JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Long Chain Alkanes in Silk Extracts of Maize Genotypes with Varying Resistance to *Fusarium graminearum*

S. SHEA MILLER,* LANA M. REID, GAIL BUTLER, SUSAN P. WINTER, AND NADIA J. MCGOLDRICK

Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6

The alkane content of the silks of nine maize genotypes was analyzed to investigate the role of silk wax in resistance to *Fusarium graminearum*. Silk samples were collected 2, 4, 6, and 8 days after silk emergence and divided into three sections: exposed silk, silk channel silk, and silk that is under the husk and overlying the kernels. Four major unbranched alkanes (C_{25}, C_{27}, C_{29}, and C_{31}) and three isoalkanes (C_{27i}, C_{29i}, and C_{31i}) were identified. Total alkane contents were highest in the exposed silk followed by the silk channel silk, with the lowest in the youngest silk closest to the kernels. In the silk channel and overlying kernel silks, the moderately resistant inbred CO272 consistently had the highest alkane content. None of the other inbreds with improved resistance had as high a level of alkanes as CO272, indicating that alkane content is not a major mechanism of resistance.

KEYWORDS: Alkanes; maize; corn; silk; *Fusarium*

INTRODUCTION

The silks of maize are often a point of first contact, or indeed, a major route of entry for both fungal and insect pests. Like all plant surfaces, the silks carry a coating of cuticular lipids which provides a two-way barrier: it prevents excessive moisture loss, and it is the first defense against attack by external agents (*1*). Studies have suggested that some component(s) of maize silk cuticular lipids may contribute to resistance to fungal or insect damage (*2*−*4*).

Fusarium graminearum Schwabe [teleomorph = *Gibberella zeae* (Schw.) Petch] is the causal agent of gibberella ear rot of maize or corn (*Zea mays* L.) in Canada, the United States, Europe, and other countries (*5*). This disease is of considerable economic importance due to the production of mycotoxins such as deoxynivalenol (DON, vomitoxin) and zearalenone. When contaminated grain is fed to livestock such as swine, these toxins result in vomiting, feed refusal, decreased weight gain, and reproductive problems (*6*). Besides causing direct and indirect economic losses, these fungi can also affect the health of grain handlers and processors. One of the major modes of entry of this pathogen into maize ears is by conidia or spores landing on the silks, germinating and then mycelia growing down the silks to the kernels and cob (*5*, *7*, *8*).

Genotypic differences in resistance of maize to infection with *Fusarium graminearum* via the silk have been reported (*9*). Little is known, however, about the underlying mechanism of this resistance. Bergvinson and Reid (*2*) have suggested that a major component of silk-based resistance may be in the wax coating of the silks, particularly in the content of long chain alkanes (C_{25}, C_{27}, C_{29}, C_{31}). Yang et al. (*3*, *4*) extracted the cuticular lipids of maize silks to determine their effect on the development of corn earworm larvae. Several classes of compounds were identified in the silk extracts, including *n*-alkanes, alkenes, aldehydes, fatty acids, and fatty alcohols. The lipid classes comprising the largest proportion of their extracts were the *n*-alkanes, with C_{27} and C_{29} being present in the highest amounts. In addition to the four alkanes identified by Bergvinson and Reid (*2*), Yang et al. (*3*) also detected C_{21}, C_{23}, and several alkanes with even numbers of carbons (C_{24}, C_{26}, C_{28}, and C_{30}). Larval growth was shown to be significantly greater when earworm larvae were reared on diets containing silks from which the cuticular lipids had been removed, than when they were reared on unextracted silks (*3*, *4*).

The studies of Bergvinson and Reid (*2*) were conducted on an Agriculture and Agri-Food Canada (AAFC) inbred maize line, CO272, which was released in 1986. During a routine screening of maize germplasm in the late 1980s, CO272 was found to possess moderate resistance to silk infection by *F. graminearum*. This inbred as well as some other sources of resistance have been used in the AAFC breeding program to develop newer inbreds with improved resistance to silk infection (*10*, *11*). The objective of the current study was to expand previous work (*2*) to examine the relationship between long-chain alkane content of silks and *F. graminearum* resistance in CO272, some of the newer AAFC ear rot resistant releases, and a selection of inbreds and hybrids varying in resistance to *F. graminearum*.

MATERIALS AND METHODS

Experimental Design and Silk Harvest. From 1997 to 1999, seven AAFC maize inbreds and two commercial hybrids were field grown

* To whom correspondence should be addressed. Telephone: (613) 759−1760. Fax: (613) 759−1701. E-mail: millers@agr.gc.ca. Centre Reference Number 03-206.

10.1021/jf0341363 CCC: $25.00 Published 2003 by the American Chemical Society
Published on Web 10/01/2003

to collect silk samples for alkane analysis. The inbreds consisted of CO272, one of AAFC's original sources of moderate silk resistance to *F. graminearum*, three inbreds with improved ear rot resistance (CO388, CO430, and CO431), and three susceptible checks (CO266, CO354, and CO359). CO388, released in 1996, was developed from the backcross of (B73 × CO272) × CO272; hence, CO272 was the source of resistance since B73 is highly susceptible (*10*). CO430 and CO431 were both released in 1999 and were developed from a synthetic population made up from five commercial hybrids with moderate levels of resistance (Pride K127, Funks G4106, Renk RK21, Northrup King NK9060, and First Line FL1656) (*11*). Since CO430 and CO431 were released in 1999, they were only available for use in the final year of this study (1999). The two hybrids used in the study were Pride K127, which has moderate resistance to *F. graminearum*, and a susceptible check, Pioneer 3751.

In early May, each genotype was planted in four-row plots in a three-replicate, randomized complete block design, on a sandy loam soil, at the Central Experimental Farm in Ottawa, Ontario, Canada. Each row consisted of 15 plants in a 3.5-m long row with 60 cm between rows. To keep track of silk maturity, individual plants in each row were tagged early in the morning on the first day of silk emergence from the primary ear. Four days after silk emergence, primary ears from each four-row plot were randomly harvested in the early morning, and transported to the laboratory within 1 h of harvesting for silk sampling. In 1997 and 1998, three ears were selected per plot, extracted, and analyzed separately, and mean values were calculated. In addition to the harvest at 4 days, three more harvest times (2, 6, and 8 days after silk emergence) were conducted on four of the seven genotypes (CO272, CO266, Pride K127, and Pioneer 3751). In 1999, all nine genotypes were harvested at all four harvest dates, but only one ear per plot was harvested per sampling time to allow for enough samples for each harvest date. The time span from 2 to 8 days after silk emergence was chosen to correspond with the peak in ear rot susceptibility for most maize genotypes (*12*). Silks were removed from the ears and divided into three sections: A, exposed silks; B, silks within the silk channel or husk collar; and C, silks inside the husk and overlying the kernels. Approximately 2 g of silk tissue from each section was weighed, placed in vials, and either extracted immediately, or frozen and stored at −20 °C until further analysis.

Extraction and Analysis of Alkanes. Long chain alkanes were extracted by washing the silks twice in 2 mL of HPLC-grade hexane. The extracts were pooled, evaporated under nitrogen, and redissolved in 1 mL of hexane containing 0.19 mg/mL of *n*-hexadecane (C_{16} alkane; Supelco) as an internal standard. Five μL of the underivatized extracts was injected into a Varian 3400 gas chromatograph equipped with a flame ionization detector and a 15-m SPB-1 thin film fused silica column (0.53 mm i.d., 0.1 μm film thickness; Supelco). The injector temperature was 300 °C, the oven temperature was programmed to ramp up 15 °C/min from 50 to 350 °C, and the detector temperature was 370 °C. Gas flow rates were set at 60 mL/min for N_2, 30 mL/min for H_2, and 300 mL/min for air. Varian Star chromatography software, Version 4.01 was used for peak integration. Peak identification was based on retention time in comparison to known standards in conjunction with mass spectrometry. For calculation of response factors, a series of alkane standards from C_{16} to C_{44} (Supelco) were used. Total alkane contents of the silk samples were calculated from GC results as the amount of hexane extract from 2 g of silks was too small for accurate weighing.

Statistical Methods. Data for each individual alkane as well as total alkanes were analyzed for each year, silk section, and harvest time. To draw general conclusions, analyses over years were also carried out for each silk section assuming a mixed model with effects for year, replicate within year, genotype, and genotype by year using the mixed procedure of SAS (*13*). Year, replicate, and interactions with them were considered random hence genotype effects in the analysis over years were compared to the genotype by year interaction. Analyses were also carried out incorporating harvest time, again as a mixed model with days after silking as a fixed effect nested within plot. Least squares means and their errors were estimated. Linear and quadratic effects over time and their interactions with genotype were examined. Where there were heterogeneous variances these were modeled as well. To further investigate the contribution of different alkanes, principal components and biplots (*14*) were plotted using SAS (*13*). Unless otherwise indicated, significance was set at the 5% level.

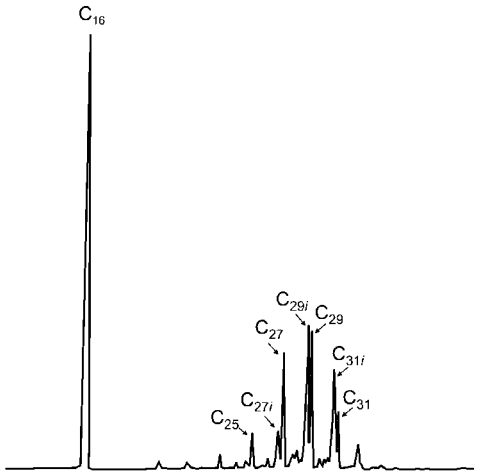

Figure 1. Chromatogram of a hexane extract of maize silk from the silk channel of CO388, with the internal standard (C_{16}) and four major alkane components (C_{25}, C_{27}, C_{29}, and C_{31}) identified as well as three isoalkanes (C_{27i}, C_{29i}, and C_{31i}).

RESULTS AND DISCUSSION

Alkane Identification. In the underivatized hexane extracts of maize silk at 4 days after silk emergence, we found four major unbranched alkanes (C_{25}, C_{27}, C_{29}, and C_{31}) in all three of the silk sections sampled (**Figure 1**). The same four alkanes were also reported as the main components in underivatized hexane extracts of maize silks from the husk collar (*2*). In addition, we have also detected measurable amounts of three isoalkanes (having the same number of carbons as the unbranched peak), in which there is a slight change in structure, probably a 2-methyl group, as the peaks were closely paired with, and chromatographed immediately prior to, the unbranched alkane peaks (**Figure 1**) (*1*, *3*). Isoalkanes were identified by GC-MS. The isoforms detected in the underivatized sample were C_{27i}, C_{29i}, and C_{31i}.

Yang et al. (*3*) extracted the cuticular lipids of whole maize silks harvested 2 days after silk emergence. Prior to analysis by GC-MS, the silks were oven-dried at 41 °C for several days, then extracted with chloroform, and derivatized. After derivatization, several classes of compounds were identified, including *n*-alkanes, alkenes, aldehydes, fatty acids, and fatty alcohols. In the present work, because the hexane extracts were underivatized, only the alkane components were detected by our GC analysis. In both our study and that of Yang et al. (*3*), C_{27} and C_{29} were present in the highest amounts. Only two isoforms were detected by Yang et al. (*3*): the C_{27i} isoform (identified as 2-methylhexacosane) and the C_{29i} isoform (2-methyloctacosane).

Total Alkane Content in Different Silk Sections. Because we wished to detect differences in alkane content that might be related to differences in levels of resistance to *F. graminearum*, but also minimize variability introduced by differences in wet weights as the silks dried down, we selected the fourth day after silk emergence (before the silks had started to senesce) for comparison of total and compositional alkane contents among genotypes. Of the five inbreds that were tested in all three years at 4 days after silk emergence, significant variation in total alkane content was found from year to year (genotype by year interaction). The hybrids were more consistent from year to year

Table 1. Total Alkane Wet Weight (μg/g) of Three Sections of Silk Tissue[a] from Seven Inbreds and Two Hybrids at 4 days Post-Silk Emergence in 1997–1999

		Year								
		1997			1998			1999		
	genotype	A	B	C	A	B	C	A	B	C
inbreds	CO272 (r)	341	241	138	146	163	98	130	180	115 (146[b])
	CO388 (r)	229	136	58	135	71	42	118	63	24
	CO430 (r)							128	103	30
	CO431 (r)							179	81	27
	CO266 (s)	181	116	60	160	67	41	429 (329[b])	110	47
	CO354 (s)	253	167	104	219	153	83	176	77	51
	CO359 (s)	56	55	37	32	45	30	41	32	27
	mean	212	143	79	138	100	59	172 (149[b])	92	46 (46[b])
	LSD[c]	68	46	18	23	20	11	124 (43[b])	32	36 (9[b])
	F-ratio[d]	25**	23**	53**	92**	77**	84**	9** (33**[b])	19.5**	8** (170**[b])
hybrids	Pride K127(r)	63	58	25	72	60	21	65	55	21
	Pioneer 3751(s)	63	39	12	54	21	9	40	29	11
	mean	63	49	19	63	41	15	52	42	16
	LSD[c]	9	30	10	55	24	8	84	51	6
	F-ratio[d]	0	8	33*	2	48*	50*	2	5	53*

[a] A = exposed silk, B = silk channel silk, and C = silk overlying kernels, under the husk. Varieties have been classed as resistant (r) or susceptible (s) based on disease ratings over several years of variety development and testing. [b] Estimates with outlier removed. [c] Least significant difference between any two means in the above column based on 2-tailed t-test. [d] Genotype F-ratio; **, $P \leq 0.01$; *, $P \leq 0.05$.

as would be expected since hybrids are usually less influenced by the environment than inbreds. Overall, the highest amount of total alkanes were found in 1997 (**Table 1**).

For most genotypes in most years, the total alkane content was generally highest on the exposed silk (section A), followed by silk channel silk (section B), with the lowest amounts usually found inside the husk on silks overlying the kernels (section C) (**Table 1**). In the exposed silk (section A), differences in total alkane among the inbred genotypes were not significant compared to the year × genotype interaction. It is apparent from **Table 1** that while the differences within each year were significant, the pattern was not the same from year to year. While the total alkane levels were highest in 1997, lower in 1998, and lowest in 1999 for CO272, CO388, and CO354, this was not the case for CO266 or CO359. Outlier data points were identified for CO266 and CO272, and while removing them had a considerable impact on the error and F-ratios (**Table 1**), it did not change the overall picture. In 1999, the errors were somewhat larger and there was a greater occurrence of outliers as only one cob was sampled per plot. While the susceptible check, CO266, was significantly lower than the resistant check, CO272, in 1997 (181 versus 341 μg/g), this was not the case in 1998 or 1999. CO388, which derives its resistance from CO272, was consistently lower than CO272 in all 3 years, significantly so in 1997. The susceptible inbred CO359 was consistently low in all three years (average 43 μg/g), but CO354, which is also susceptible, had a fairly high alkane content in all three years (216 μg/g). Increased variability in this section of silk tissue is to be expected since this is the section most exposed to environmental influences.

In the silk channel tissue samples (section B), alkane quantities were generally smaller, and while there was still interaction of genotype with year, the genotype effect was significantly greater in the overall analysis. CO272, the moderately resistant check, had the highest levels of total alkane, while CO359, a susceptible inbred, had the lowest. CO388, the resistant inbred related to CO272, was intermediate but still significantly less than CO272. CO266 and CO354, susceptible inbreds, were also intermediate with significantly less total alkane than CO272. This confirms preliminary results on the wax content of CO272 and CO266 at the husk collar (*2*).

There were no significant interactions with year for the samples from silk overlying the kernels (section C); thus, we saw a very similar pattern each year for the five inbreds included in all three years. As with the silk channel silk, the resistant check CO272 had a high total alkane content, while levels for the susceptible inbred, CO359, were consistently low across years. Once again, CO388 had significantly less total alkane than CO272 in each year; thus, it is doubtful that silk alkanes are important in the resistance observed in CO388 despite its derivation from CO272. CO388 is a very morphologically distinct genotype from CO272 with a much more robust growth habit, presumably inherited from the B73 side of its pedigree. Studies on *Aspergillus flavus*, which causes major problems in maize in the United States, suggest that silk-based resistance to fungal pathogens may have several components. *A. flavus* growth is inhibited by aqueous extracts of silks of resistant varieties (*15*), and volatiles emitted from silks of different maize genotypes have been reported to affect both fungal growth and mycotoxin production (*16*).

In 1999, we tested two additional moderately resistant inbreds (CO430 and CO431), which have Pride K127 in their pedigree. At each of the three silk sections, CO430 and CO431 had similar total alkane values ($P > 0.5$) that were higher than Pride K127 but less than the resistant check CO272 (significantly so in sections B and C), and not significantly greater than the susceptible inbred. Thus, it is apparent that alkanes do not contribute to the resistance of CO430 and CO431. Some recent studies with these inbreds indicate that resistance may be associated with compounds in the kernels rather than in the silks (*17*). In addition, differences in alkylresorcinol compounds in the pericarp wax were found in maize genotypes resistant and susceptible to *A. flavus* (*18*).

Silks from the two hybrids had quite low alkane contents compared to most of the inbreds and there was no significant ($P > 0.25$) year by genotype interaction for any silk section sampled (**Table 1**). In section A silk, the two hybrids did not have significantly different alkane contents in any of the three years, although there was a definite trend for higher alkane contents in the more resistant hybrid, Pride K127. A similar effect which was significant over 3 years was seen in silk sections B and C as well.

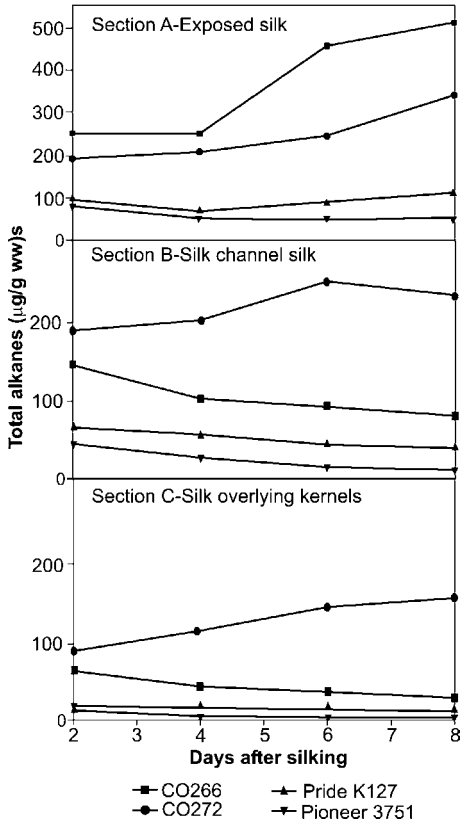

Figure 2. Total alkane profiles of two maize inbreds (CO272 and CO266) and two maize hybrids (Pride K127 and Pioneer 3751) over time averaged over three years, for silk tissue sections A (exposed silk), B (silk channel silk), and C (silk overlying kernels). Standard error of the difference between the inbreds (hybrids) at the same time point is 117 (20) for section A, 24(7) for section B, and 14(3) for section C.

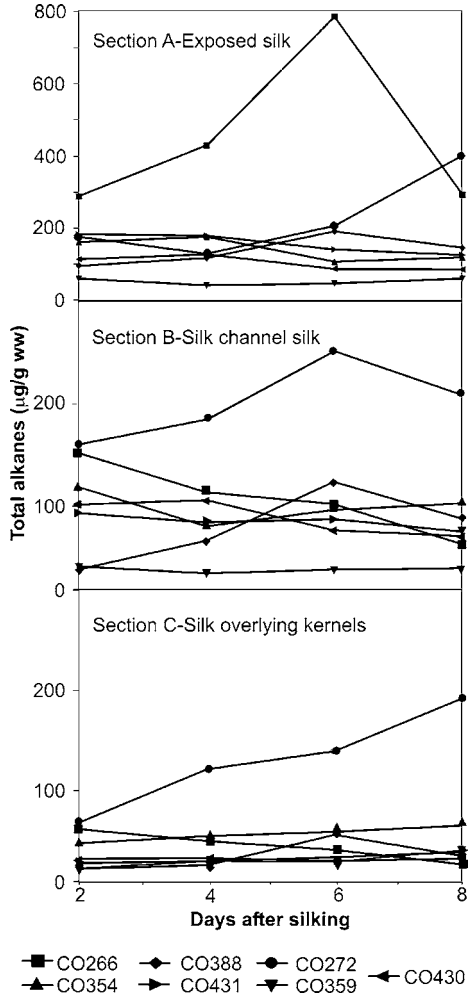

Figure 3. Total alkane profiles of seven maize inbreds (CO272, CO388, CO430, CO431, CO266, CO354, and CO359) in 1999 for silk tissue sections A (exposed silk), B (silk channel silk), and C (silk overlying kernels). Standard error of the difference between the inbred least squares means for section A at days 2, 4, 6, and 8 is 25, 56, 33, and 29, respectively. The standard error of the difference between any two means on the same day is 16 and 14 for sections B and C, respectively.

To investigate the change in total alkanes with time, we first examined the change in total alkane content from 2 to 8 days after silk emergence in the varieties examined over time in each year: CO272, CO266, Pride K127 and Pioneer 3751. Since the effects discussed were significantly greater than the year-to-year variability, data were analyzed over years for each silk section but separately for inbreds and hybrids. The total content of alkanes on the exposed silk in section A increased with time from day 2 until day 8 for the inbreds CO272 and CO266, significantly so for the latter (**Figure 2**a). Although the alkane level of CO266 was consistently greater than that of CO272, the overall difference between the two inbreds was not significant ($P = 0.06$). In contrast, the alkane content in the silk channel tissue (section B) was significantly higher in the more resistant inbred CO272 than in the susceptible inbred CO266 (**Figure 2b**). The pattern over time is also different for the two inbreds ($P = 0.01$); the alkane content for CO272 increased from day 2 until day 6, while the alkane content for CO266 decreased. In the silk overlying the kernels (position C), the pattern was similar to the silk channel tissue with the alkane content for CO272 increasing and CO266 decreasing through day 8 (**Figure 2c**).

As with the results at 4 days after silk emergence, the alkane contents at 2, 6, and 8 days were much lower in both of the hybrids than those found in the inbreds (**Figure 2**). In the exposed silk (section A), the difference between the two hybrids was not significant, but the difference in the slopes was; the total alkane content for Pioneer 3751 decreased slightly over time, while Pride K127, the more resistant hybrid, showed a slight increase after day 4 (**Figure 2a**). In section B silk (**Figure 2b**), the alkane content was significantly higher for Pride K127, but there was a significant linear decrease over time for both hybrids. Similarly for position C silk, Pride K127 showed a significantly higher alkane content (**Figure 2c**) and again there was a significant decrease over time for both hybrids.

In 1999, all of the inbreds in the study were sampled at 2, 4, 6, and 8 days after silk emergence (**Figure 3**). There were significant differences in average alkane content among the different inbreds as well as different patterns over time in all three sections. The alkane content on exposed silk of the susceptible inbred CO266 was 50% higher than any of the other inbreds (**Figure 3a**), and increased with time until day 6, then decreased to below the level of the more resistant inbred CO272. The alkane content on the silk of CO272 increased with time from day 2 until day 8, but only surpassed that of CO266 on day 8. All other inbreds sampled had levels below CO266 at all harvest times, although there was some variation in the time of peak alkane content.

For silk within the silk channel (**Figure 3b**), although the alkane contents of CO272 and CO266 were at the same level on day 2, CO266 levels decreased steadily through day 8, while CO272 levels increased to day 6, and then declined slightly by day 8. From day 4 to day 8, CO272 had a significantly greater

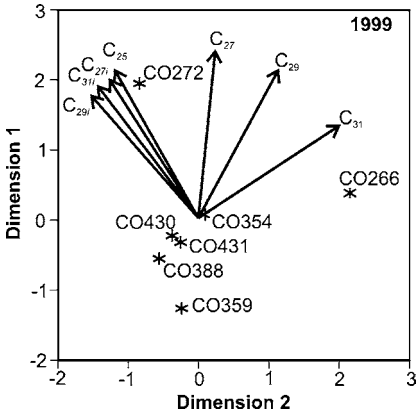

Figure 4. Biplot of the first two principal components based on the 1999 values for each of the four major alkanes and the three isoforms from silk channel silk tissue (section B) collected 2, 4, 6, and 8 days after silking from seven maize inbred lines. Vectors represent averages over days 4, 6, and 8.

alkane content than all of the other inbreds. Interestingly, the pattern of the alkane content of the related resistant inbred, CO388, was the same as that of CO272, although the levels were much lower. There was no significant difference between the alkane contents of CO430 and CO431, two inbreds derived from the same synthetic of commercial hybrids.

For silk overlying the kernels (**Figure 3c**), CO272 again started at the same level as CO266 on day 2, but increased through day 8, while CO266 decreased. Again, CO272 had a significantly higher alkane content than all the other inbreds from day 4 through 8.

Alkane Components. To further investigate the components of total alkane, we focused our attention on silk channel silk (section B), which was generally less variable than section A and had similar patterns but higher alkane levels than C. Principal component analysis was carried out for the inbreds using data obtained from 1999 silk channel silk (section B) samples as an exploratory tool to look at the different components of total alkane that are important for distinguishing among the inbred lines. A biplot of this analysis includes vectors representing each of the variables in the analysis and allows a visual examination of the relationship of these variables to each other; the smaller the angle between two vectors the larger the correlation between the two variables. The variables included in the analysis were means for the seven inbreds for the four major unbranched alkanes (C_{25}, C_{27}, C_{29}, and C_{31}) and the three isoforms (C_{27i}, C_{29i}, and C_{31i}) at each harvest time (2, 4, 6, 8 days after silking). The first principal component accounted for 59% of the variation and was highly correlated with total alkane (averaged over components and time, $r = 0.99$). It clearly separated CO272 from the remainder of the inbreds, even from CO388, a line that was partly derived from it (**Figure 4**). The second principal component accounted for an additional 31% of the variation. This component separated CO266 from the remainder of the inbreds. It was correlated with the total values for C_{31} ($r = 0.84$) that were high for CO266 in each year. It was also correlated with the slope over time ($r = -0.80$) which, as mentioned previously, was negative for CO266 (**Figure 3b**). We found that days 4, 6, and 8 for each of the unbranched alkanes clustered together in the biplot and were highly correlated ($r > 0.88$), while the different unbranched alkanes were not closely correlated to each other. The three isoforms C_{27i}, C_{29i}, and C_{31i} for each of days 4, 6, and 8 were all highly correlated to each other ($r > 0.82$) with the exception of C_{31i} day 4. Vectors representing the average for days 4, 6, and 8 for each of C_{25}, C_{27}, C_{29}, C_{31} and the isoforms are included in **Figure 4**. The vector for C_{31} is almost perpendicular to C_{25}, indicating little correlation between the two ($r = 0.03$), with C_{27} and C_{29} in between. When we conducted a similar analysis on the smaller group of inbreds sampled in 1997 and 1998, a similar pattern was seen in subsequent principal component analysis of day 4 data (**Figure 5a,b**). C_{25} and the isoform vectors were close to CO272 (**Figures 4** and **5**) indicating that higher values for these components were associated with CO272.

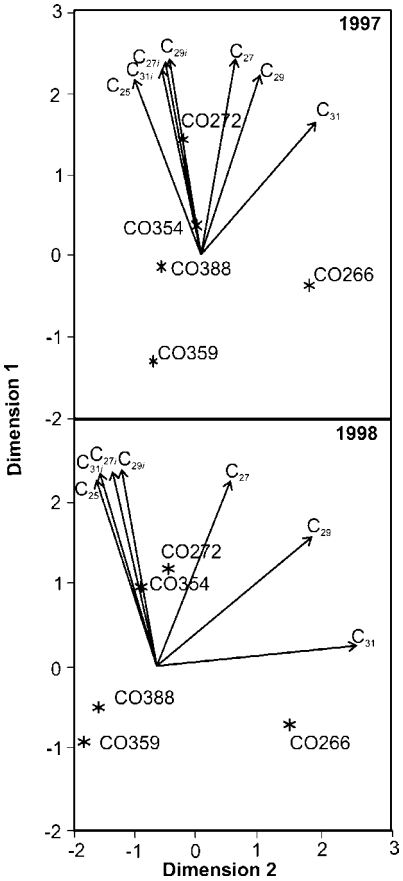

Figure 5. Biplot of the first two principal components based on the 1997-(a) and 1998(b) values for each of the four major alkanes and the three isoforms from silk channel silk tissue (section B) collected at 4 days after silking from five maize inbred lines.

A mixed model analysis of variance of the inbreds for each component separately over the three years for day 4 indicated significant variability over years (genotype by year interaction was significantly greater than the within year error) for each component. The least-squares means for the inbreds are presented in **Figure 6**. Differences among genotypes were significant for each component of total alkane (C_{25}, C_{27}, C_{29}, C_{31}, C_{27i}, C_{29i}, and C_{31i}). As can be seen in **Figures 4** and **5**, the two susceptible inbreds, CO354 and CO359 separated in the biplots; they were significantly different for all the components. CO354 clustered closer to the less susceptible inbreds, suggesting some other resistance mechanism must be in play. CO388, a line that was partly derived from CO272, was significantly different from CO272 for every component except C_{31} ($P = 0.08$) and yet not significantly different than the susceptible CO359 for any component. From **Figures 4** and **6**, we can see that it is the second axis (related to C_{31}) that separates CO272 and CO388 from the susceptible CO266. CO430 and CO431 clustered together in the biplot and were not significantly different for any of the components, not surprising considering they were selected from the same synthetic population.

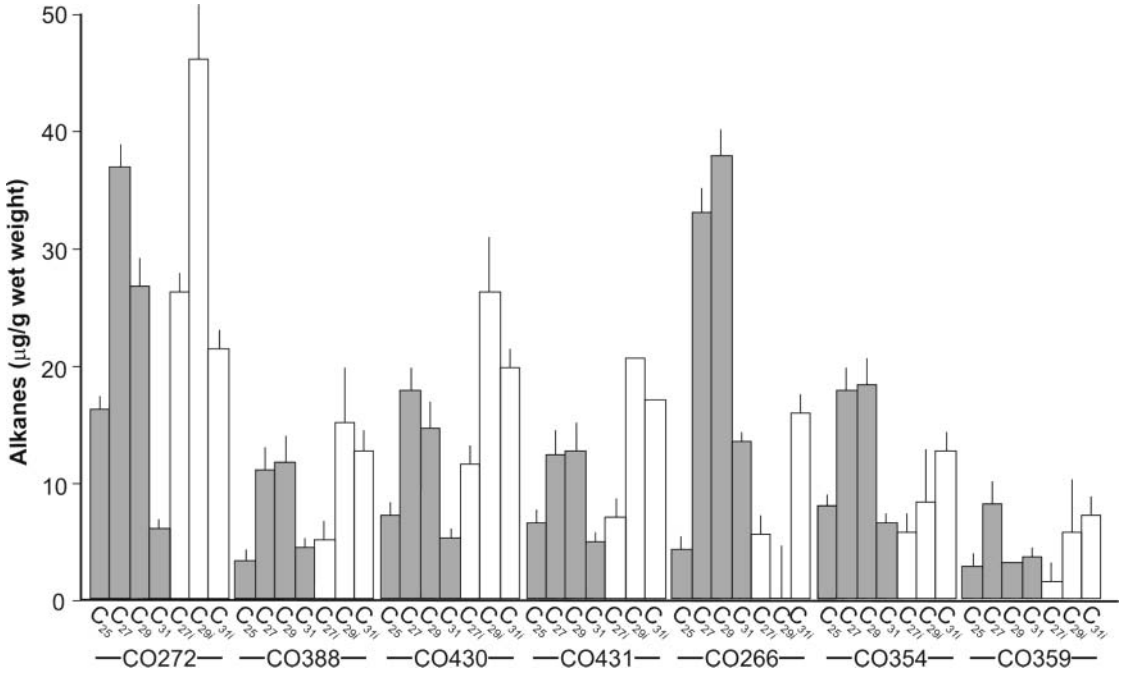

Figure 6. Alkane component least-squares means for CO272, CO388, CO266, CO354, and CO359 (1997–1999) and for CO430 and CO431 (1999) from silk channel silk tissue (section B) harvested for days after silk emergence. Error bars represent the estimated standard error of the mean.

Table 2. Alkane Wet Weight (μg/g) for Silk Channel Silk (section B) Harvested 2, 4, 6, and 8 days Post-Silk Emergence from a Moderately Resistant Maize Hybrid (Pride K127) and a Susceptible Hybrid (Pioneer 3751)

		alkane component											
	harvest time	C_{25}		C_{27}		C_{29}		C_{31}		total isoforms		total alkanes	
year	(days)	K127	P3751	K127	P3751	K127	P3751	K127	P3751	K127	P3751	K127	P3751
1997	2	0.0	4.2	9.0	12.5	8.9	10.9	0.5	2.2	37.8	33.2	56.2	63.0
	4	0.6	1.0	9.6	10.9	9.0	10.7	1.6	2.4	37.7	14.2	58.4	39.1
	6	1.5	0.0	10.1	8.1	9.7	9.5	2.4	0.9	35.7	5.5	59.5	23.9
	8	0.6	0.0	13.2	6.7	12.7	9.0	3.7	2.4	24.0	3.3	54.1	21.4
	LSD[a]	1.4		2.7		1.7		2.6		7.4		9.9	
	linear contrast	NS	**	**	**	**	*	*	NS	**	**	NS	**
1998	2	4.2	2.6	11.4	8.0	10.9	8.1	4.2	3.1	38.5	16.5	69.2	38.3
	4	2.8	0.2	12.2	6.1	11.9	7.1	4.8	3.1	28.4	4.9	60.1	21.3
	6	0.3	0.0	9.5	3.4	10.8	5.8	4.4	3.1	14.2	0.6	39.2	13.0
	8	0.5	0.0	9.7	1.7	11.4	4.6	4.7	2.9	10.2	0.0	36.5	9.3
	LSD[a]	0.8		1.4		0.6		0.5		10.4		11.9	
	linear contrast	**	**	**	**	NS	**	NS	NS	**	**	**	**
1999	2	4.1	3.9	12.6	8.5	11.7	4.9	4.6	2.8	42.7	13.9	75.7	34.0
	4	2.8	2.3	12.8	7.8	14.1	0.0	5.2	4.0	20.4	14.8	55.3	28.9
	6	1.8	0.6	7.1	4.0	12.0	0.0	4.9	3.1	8.7	7.8	34.6	15.6
	8	1.0	0.6	8.8	2.8	12.3	0.0	4.1	3.6	3.8	5.9	30.0	12.9
	LSD[a]	1.2		3.9		4.6		1.4		9.4		16.2	
	linear contrast	**	**	*	**	NS	*	NS	NS	**	*	**	**

[a] Least significant difference between any two means of the same genotype in either of the above columns based on 2-tailed t-test: **, $P < 0.01$; *, $P < 0.05$; NS, no statistical differences.

A mixed model analysis of variance of the hybrids at position B for each component separately over the three years for all days indicated significant variability over years (genotype by year by day interaction was significantly greater than the within year error) for all components with the exception of C_{31} and C_{31i}. As seen for total alkane (**Table 1**, **Figure 2b**), the component alkane levels were generally greater in the moderately resistant genotype Pride K127 than the more susceptible hybrid Pioneer 3751. Exceptions to this were the unbranched alkanes in the early harvest days in 1997 (**Table 2**); there was a significant genotype by year interaction as a result for C_{27} and C_{29}. C_{25} and the isoform vectors (C_{27i}, C_{29i}, and C_{31i}) were similar to each other and total alkane in the pattern over time, showing significant decreases for both genotypes. C_{27}, C_{29}, and C_{31} showed somewhat different patterns with Pride K127 silk channel tissue, averaged over years, maintaining or increasing in alkane content over days. However, for the unbranched alkanes there was variability in the pattern over years with more positive slopes over time for Pride K127 in 1997 than in 1998 and 1999 (**Table 2**).

CONCLUSIONS

The results of this study confirmed previous findings (*2*) that higher wax or alkane contents were present in the silk channel

or husk collar silk tissue of the moderately resistant inbred CO272 than in the susceptible inbred, CO266. Alkane levels in these two sections for CO272 increased after silk emergence but decreased in CO266. However, it was clear from a further examination of other inbreds with improved resistance to ear rot that high alkane contents are not always associated with increased resistance. An inbred line CO388, which was derived from CO272, did not have high alkane levels despite having moderate resistance. In addition, two resistant inbreds, CO430 and CO431, of a different genetic background than CO272 did not have high alkane contents. Clearly, there is more than one mechanism of resistance to ear rot in maize; high alkane contents may play a role in some genotypes but not others.

ACKNOWLEDGMENT

We thank Laura Visentin, Janet Gale, and James Powers for excellent technical assistance, and extend special thanks to David Bergvinson for many helpful discussions. The assistance of AAFC's maize breeding and pathology crew is greatly appreciated. This research was funded in part by the Ontario Corn Producers' Association and the Agriculture Agri-Food Canada Matching Investment Initiative.

LITERATURE CITED

(1) Misra, S.; Ghosh, A. Analysis of epicuticular waxes. In *Essential Oils and Waxes*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, 1991; pp 205−229.

(2) Bergvinson, D. J.; Reid, L. M. Phytochemical mechanisms of resistance to ear rots. *Maize Genet. Cooperation Newsl.* **1995**, *69*, 114−115.

(3) Yang, G.; Wiseman, B. R.; Espelie, K. E. Cuticular lipids from silks of seven corn genotypes and their effect on development of corn earworm larvae [*Helicoverpa zea* (Boddie)]. *J. Agric. Food Chem.* **1992**, *40*, 1058−1061.

(4) Yang, G.; Wiseman, B. R.; Espelie, K. E. Effect of cuticular lipids from silks of selected corn genotypes on the development of corn earworm larvae. *J. Entomol. Sci.* **1994**, *29*, 239−246.

(5) Sutton, J. C. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Can. J. Plant Pathol.* **1982**, *4*, 195−209.

(6) Vesonder, R. F.; Ellis, J. J.; Rohwedder, W. K. Elaboration of vomitoxin and zearalenone by *Fusarium* isolates and the biological activity of *Fusarium*-produced toxins. *Appl. Environ. Microbiol.* **1981**, *42*, 1132−1134.

(7) Hesseltine, C. W.; Bothast, R. J. Mold development in ears of corn from tasseling to harvest. *Mycologia* **1977**, *69*, 328−340.

(8) Koehler, B. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. *J. Agric. Res.* **1942**, *64*, 421−442.

(9) Reid, L. M.; Mather, D. E.; Hamilton, R. I.; Bolton A. Genotypic differences in the resistance of maize silk to *Fusarium graminearum*. *Can. J. Plant Pathol.* **1992**, *14*, 211−214.

(10) Reid L. M.; McDiarmid, G.; Parker, A. J.; Woldemariam, T.; Hamilton, R. I. CO388 and CO389 corn inbred lines. *Can. J. Plant Sci.* **2001**, *81*, 457−459.

(11) Reid, L. M.; McDiarmid, G.; Parker, A. J.; Woldemariam, T.; Hamilton, R. I. CO430, CO431 and CO432 corn inbred lines. *Can. J. Plant Sci.* **2001**, *81*, 238−284.

(12) Reid, L. M.; Bolton, A. T.; Hamilton, R. I.; Woldemariam, T.; Mather, D. E. Effect of silk age on resistance on of maize to *Fusarium graminearum*. *Can. J. Plant Pathol.* **1992**, *14*, 293−298.

(13) *SAS/STAT User's Guide*, Version 8; SAS Institute Inc.: Cary, NC, 1999.

(14) Jolliffe, I. T. *Principal Component Analysis*; Springer-Verlag: New York, 1986; 257 pp.

(15) Neucere, J. N. Inhibition of *Aspergillus flavus* growth by silk extracts of resistant and susceptible corn. *J. Agric. Food Chem.* **1996**, *44*, 1982−1983.

(16) Zeringue, H. J. Identification and effects of maize silk volatiles on cultures of *Aspergillus flavus*. *J. Agric. Food Chem.* **2000**, *48*, 921−925.

(17) Bily, A.; Ramputh, A.; Regnault-Roger, C.; Garcia, L. S.; Bergvinson, D.; Reid, L. M.; Arnason, J. T.; Philogene, B. J. R. Diferulic acids (DFA) profiles of maize grain: are the same factors operational for *Sitophilus zeamais* (Motsch.) and *Fusarium graminearum*? *Abstract. Entomological Society of America*, Dec 5−11, 2000, Montreal, Quebec.

(18) Gembeh, S. V.; Brown, R. L.; Grimm, C.; Cleveland, T. E. Identification of chemical components of corn kernel pericarp wax associated with resistance to *Aspergillus flavus* infection and aflatoxin production. *J. Agric. Food Chem.* **2001**, *49*, 4635−4641.

Received for review February 12, 2003. Revised manuscript received August 6, 2003. Accepted September 4, 2003.

JF0341363