Maize-Silk Maysin Data: Comparison of Interpretations of Quantifications by Spectrophotometry and HPLC

N. W. Widstrom,* M. E. Snook,‡ W. W. McMillian, A. C. Waiss, Jr.,§ and C. A. Elliger§

Insect Biology and Population Management Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 748, Tifton, Georgia 31793

Maysin in maize silks is antibiotic to the corn earworm. Our objective was to compare spectrophotometric and reversed-phase HPLC methods of quantifying maysin concentrations. HPLC assay has eliminated abnormally high readings obtained when the spectrophotometric method was used. Maysin of three maize populations, and parents and related generations of two hybrids, was analyzed by both methods and compared. Spectrophotometrically analyzed maysin concentrations of individual silks were similar, but variances differed from those analyzed by HPLC. Spectrophotometric values were scattered for SC235×F44 and its related populations in 1983, but 1988 HPLC determinations produced interpretable data. Data from F6×F44 populations in 1983 suggested that F6 has a gene for reducing maysin content, but 1988 HPLC distributions indicated that the gene in F6 is dominant for low maysin content. The changes in interpretation illustrate the importance of using an accurate method for measuring silk maysin concentration.

The presence of antibiotic chemicals in plant tissue, such as those found in cotton (*Gossypium hirsutum* L.; Chan et al., 1978), can be critical to the suppression of plant pests. The identification of maysin, a C-flavonol glycoside found in certain maize (*Zea mays* L.) silks (Waiss et al., 1979), kindled an interest in control of the corn earworm [*Heliothis zea* (Boddie)] in corn. Maysin impedes larval growth and development in the corn earworm and opens a new avenue for reducing earworm damage in corn through the assistance of naturally occurring chemicals.

The presence of a "lethal" factor in certain corn silks was suggested by Walter (1957, 1962). The effects of antibiosis on corn earworm larvae by corn silks were later demonstrated by Straub and Fairchild (1970) and Wiseman et al. (1976, 1984). When maysin was initially identified, relative amounts in corn silks were routinely quantified by using UV absorption at a wavelength of 352 nm (Widstrom et al., 1982). This procedure proved effective for making initial determinations of the genetic basis for genotype differences (Widstrom et al., 1981) and even for differentiating among corn genotypes at varying locations (Widstrom et al., 1982).

Failure to show a significant relationship between maysin content in oven-dried silks and corn earworm larval growth in oven-dried silks (Wiseman et al., 1985) suggested that maysin may be only a minor component of resistance to the corn earworm. Alternatively, the method for determining relative maysin content may have given erroneous results, especially with certain genotypes. Spurious or erratic readings seemed to occur most frequently for genotypes known to be high in anthocyanins and flavonoid compounds. Further evidence of erroneous maysin content determination resulted when 20% of the plants in some F_2 distributions were apparent transgressive segregates, making genetic interpretation of the data difficult or impossible (Widstrom et al., 1983).

Reassessment of the spectrophotometric technique led to the conclusion that certain UV-absorbing compounds were causing the erroneous values (Snook et al., 1989) obtained for several of the genotypes we were studying. This reassessment led to the development of a reversed-phase high-performance liquid chromatographic procedure for determining maysin content of silks (Snook et al., 1989). The HPLC method was found to provide silk maysin values that were significantly correlated with larval weights in diet studies (Wiseman, 1989, unpublished data). The objective of this study was to compare silk maysin values of corn genotypes determined by spectrophotometry and chromatography and to compare conclusions regarding inheritance of silk maysin content measured by both methods.

‡ Richard Russell Research Center, USDA—ARS, Box 5677, Athens, GA 30613.

§ Western Regional Research Center, USDA—ARS, 800 Buchanan Street, Albany, CA 94710.

MATERIALS AND METHODS

When highly inflated values for individual silk maysin content began to appear unexpectedly in our screening tests, two selections (flavonoids G12# and G21#) typical for the inflated values were chosen for comparison with our high-maysin check, Zapalote Chico 2451# (P) C3. This check, referred to as ZC, also produced some high, unexpected values for silk maysin when evaluated spectrophotometrically. The flavonoids and ZC were grown, and individual silks sampled in 1984, 1985, and 1988, for comparison of statistical parameters obtained from distribution of maysin content for individual silks.

The inbreds F6, F44, and SC235 were chosen for testing the inheritance of maysin content of silks, on the basis of previous results from screening tests (Widstrom et al., 1983). These inbreds, the crosses F6×F44 and SC235×F44, and their F_2s and backcrosses were grown and individual silks sampled in 1983. When the inflated, unexpected values for our check were verified, we decided not to publish these 1983 data until an adequate method of analysis could be developed. The new method was reported by Snook et al. (1989). The F6×F44 and SC-235×F44 experiments were repeated in 1988, using the new method so that the methods for measuring maysin could be compared. The inheritance of maysin content was examined from content of individual silks, and the distributions were used for comparing measurement methods and for comparing the testing of inheritance under both systems.

Unpollinated 5-day-old exposed silks of individual plants were removed, and those remaining in the silk channel to the tip of the ears were collected and freeze-dried immediately in 1983, 1984, and 1985. Freeze-dried samples were processed at the USDA—WRRC at Albany, CA, and were analyzed for maysin content according to the spectrophotometric procedures given

Table I. Parameter Estimates Obtained from Individual Plant Values for Maysin Content of Silks in Three Partially Inbred Populations Using Spectrophotometric Analyses for 1984–1985 and HPLC Procedures in 1988

			maysin content, % dry wt		
population[a]	eval method[b]	N	mean	variance	range
ZC 2451# (P) C3	S	12	8.17	11.402	1.59–13.70
	C	45	3.40	2.545	0.96–8.13
flavonoid G12#	S	10	12.86	5.363	9.70–16.15
	C	45	3.71	1.905	0.86–7.51
flavonoid G21#	S	10	7.28	29.949	0.11–15.04
	C	31	3.62	3.409	0.03–7.33

[a] ZC 2451# (P) C3 is a selection from Zapalote Chico (PI 217413) that is nearly homozygous. Flavonoids G12# and G21# are populations selected from W23×K55 background, with and without a kernel pigment intensifier gene, respectively. [b] S, spectrophotometric evaluation at 352 nm UV; C, chromatographic evaluation by HPLC.

by Waiss et al. (1979). Spectrophotometric absorbance was recorded at 352 nm.

Unpollinated 3-day-old silks of individual plants were excised at the tip of the ear, frozen immediately, and later submerged in methanol for shipment to the USDA—Richard Russell Agricultural Research Center at Athens, GA, in 1988. These samples were processed and analyzed according to the procedures developed by Snook et al. (1989). Means, variances, and ranges were calculated for all plants tested under each evaluation procedure. The distributions of individual silk maysin values for all plants in each grouping were studied for segregation that would indicate the inheritance of silk maysin content. Conclusions regarding inheritance under both systems of measurement were compared by using χ^2 techniques (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Most samples that were analyzed prior to the 1988 study were obtained from bulks of five or more silks, masking the effect of individual silks that may have had compounds causing inflated values. Spectrophotometrically determined single-silk analyses, however, gave inflated estimates for some individual silks of certain corn populations and inbred lines. We concluded that UV-absorbing compounds other than maysin were interfering with the analyses.

High values for maysin in individual silks occurred in our high-maysin check population (ZC), from which maysin was isolated (Table I). The partially inbred ZC population, though not genetically homozygous, was not expected to have a large mean and wide range for maysin content. Even larger means and variances for maysin content were realized among individual silks of other populations, especially flavonoid G21#. We expect that ZC silks may contain a much higher proportion of compounds that are biochemically more closely related to maysin than silks from either of the flavonoid selections. Bioassays suggest that ZC has more antibiotic compounds than G12# and G21# (Wiseman et al., 1985).

The chromatographic analysis indicated a similar level of maysin for ZC and the two flavonoids (Table I), even though ZC has a more pronounced antibiotic effect (Wiseman et al., 1985). It is possible that compounds closely related to maysin or to its degradation products also produce some antibiotic effect, thus enhancing the performance of ZC in laboratory bioassays. The trend of similar but smaller variances for the flavonoids when analyzed by HPLC, than when analyzed spectrophotometrically, seems to carry no detectable biological significance when maysin content alone is considered.

As was the case with the populations, variation among individual silks of inbreds was not expected to be high. Surprisingly, individual silk maysin values for SC235 and F44 each had ranges with a more than 10-fold difference from low to high (Table II). Only F6, with a very low content of maysin and other related compounds, failed to give rise to one or more inflated values. In F44, the high variance of maysin content of individual silks was largely attributable to several very low values, while SC235 had numerous and scattered high values. Since these inbreds have never been selected for maysin content, it is possible that plants may still be heterozygous for genes controlling maysin concentration. The resultant distributions of single-silk spectrophotometric values in 1983 for the parents and related generations of the SC235×F44 cross could not be given a simple genetic explanation. Inspection of 1983 spectrophotometric data for the F6×F44 cross suggests that F44 has a gene for high-maysin content. Numerous 1983 silk maysin values of backcrosses, F_1, and F_2 were skewed and/or scattered in the distributions. Skewed and scattered characteristics in the distributions should not occur if a single gene is conditioning the maysin content. In any case, the 1983 spectrophotometric data are inconsistent and hinder genetic interpretation.

Table II. Parameter Estimates Obtained from Individual Plant Values for Maysin Content of Silks in Two Maize Crosses and Their Related Populations Using Spectrophotometric Analyses in 1983 and HPLC Procedures in 1988

			maysin content, % dry wt		
population	eval method[a]	N	mean	variance	range
SC235×F44	S	15	1.58	1.538	0.55–3.43
SC235×F44	C	15	1.63	0.124	0.58–2.16
SC235	S	20	1.26	1.203	0.33–4.09
SC235	C	15	0.92	0.228	0.08–1.58
F44	S	21	0.70	0.422	0.25–2.92
F44	C	15	2.36	0.152	1.83–3.08
BC_1	S	30	2.10	1.677	0.34–4.31
BC_1	C	42	1.16	0.476	0.08–3.66
BC_2	S	31	1.48	1.201	0.44–3.73
BC_2	C	42	1.83	0.698	0.42–4.07
F_2	S	52	0.91	0.478	0.14–2.56
F_2	C	80	0.87	0.209	0.08–2.41
F6×F44	S	15	0.303	0.0147	0.16–0.68
F6×F44	C	15	0.332	0.0228	0.09–0.56
F6	S	15	0.124	0.0002	0.09–0.14
F6	C	13	0.124	0.0090	0.05–0.40
F44	S	21	0.703	0.4224	0.25–2.92
F44	C	15	2.234	0.4658	0.48–3.35
BC_1	S	30	0.290	0.0839	0.07–1.17
BC_1	C	39	0.391	0.0650	0.03–0.97
BC_2	S	30	0.539	0.2264	0.21–1.90
BC_2	C	41	0.712	0.4603	0.07–2.57
F_2	S	50	0.602	0.4045	0.007–2.48
F_2	C	74	0.717	0.5618	0.01–4.89

[a] S, values determined spectrophotometrically at 352 nm; C, values determined chromatographically by HPLC.

In contrast to 1983 data, analyses of the 1988 data from the individual silk HPLC data distributions of the F6×F44 cross and related generations suggested that low maysin content is controlled by a single dominant gene in F6. Clear-cut truncation points for individual classes for maysin content were not obvious in the segregating population distributions, but were determined primarily by inspection of the means and distributions of the parents, F_1 and BC_1, which should not be segregating under the hypothesis being tested. χ^2 values for 3:1 segregation in F_2 and 1:1 in BC_2 were 1.80 and 3.51 ns, respectively. Under the assumption that SC235 also carries the dominant gene in F6, the chromatographic data for the SC235×F44 populations did not fit the hypothesis when the F_2 and BC_2 populations were tested for 3:1 and 1:1 segregation for low and high maysin content, respectively. The F_2 contained too few high-maysin segregates, while dominance in SC235 was evidently not strong enough to place all F_1 and BC_1 silks in the low-maysin category.

Since maysin values obtained by the chromatographic method are known to have improved accuracy for maysin determination (Snook et al., 1989), the genetic interpretation for F6 obtained from the 1988 data for F6 is believed to be the correct one. That the crosses tested in 1988 (SC235×F44 and F6×F44) give genetic interpretations compatible with each other is of greater significance, and only F6 is assumed to have a dominant gene for low-maysin content. The 1988 HPLC results contradict our 1983 hypothesis of a gene in F44 contributing to high-maysin content.

On the basis of comparisons with standard concentrations, Snook et al. (1989) clearly demonstrated that their HPLC procedure accurately assayed the maysin content of silks in the presence of other closely related compounds. Some of these closely related compounds are believed to make a contribution to corn earworm resistance, but maysin is presently believed to be the major component of chemical resistance. Snook et al. (1989) also estimated that silk levels of 2% dry weight may be needed to impart corn earworm resistance. All the populations (Table I) and the inbred F44 (Table II) exceeded 2% maysin on a dry weight basis. As also stated by Snook et al. (1989), the new HPLC silk maysin determinations are expected to relate much better to antibiotic effects exhibited by larvae of the corn earworm.

LITERATURE CITED

Chan, B. G.; Waiss, A. C., Jr.; Lukefahr, M. Condensed tannin, an antibiotic chemical for *Gossypium hirsutum*. *J. Insect Physiol.* **1978**, *24*, 133–8.

Snook, M. E.; Widstrom, N. W.; Gueldner, R. C. A reversed phase, high performance liquid chromatographic procedure for the determination of maysin in corn silks. *J. Chromatogr.* **1989**, *477*, 439–47.

Steel, R. G. D.; Torrie, J. H. *Principles and procedures of statistics*; McGraw-Hill: New York, 1960.

Straub, R. W.; Fairchild, M. L. Laboratory studies of resistance in corn to the corn earworm. *J. Econ. Entomol.* **1970**, *63*, 1901–3.

Waiss, A. C., Jr.; Chan, B. G.; Elliger, C. A.; Wiseman, B. R.; McMillian, W. W.; Widstrom, N. W.; Zuber, M. S.; Keaster, A. J. Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. *J. Econ. Entomol.* **1979**, *72*, 256–8.

Walter, E. V. Corn earworm lethal factor of sweet corn. *J. Econ. Entomol.* **1957**, *50*, 105–6.

Walter, E. V. Source of earworm resistance for sweet corn. *Proc. Am. Soc. Hortic. Sci.* **1962**, *80*, 485–7.

Widstrom, N. W.; Wiseman, B. R.; McMillian, W. W.; Elliger, C. A.; Waiss, A. C., Jr. Variations among corn silk genotypes for maysin, a compound antibiotic to the corn earworm. In *Agronomy Abstracts ASA*; American Society of Agronomy: Madison, WI, 1981; p 76.

Widstrom, N. W.; Waiss, A. C., Jr.; McMillian, W. W.; Wiseman, B. R.; Elliger, C. A.; Zuber, M. S.; Straub, R. W.; Brewbaker, J. L.; Darrah, L.; Henson, A. R.; Arnold, J. M.; Overman, J. L. Maysin content of silks of nine maize genotypes grown in diverse environments. *Crop Sci.* **1982**, *22*, 953–5.

Widstrom, N. W.; Wiseman, B. R.; McMillian, W. W.; Elliger, C. A.; Waiss, A. C., Jr. Genetic variability in maize for maysin content. *Crop Sci.* **1983**, *23*, 120–2.

Wiseman, B. R.; McMillian, W. W.; Widstrom, N. W. Feeding of corn earworm in the laboratory on excised silks of selected corn entries with notes on *Orius insidiosis*. *Fla. Entomol.* **1976**, *59*, 305–8.

Wiseman, B. R.; Widstrom, N. W.; Waiss, A. C., Jr. Resistance to corn earworm larvae in corn silks as related to larval growth suppression and recovery. *J. Agric. Entomol.* **1984**, *1*, 53–7.

Wiseman, B. R.; Widstrom, N. W.; McMillian, W. W.; Waiss, A. C., Jr. Relationship between maysin concentration in corn silk and corn earworm (Lepidoptera: Noctuidae) growth. *J. Econ. Entomol.* **1985**, *78*, 423–7.

Received for review April 3, 1990. Accepted July 2, 1990.

Registry No. Maysin, 70255-49-1.