Chemical and Genetic Attributes of a Maize-Silk Olfactory Trait

N. W. Widstrom,*,† R. A. Flath,‡ and P. J. Loesch, Jr.§

Insect Biology and Population Management Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Tifton, Georgia 31794, Western Regional Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Berkeley, California 94710, and U.S. Department of Agriculture, Agricultural Research Service, Columbia, Missouri 65211

A maize inbred extracted from an Iowa synthetic ("BSBB") produces silks with an odor that is easily detectable by most humans. The inbred did not demonstrate greater field resistance to ear-feeding by insects than sister lines from the same synthetic. The odorous trait was determined to be inherited as a recessive trait but could not be positively associated with a single gene when the odorous line was crossed with unrelated normal lines. Failure to produce a single-gene Mendelian ratio for F2 and backcross generations was attributed to errors in classification for a subjectively evaluated trait and the seeming incomplete penetrance of the trait, possibly due to the presence of modifying genes. Eleven compounds that differed from those obtained from nonodorous sister lines were separated and identified by headspace trapping and analysis by gas chromatographic and mass spectrometric techniques. A five-member panel clearly identified the odorous compound as indole.

Keywords: *Corn; Zea mays L.; corn earworm; Helicoverpa zea (Boddie); aroma; headspace volatiles*

INTRODUCTION

The notion that plant volatiles are important in attracting insects to corn (*Zea mays* L.) plants is not a new concept. McColloch (1922) suggested that the odor of corn plants is attractive to moths of the corn earworm, *Helicoverpa zea* (Boddie). The results of experiments reported by Dethier (1937) demonstrated that lepidopterous larvae use olfaction and gustation responses to determine their food choices. He later stated that odor is important for recognition of food plants by phytophagous insects (Dethier, 1941). These studies led Kennedy (1965) to generalize that insects may find their host plants by chemotropism. Starks et al. (1966) demonstrated that various corn plant extracts elicit an olfactory response by corn earworm moths.

The routine examination of volatiles was begun when technology became available to identify and quantify the compounds eminating from a source (Flora and Wiley, 1974). Buttery et al. (1978) identified numerous potential corn earworm attractants from the volatiles of corn kernels and husks. Flath et al. (1978) identified a similar series of volatiles from corn silks, and Buttery et al. (1980) followed with a study of volatile compounds from corn tassels. Several compounds identified from corn tissue were tested against the *Aspergillus* group of fungi by Wilson et al. (1981) as a result of reports by Moore-Landecker and Stotsky (1974) and Nandi (1977) that volatile metabolites from bacteria and germinating seeds are able to affect growth and development of fungi, such as those that are associated with field and stored wheat grain. Some of the corn tissue volatiles, notably β-ionone, noticeably influence sporulation and morphology of *Aspergilli* (Wilson et al., 1981).

The motivation for initiation of the research reported in this paper was provided by the late Dr. P. J. Loesch, U.S. Department of Agriculture (USDA) Research Geneticist at Columbia, MO, and later at Ames, IA. He noted a distinct difference in human olfactory response to the silks among S5 sister lines of the Iowa opaque-2 broad base synthetic "BSBB". Our objective was to determine if the silks of lines with distinctively different odors have biological significance in resistance to insects, and also to determine the chemical responsible for olfactory responses by humans.

MATERIALS AND METHODS

Plant Materials for Field Evaluation. The selections with normal silk odor and with the "flower-like smell" were S5s from the BSBB population. Two selections from each class were increased, and for identification purposes, were labeled 4-2 and 5-2, "normal", and 0-2 and 1-2, "odorous", respectively. Seed of parents, F_1, F_2, and backcrosses were obtained for each of two normal × odorous pairs of lines. These two sets were planted and tested for silk odor in 1985. Although neither odorous parent could be confirmed as homozygous for the odorous trait, the most promising of the two sets was grown and evaluated again in 1986. The odorous line (1-2) was then subjected to two more generations of selfing and evaluation to increase homozygosity for the odorous trait. The odorous and normal sister lines were repeatedly evaluated for differences in susceptibility to damage by corn earworm in the field during their development.

The odorous line (1-2) was then crossed to two unrelated normal lines ("CI38B" and "GT114"); also, F2 and first backcross seed were obtained for evaluation in 1992 as sets after the fashion of the 1985 and 1986 tests. Selfed families of the backcrosses to the odorous line, 1-2, were evaluated in 1993 to assist in determining inheritance of the trait. Evaluations were made on windless days by technicians with the ability to detect the unique aroma of "odorous" silks. All ear shoots were covered with shoot-bags to prevent pollination and allow accumulation of aroma, which was enhanced by the morning sun. These conditions provide ideal circumstances for identification of odorous silks.

* Author to whom correspondence should be addressed [tel (912) 387-2341, fax (912) 387-2321, e-mail NWIDSTRO@TIFTON.CPES.PEACHNET.EDU].

† Insect Biology and Population Management Research Laboratory.

‡ Western Regional Research Laboratory.

§ Deceased. Formerly with the USDA, Columbia, MO.

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An error rate indicating failure to properly classify individual silks was not determined, but some allowance for classification errors must be assumed for our subjective system. Rigid statistical procedures were, therefore, not applied to segregation ratios of determined classes, and discussion was based on nearness to expected ratios for genetic models.

Silk Collections for Chemical Analysis. Approximately 100 plants each of the normal (5-2) and odorous (1-2) sister lines were grown in separate greenhouses during the winter of 1988−89. Nonpollinated silks were excised at 3−5 days after emergence and bulked to obtain 320 g of 5-2 and 399 g of 1-2. Bulked samples were immediately frozen and shipped by overnight express to the USDA, Agricultural Research Service (ARS), Western Regional Research Center (WRRC) in Albany, CA.

The same sister lines (5-2 and 1-2) were field-grown during the summer growing season of 1989. Approximately 5 kg of intact fresh ears were harvested from each line when silks were 3−5 days old. The fresh, unfrozen ears were placed in cold boxes with blue ice and shipped by overnight express to the WRRC in Albany, CA.

Headspace Trapping and Analysis. A headspace gas sample was collected from each of two batches of silks, odorous and nonodorous. Silk samples (odorous silk, 399 g; nonodorous silk, 320 g) were placed in clean 5-L round-bottom resin flasks. The flask heads were fitted with two o-ring-sealed thermometer adapters. Through one of these adaptors, a 0.25-in. o.d. Teflon tube extended to the bottom of each flask, leading from a large activated charcoal trap. The other adapter supported a glass tube trap containing 6.5 g of 60/80 mesh Tenax adsorbent in a bed 2.2 cm in diameter × 12 cm long. The exit end of each trap was connected with additional Teflon tubing to a stainless steel bellows pump fitted with a needle valve on the exit port, permitting adjustment of the pump (and chamber) air flow to 1.1 L/min. After an initial 5-min purge interval, with an empty tube in place of the Tenax trap, the tube was replaced with a clean Tenax trap, and trapping was continued for 90 min at 1.1 L/min (99 L total). This sequence was also followed with a clean empty sample chamber and plumbing before any silk samples were run to provide a system "blank" in a third Tenax trap.

Each of the three traps received 15 μg each of the internal standards cycloheptanone and cylodecanone in ether solution, then was backflushed with 75 mL of freshly distilled ether. Each rinse solution was concentrated by careful distillation for subsequent gas chromatography/mass spectrometry (GC/MS) examination.

A Finnigan 4500 GC/MS interfaced with an INCOS data system was used for concentrate analyses. A cool on-column injector (SGE, Inc.) led to a bonded crosslinked DB-1 methylsilicone capillary column (J&W Scientific), which was programmed from 50−250 °C at 4 °C/min and at a constant head pressure of 15 psi. The exit end of the column led directly to the ion source of the quadrupole mass spectrometer [1 s scan; 33−350 atomic mass units (amu); 70 eV; electron-impact ionization]. Initial component identifications were based on comparisons of mass spectra of components with those of authentic samples, contained in a reference library, followed by subsequent verification by matching experimental component peak retention indices (RI) with those of authentic samples determined under the same GC conditions.

A second pair of corn samples consisted of intact immature ears, still retaining husks and emerging silks. Ears were ≈22 cm long and 2.5−3.0 cm in maximum diameter. A 22-L resin kettle was used as the sampling chamber; all other features were identical to the apparatus just described. Each of the two immature ear samples (odorous, 72 ears, 5.11 kg; nonodorous, 88 ears, 4.82 kg) was treated in the same manner. Purified air flow through the chamber was initially set at 0.44 L/min. A corn sample was placed in the chamber, and headspace volatiles were swept into a clean Tenax trap for 3 h at 0.44 L/min (79.2 L total). A clean trap was substituted, the flow was reduced to 0.25 L/min, and sampling was continued for 18 h at 0.25 L/min (270 L total).

Table 1. Olfactory Responses to Individual Silks of Parents, F1s, F2s, and Backcrosses of Odorous and Normal Experimental Lines Extracted from the BSBB Population in 1985 and 1986

	number of individual plant olfactory determinations			
	1985		1986	
pedigree	odorous	normal	odorous	normal
experimental 4-2 (normal)	0	41		
experimental 0-2 (odorous)	9	29		
4-2 × 0-2 (F1)	1	107		
(4-2 × 0-2) ⊗ (F2)	63	414		
(4-2 × 0-2) × 4-2 (BC1)	16	313		
(4-2 × 0-2) × 0-2 (BC2)	66	263		
experimental 5-2 (normal)	1	23	0	12
experimental 1-2 (odorous)	24	14	36	0
5-2 × 1-2 (F1)	0	108	5	37
(5-2 × 1-2) ⊗ (F2)	98	371	62	320
(5-2 × 1-2) × 5-2 (BC1)	11	306	5	313
(5-2 × 1-2) × 1-2 (BC2)	47	266	73	275

The traps were extracted in the same manner as just outlined, yielding a total of four concentrates, two from each corn ear sample. Each concentrate was adjusted in volume with freshly distilled pentane so 50 μL of solution represented 1 ear equivalent.

The concentrates were examined by GC/MS, with the instrumentation and settings already noted. The two concentrates representing the 18-h trappings proved most useful for comparison purposes.

RESULTS AND DISCUSSION

The first field determinations among segregating plant populations were made in 1985. The plants evaluated were derived from crosses between the original S5 lines received from Dr. Loesch. The results (Table 1) suggest that the original lines were not homozygous or that we were unable to identify all the individual plants with odorous silks in the field. The clearest evidence of misclassification was provided by approximately one-third ("experimental 1-2") to three-fourths ("experimental 0-2") of silks from plants of lines classified as odorous being determined to be of the normal type. Additionally, the segregation of F2, BC1, and BC2 plants gave ratios that yielded no obvious genetic interpretation.

A cross between another plant of experimental 1-2 and experimental 5-2, along with its segregating F2 and backcrosses was evaluated in 1986 with better results. Both lines in the 1986 test were uniform in their classification, but no clear explanation was revealed by the segregation observed in the F2 and backcrosses. The results in 1985 and 1986 indicated that the odorous trait was recessive because the F1 produced few if any odorous progeny and odorous silks were always in the minority for F1, F2, and backcrosses. At this point, it was decided to conduct further selfing in the parents to increase confidence that genes for the odorous trait were homozygous and that others, possibly interfering with proper classification of silks, were purged from the plants used for crossing to develop the next round of field testing.

The additional selfing of experimental 1-2 produced an inbred that was very uniform for the odorous trait in 1989. This inbred and related sister lines without the odorous trait were evaluated for resistance to ear-feeding insects. All lines from the BSBB population proved to be susceptible to ear feeding, even though plants occurred in those lines that exceeded 2% silk maysin on a dry weight basis (Snook et al., 1989). This

Table 2. Olfactory Responses for Individual Silks of Parents, F1s, F2s, and Backcrosses of Odorous Experimental Line 1-2 with Inbreds GT114 and CI38B

	number of individual plant olfactory determinations	
pedigree	odorous	normal
experimental 1-2 (odorous)	30	0
GT114 (normal)	0	45
1-2 × GT114 (F1)	0	63
(1-2 × GT114) ⊕ (F2)	23	190
(1-2 × GT114) × 1-2 (BC1)	29	72
(1-2 × GT114) × GT114 (BC2)	0	106
experimental 1-2 (odorous)	42	9
CI38B (normal)	0	49
1-2 × CI38B (F1)	1	57
(1-2 × CI38B) ⊕ (F2)	61	177
(1-2 × CI38B) × 1-2 (BC1)	47	89
(1-2 × CI38B) × CI38B (BC2)	5	118

level of maysin will affect larval growth in corn earworm when husks are tight enough to force feeding on the silks before entry into the ear. All BSBB lines have loose husks that allow immediate access to kernels on the ear. Inability to quantify odor of silk masses from individual plants has prevented the determination of a potential relationship between odor and maysin content of silks.

The odorous line was crossed to two unrelated normal lines ("CI38B" and "GT114"), and the populations developed from these crosses were evaluated in 1992 (Table 2). The evidence reaffirmed the odorous trait as recessive, but did not confirm the trait as being controlled by a single gene. A single-gene recessive explanation seems most probable, and deviations from expected ratios may be accounted for by one or more of three possible explanations: (1) the classification system may be too subjective to accurately identify all silks of the odorous class; (2) modifier genes, incomplete penetrance, or presence of other chemical compounds may be interfering with proper classification; and (3) the genetic system controlling the odorous trait is too complicated for single genetic ratio explanations. The existing data suggest that inheritance of the trait is primarily controlled by a single recessive gene in the presence of modifiers that influence expression of the trait. Also, the possibility of misclassification remains when determinations are based on a subjective scale.

Chi-square values for segregation of the F2 and BC1 generations of the CI38B and GT114 crosses confirm a single gene recessive when penetrance values are assumed for the recessive genotypes to be 0.85 and 0.50, respectively. The evaluation of families from selfs of the backcross to the odorous parent suggest a recessive hypothesis with incomplete penetrance, because 12 of 22 and 11 of 17 selfed BC1 families segregated for the odorous trait in 1993 tests.

The first attempt at sampling and shipping silks for analysis was something less than successful. Samples excised and frozen for shipment proved unsatisfactory. Physical collapse of the silk cell structure and subsequent coalescing of cell contents may have prevented the extraction of volatiles responsible for silk odor. Some differences in appearance and volatile identification occurred, but they were not distinctive enough to merit consideration as the cause of odorous silks of experimental 1-2.

Our second attempt at sampling and shipment with whole ears of intact silks in an unfrozen condition was more encouraging than our first attempt. The unfrozen samples of our second shipment more nearly mimicked their natural state during field testing when they were extracted for volatiles. The following differences were notable when chromatogram printouts for the odorous and normal silks were superimposed and compared. (a) normal silks were much higher in sesquiterpene hydrocarbons than odorous silks; (b) odorous silks had much higher concentrations of ethyl acetate than normal silks; and (c) other compounds demonstrating lesser differences and/or concentrations in normal silks than in odorous silks included 2-propyl acetate, 1-propyl acetate, 2-methyl-1-propyl acetate, 3-methyl-1-butyl acetate, 2-methyl-1-butyl acetate, 1-pentyl acetate, (*Z*)-3-hexen-1-yl acetate, 1-hexyl acetate, indole, and *n*-tridecane.

Small samples of ethyl acetate and the 10 compounds just listed were shipped to Tifton for possible identification of the unique compound of the odorous inbred line. A blind test was constructed for a five-person panel to determine if one or more of the compounds listed was responsible for the unique smell of odorous silks. The panel consisted of individuals with field and greenhouse experience over a period of at least 3 years at identification of odorous silks. The test was replicated with the following results: (a) four of five panel members independently identified indole as the distinguishing compound for odorous silks in both replicates; and (b) one of the least experienced raters indicated in one replicate that ethyl acetate was a possible candidate, but chose indole in the other replicate.

Subsequent testing by panel members, when direct comparisons of field-grown samples and purified vial samples were available, have repeatedly identified indole as the compound distinguishing odorous silks from normal. It is possible that ethyl acetate, as a dominating component of odorous silks, could interfere with classification of odorous plants when it is segregating in conjunction with indole in F2 and backcross populations. At this point, no biological significance in terms of resistance to insects has been attached to indole content of silks.

We have targeted resistance to insect or infection to *Aspergillus flavus* as having a possible relationship to indole content because we happen to be working with those plant traits. Several possibilities exist regarding the biological significance of odorous silks, the most obvious being that the trait is related to the production of indole acetic acid (IAA) in the silks. Because IAA, a common natural growth regulator or auxin, may be involved, it should provide an interesting area for investigation by physiologists. One further evidence for the possibility of blockage of IAA synthesis and the accumulation of indole in odorous silks is a corresponding increase in other acetic compounds, such as the 10 listed in our previous discussion. Investigations using these observations and ideas as a premise seem a logical approach for subsequent studies.

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