% of the surface lipids of the corn silks, with the exception of genotype GT202wx (2.5%) (Table II).

The mean weight of larvae reared on diet containing unextracted silks (284 mg) was significantly less than the average mean weight of larvae reared on diet containing extracted silks (317 mg). Larvae reared on artificial diet to which chloroform-extracted corn silks had been added weighed more than those larvae which had been reared on diet plus unextracted silks for five of the seven genotypes. The largest differences were observed for corn earworm larvae reared on diet plus silks from GT202wx, GT113, and Antigua 2D-118 (Table III). Corn earworm larvae that were reared on artificial diet containing unextracted corn silks weighed significantly less after 8 days than those larvae reared on control diet (Table III). The one exception to this decrease in weight of larvae was for those insects reared on silks from genotype GT201wx.

DISCUSSION

The cuticular lipid compositions found for the silks of the seven corn genotypes are very different from that which has been previously reported for the foliage of corn (Avato et al., 1985, 1990; Blaker and Grayson, 1988). The most distinct features of the silk lipids were the high proportion of alkenes (3–15%) and the relatively low amounts (0–3%) of free fatty alcohols (Table II). Fatty alcohols comprise 15% of the cuticular lipids of corn foliage, and alkenes have not been reported to be present in the surface wax of the leaves. The *n*-alkanes found in the foliar cuticular lipids of corn have a longer average chain length (C_{31} is the most dominant alkane) than those which are found in the lipids extracted from the corn silks (C_{27} and C_{29}).

It is common for the composition of cuticular lipids from the same plant to vary from one plant part to another (Baker, 1982; Jeffree, 1986), and it has been suggested that these variations may influence the behavior of herbivorous insects (Espelie et al., 1991). Cuticular lipids have been examined from several regions of the corn plant. The cuticular lipid extract of corn husks has a very low proportion of alkanes (4%) and fatty alcohols (not detected) but a large amount of sterols (21%) (Bianchi and Avato, 1984). The surface lipids extracted from corn pollen have a composition similar to that found for the corn silks. The lipids extracted from the pollen of four corn genotypes had a large amount of alkenes (9–22%) and slightly smaller proportions of *n*-alkanes (Bianchi et al., 1990). Triterpenols and esters of triterpenols were major components (up to 40%) of the pollen lipids. Two of the major triterpenols found in pollen lipids, α-amyrin and β-amyrin, were also detected in the lipids of the corn silks, but for each genotype they comprised less than 0.1% of the total cuticular lipids.

Maysin, a flavone glycoside that inhibits growth of lepidopteran larvae, has been shown to be present in silks of corn genotypes which are resistant to feeding by corn earworm (Waiss et al., 1979). However, maysin content in corn silks did not correlate well with corn earworm resistance (Henson et al., 1984; Wiseman et al., 1985) until recently (Snook et al., 1989; Wiseman, unpublished results).

The cuticular lipids of the corn silks may contribute to the inhibition of corn earworm growth observed when silks were added to the insect diet. Increased larval growth on diet that contained silks from which the lipids had been removed supports this possibility (Table III). Cuticular lipids from the leaves of corn had a similar effect on the growth of fall armyworm, *Spodoptera frugiperda* (Yang et al., 1991). The growth of fall armyworm larvae was enhanced when they were reared on meridic diet containing corn foliage from which the cuticular lipids had been extracted compared to when the larvae were reared on diet containing unextracted foliage. Fall armyworm growth was also inhibited when the cuticular lipid extracts were added to meridic diet (Yang et al., 1991). However, the addition of individual cuticular lipid components (*n*-alkanes, fatty alcohols, aldehydes, and fatty acids) to the diet enhanced the growth of fall armyworm (Yang, Wiseman, Isenhour, and Espelie, unpublished results).

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Registry No. Nonanoic acid, 112-05-0; decanoic acid, 334-48-5; tridecanol, 10486-19-8; hendecanoic acid, 112-37-8; tetradecanal, 124-25-4; dodecanoic acid, 143-07-7; pentadecanal, 2765-11-9; tridecanoic acid, 638-53-9; hexadecanal, 629-80-1; tetradecanoic acid, 544-63-8; heptadecanal, 629-90-3; pentadecanoic acid, 1002-84-2; octadecanal, 638-66-4; hexadecenoic acid, 373-49-9; hexadecanoic acid, 57-10-3; heneicosane, 629-94-7; nonadecanal, 17352-32-8; heptadecanoic acid, 506-12-7; octadecadienoic acid, 60-33-3; eicosanal, 2400-66-0; octadecenoic acid, 112-80-1; octadecanoic acid, 57-11-4; tricosane, 638-67-5; heneicosanal, 51227-32-8; tetracosane, 646-31-1; docosanal, 57402-36-5; eicosanoic acid, 506-30-9; pentacosene, 30551-31-6; pentacosane, 629-99-2; tricosanal, 72934-02-2; heneicosanoic acid, 2363-71-5; hexacosene, 64808-91-9; hexacosane, 630-01-3; tetracosanal, 57866-08-7; docosanoic acid, 112-85-6; 2-methylhexacosane, 1561-02-0; heptacosene, 67537-80-8; heptacosane, 593-49-7; tetracosanol, 506-51-4; octacosene, 38888-69-6; octacosane, 630-02-4; hexacosanal, 26627-85-0; tetracosanoic acid, 557-59-5; pentacosanal, 26040-98-2; 2-methyloctacosane, 1560-98-1; nonacosene, 77046-61-8; nonacosane, 630-03-5; hexacosanol, 506-52-5; triacontane, 638-68-6; octacosanal, 22725-64-0; hentriacontene, 77046-64-1; hentriacontane, 630-04-6.