Laboratory Bioassays for Evaluating Sweet Corn Antibiosis on European Corn Borer (Lepidoptera: Pyralidae) Larval Development[1]

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ABSTRACT European corn borer (*Ostrinia nubilalis* Hübner) can severely affect commercial sweet corn quality during years of heavy infestation. The isolation and identification of allelochemicals in sweet corn which detrimentally affect *O. nubilalis* may enhance breeder selection for greater ear feeding resistance, thus reducing the need for insecticide application. Field selection techniques for improving plant resistance to *O. nubilalis* cannot easily distinguish between plant tolerance or antibiosis. A laboratory bioassay incorporating ear tissues from field resistant and susceptible sweet corn genotypes into a nutritionally complete *O. nubilalis* larval diet was developed as an initial step to facilitate the isolation and identification of potential chemical resistance factors in sweet corn. Neonates reared for 7 d on a meridic diet with limited fungal and bacterial contaminant control agents weighed more than larvae grown on a comparable diet with high levels of contaminant control (5.96 and 2.46 mg, respectively). Silk tissue from several sweet corn genotypes significantly reduced larval weight and increased total larval development time compared with kernel tissue. Silk tissues incorporated on a weight basis had volumes about 3 x that of an equal weight of kernel tissues. However, tissues incorporated into a specific diet volume on a weight or volume basis usually did not alter larval weight or time to pupation within a genotype. Incorporation on a weight basis was most time efficient. Future bioassays screening for antibiotic effects of sweet corn tissue on *O. nubilalis* development should utilize a diet with limited contaminant control agents, incorporate tissue on a weight basis, and focus on silk tissue.

KEY WORDS *Ostrinia nubilalis*, European corn borer, *Zea mays*, allelochemicals, antibiosis, host plant resistance

European corn borer, *Ostrinia nubilalis* (Hübner), ear feeding damage in sweet corn is a perennial problem in the United States. Minnesota ranks second in U. S. sweet corn production for processing and spends approximately $4 million

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annually to minimize *O. nubilalis* damage, primarily with insecticidal control (Gingera et al. 1993) and removal of infested ears and insect parts in processing factories (Noetzel et al. 1985). Insecticide application is a major production cost which could be reduced by using resistant hybrids. Although most commercial sweet corn hybrids are highly susceptible to ear feeding by *O. nubilalis*, sources of resistance have been identified (Davis and Grier 1978, Davis et al. 1994, Lamb et al. 1994) and recently released to the seed industry (Davis et al. 1993).

Recently, Bolin et al. (1996) demonstrated the commercial potential of selected *O. nubilalis* resistant sweet corn hybrids for the midwestern U.S. The mechanism(s) of ear feeding resistance in these genotypes are unknown but may result from morphological or chemical features. Morphological features, such as tight, narrow, ear shoot silk channels or tough husks, may protect kernel tissue from insect feeding but not reduce insect survival rates. This form of resistance is termed tolerance (Painter 1951). Alternatively, chemical resistance which limits insect survival or development is referred to as antibiosis (Painter 1951).

Field experiments have identified morphological and/or allelochemical factors that mitigate *O. nubilalis* damage, but they do not distinguish the effect of either component. Grier (1981) indicated that decreased ear damage occurred with increased silk channel length ($r = -0.42$) and wider silk channel diameter ($r = -0.36$). Greater ear feeding damage in 21 *O. nubilalis* resistant breeding lines occurred with shorter silk channel lengths (Davis et al. 1992). Grier (1981) and Davis et al. (1992) evaluated morphological resistance components in these experiments, although the possibility of a chemical component could not be excluded. Joyce and Davis (1995) experimentally modified the morphological component in several genotypes by mechanically reducing silk channel length. Most genotypes had greater ear feeding damage when ear shoot tips were shortened. However, the modified ears maintained a resistance level beyond that of the susceptible control, 'Jubilee', indicating that other resistance mechanisms existed in the screened germplasm.

A variety of laboratory bioassay experiments have been used to identify chemical resistance factors without the confounding morphological resistance components (e.g., Smith et al. 1994, Wiseman and Carpenter 1995). As an initial step to facilitate isolation and identification of potential chemical resistance factors in sweet corn, we developed a laboratory bioassay incorporating sweet corn ear tissue into a nutritionally complete *O. nubilalis* larval diet. Tissues from sweet corn genotypes, shown in field experiments to be resistant or susceptible to ear feeding by *O. nubilalis*, were used. Three bioassay experiments were conducted to identify: (1) a basal diet, (2) ear tissue(s), and (3) a tissue concentration that allowed separation of *O. nubilalis* resistant and susceptible sweet corn genotypes. Protocols defined in these experiments will be used in future assays to screen additional sweet corn genotypes and tissue extracts for antibiotic effects on larval development.

Materials and Methods

The following procedures were used in each of three bioassay experiments. Field resistant and susceptible sweet corn genotypes were grown in 1993 and 1994. Cultural practices common to dry-land commercial sweet corn production in

the Midwest were followed, except that no insecticides were applied. To obtain the maximum amount of silk tissue and to limit tissue age effects on larval development (Johnson 1980), silk tissue of each genotype was bulk harvested 5 to 7 d after 50% silking, stored at -20° C, and subsequently lyophilized. Because field selection for improved plant resistance to *O. nubilalis* occurred at the commercial processing stage (Davis et al. 1992, 1994), kernel tissue at this stage, about 70% moisture, was cut from cobs, bulked within genotype (≥ 15 ears/genotype), stored, and prepared as described for silk tissue. Dried tissues were ground to 0.6 mm and incorporated into nutritionally complete meridic diets for laboratory rearing of *O. nubilalis* larvae.

Ostrinia nubilalis neonates from a laboratory colony were placed onto diet + tissue combinations and reared in growth incubators set at 25° C, continuous light and 75 to 80% RH. To limit the effects of light and temperature variations within the chamber, trays containing individual treatment combinations were rotated daily from chamber top to bottom and side to side. At 7 or 10 d intervals, larval weights and number of living larvae were recorded to estimate larval developmental changes. Time to pupation also was noted as an additional estimate of larval developmental change. For the 7 d intervals, relative growth rates between each weight measurement were calculated by using the formula described by Waldbauer (1968). A mean neonate weight of 0.62 mg, as determined from the collective weight of 50 neonates, was used as an initial larval weight. The first bioassay (1993 field tissues) was designed to determine if diet affected larval development. The second bioassay (1993 field tissues) was developed to determine if silk or kernel tissue from individual genotypes differentially affected larval development. The third bioassay (1994 field tissues) investigated how tissue concentrations in the diet affected larval development.

Bioassay 1. In the first bioassay, a single field resistant genotype, MN365, was used to compare two nutritionally complete *O. nubilalis* larval diets routinely used at the University of Minnesota. A split-plot design was used with diets (Diet 1 and Diet 2) as main plots and ear tissues (silk and kernel) as subplots. Diet 1 contained Fumidil B (Mid-Continent Agrimarketing, Inc., Overland Park, KS, 500 mg/kg), Aureomycin (American Cyanamid Company, Wayne, NJ), sorbic acid, and methyl-p-hydroxybenzoate to limit protozoa diseases, bacterial, fungal, and fungal contamination, respectively (Guthrie 1972, Reed et al. 1972, See Table 1). Diet 2 did not contain Fumidil B or Aureomycin but did contain sorbic acid and methyl-p-hydroxybenzoate (Table 1). Diet additives may affect larval development in some species (Chippendale and Beck 1964, Dunkel and Read 1991, Lewis and Lynch 1970, Lewis et al. 1971, Schroeder et al. 1986, Vanderzant 1974, Wiseman et al. 1984).

Though the mixing protocols were similar, each diet was individually prepared. Plastic 35-ml cups were sterilized with 95% ethanol and the work area with 10% bleach, zephiran chloride, and 95% ethanol in that order. The dry ingredients, except for methyl-p-hydroxybenzoate and choline chloride, were weighed and stored at 4° C until diet preparation. Just before mixing the diets, methyl-p-hydroxybenzoate and choline chloride were added to the dry ingredients listed in Table 1.

Diets were prepared by heating 1000 ml deionized water and agar to 90° C with constant stirring, then incorporating ingredient(s) no. 3 for Diet 1 and nos. 3 to 7 and 15 to 19 for Diet 2. The ingredients were thoroughly mixed and cooked for 15

Table 1. Ingredients for 2 meridic diets for rearing European corn borer larvae. Diet 1 is modified from Guthrie (1972) and Reed et al. (1972). Diet 2 is modified from Adkisson et al. (1960), Chippendale and Beck (1964), and Vanderzant et al. (1962).

Ingredients	Diet 1 (% W/W)*	Diet 2 (% W/W)*
1) Deionized Water	84.95	82.55
2) Agar	1.92	1.99
3) Wheat Germ	3.57	3.98
4) Casein	3.02	2.79
5) Cholesterol	0.22	0.24
6) Sorbic Acid	0.06	0.10
7) Methyl-p-hydroxybenzoate	0.14	0.10
8) Vitamin Diet Fortification Mixture	0.60	1.00
9) Ascorbic Acid	0.85	0.45
10) Dextrose	2.75	
11) Salt Mixture No. 2 U.S.P.	0.99	
12) Fumidil B (500 mg/kg)**	0.05	
13) Propionic Acid Mixture	0.59	
14) Aureomycin†	0.29	
15) Sucrose		2.76
16) Cellulose		2.39
17) Linseed Oil		0.02
18) Wesson Salt Mixture		0.80
19) Choline Chloride		0.17
20) Wheat Germ Oil		0.26
21) Sodium Alginate		0.40

*W/W = ingredient percentage on a weight/weight basis.
**(Mid-Continent Agrimarketing, Inc., Overland Park, KS).
†(American Cyanamid Company, Wayne, NJ).

minutes at 90° C. Heat was removed and the mixture cooled to 54° C with constant stirring. The remaining ingredients, nos. 4 to 14 for Diet 1 and nos. 8, 9, 20, and 21 for Diet 2 (Table 1), were blended with 225 ml deionized water for 1 minute and added to the cooled diet mixtures. The blender was rinsed with a second 225 ml deionized water and this rinse water was added to the diets. Individual diets were stirred an additional 2 to 3 minutes.

Prepared diets were equally divided before incorporating the dried ground corn tissues (1 g dried corn tissue/20 ml diet) with a hand whisk. About 20 ml of the molten diet + tissue was quickly poured into sterile 35-ml cups. Any excess diet was removed from the sides of the cups after the diet had solidified. Because tissue quantities were limiting, the 6 treatment combinations included 29 to 45 cups over three replications. Poured cups were surface sterilized with UV light for 1 hour and stored overnight in sealed containers at 4° C before infesting with two *O. nubilalis* neonates per cup. The controls were diet without tissue additions.

Infested cups were capped, placed in the growth incubator, and held at the environmental regime described above. Larvae were transferred to fresh diet + tissue at 7 d intervals to minimize any confounding effects caused by fungal or bacterial growth on Diet 2.

The experimental layout and model statements of a split-plot design were as described by Little and Hills (1978). Data were analyzed by using the general linear models procedure of SAS software (SAS Institute 1990). An initial analysis of main effects indicated the cups did not differ ($P \geq 0.05$). To simplify the model statement, this variable was included in the error term when testing other main effects and interactions. A protected LSD was used to separate means.

Bioassay 2. The second bioassay design was similar to that of Bioassay 1 except that only Diet 2 was used, four sweet corn tissue sources (genotypes) were tested, and individual treatment combinations had from 20 to 45 cups over three replications. Two controls, diet alone and diet + cellulose, were included. Cellulose was added to the control diet as an indicator of nutritional dilution effects when tissues were added to the diet. A split-plot design with genotypes MN3152, MN3153, 'Apache', and 'Jubilee' as main plots and tissues (silk or kernel) as subplots was used. Of the four genotypes used as tissue sources, MN3153 inbred and 'Apache' were field resistant to *O. nubilalis* feeding, while MN3152 inbred and 'Jubilee' were field susceptible (Davis et al. 1994, 1996). Because diet contamination was not observed in Bioassay 1 and ear tissue quantities were limited, the 7 d interval between larval measurements was increased to 10 d. The interval increase biased relative growth rate calculations due to the presence of late instar larvae in some treatments. Incorporation protocols, incubation regimes, data collection, and data analysis were as described for Bioassay 1.

Bioassay 3. The design and protocols of the third bioassay were as described for Bioassay 2, except that main plots were tissue concentrations and subplots were tissues. Two tissue concentrations, as determined by (1) tissue-to-diet weight/volume (W/V) (1 g/20 ml) or (2) tissue-to-diet volume/volume (V/V) (4 ml/20 ml), were used for each genotype + tissue combination. Previous assays with Lepidoptera incorporated corn tissues on a tissue weight-to-diet volume basis (Wiseman and Isenhour 1991, Johnson 1980). Tissue density, however, affects volume inversely and incorporating tissues on a weight basis may differentially affect the nutritional quality of the diet as a specific weight of silk tissue has a volume about three times that of an equal weight of kernel tissue. The third bioassay examined the hypothesis that incorporation of large volumes of tissue may dilute the nutritive value of diets and contribute to larval weight variations. A diet + cellulose control was used. Individual treatment combinations varied from 27 to 55 cups over two replications. In an effort to account for larval weight variability attributed to sex, each cup was infested with a single larvae. Pupae were held at the environmental regime listed earlier and adults were sexed upon emergence. Data were collected and analyzed as above, and by sex.

Results and Discussion

Bioassay 1. Larval survivability did not differ at 7 or 14 d for either diet, but did differ for tissue ($F = 40.23$; df = 2, 256; $P \leq 0.001$ and $F = 39.66$, df = 2, 256; $P \leq 0.001$ for 7 and 14 d, respectively). This difference, however, was due to limited

silk quantities, which resulted in silk tissue treatment combinations having fewer larvae (≈ 20/replication) than kernel tissue and control treatment combinations (≈ 30 and 30/replication, respectively).

Larval weights at 7 ($F = 35.85$, df = 2, 213; $P \leq 0.001$) and 14 d ($F = 32.11$, df = 2, 198; $P \leq 0.001$) and relative growth rates at 7 ($F = 25.25$, df = 2, 213; $P \leq 0.001$) and 14 d ($F = 8.36$; df = 2, 196; $P \leq 0.01$) significantly differed on various diet + tissue combinations (Table 2). At 7 d, larvae reared on Diet 1 + kernel and Diet 1 + silk were heavier than larvae on Diet 1 alone (Table 2). Larvae reared on Diet 2 + silk for 7 d weighed less than those on the Diet 2 + kernel or Diet 2 alone (Table 2). Weight trends observed within each diet at 7 d also were noted at 14 d (Table 2).

When error terms were pooled, the main effect diet was significant beyond any interactions for larval weight at 7 ($F = 339.83$; df = 1, 206; $P \leq 0.001$) and 14 d ($F = 52.34$; df = 1, 191; $P \leq 0.001$) and time to pupation ($F = 96.95$; df = 1, 169; $P \leq 0.001$). Though significant, the Diet 1 effects on larval weight were not as dramatic as those with Diet 2. During the first 7 d, the relative growth rate of larvae on Diet 2 was larger than on Diet 1 ($F = 178.94$; df = 1, 206; $P \leq 0.001$), This was reflected in the overall average 7 d larval weights on Diet 2 versus Diet 1 (5.96 and 2.46 mg, respectively). During the next 7 d, the relative growth rate of larvae on Diet 1 was largest ($F = 73.87$; df = 1, 189; $P \leq 0.001$); however, the larvae on Diet 1 still weighed less than larvae on Diet 2 (55.62 and 80.87 mg, respectively). The initial reduction in growth seen on Diet 1 carried through to pupation.

While few of the larvae grown on the Diet 1 + tissue combinations (35.6%) had begun pupation at 14 d, several of the larvae grown on Diet 2 + tissue combinations had pupated (53.5%). For diet + kernel and diet alone, larvae on Diet 2 required fewer days to pupation than larvae exposed to Diet 1 ($F = 96.95$; df = 1, 169; $P \leq 0.001$; Table 2). As silk tissue quantity was limited, times to pupation were not obtained for treatment combinations including silk tissues.

The data show that faster larval development occurred, as measured by larval weight and time to pupation, on Diet 2 than on Diet 1. Additionally, the differences among diet + tissue combinations were more readily detected on Diet 2. Thus, for the remaining two bioassays, Diet 2 was used to screen for tissue effects on *O. nubilalis* larval development.

Bioassay 2. Larval survival at 10 d and pupation did not differ by genotype ($P = 0.43$) or tissue ($P = 0.91$). However, interactions between two tissues (silk and kernel) and four sources (MN3152, MN3153, 'Apache', and 'Jubilee') differentially affected 10 d larval weight ($F = 20.19$; df = 3, 323; $P \leq 0.001$). Ten-d larval weights on diet + kernel tissue from MN3152, MN3153, and 'Apache' did not differ from the diet + cellulose control (Fig. 1). However, diet containing 'Jubilee' kernel tissue lowered larval weight compared to the controls. Diet incorporated with silk tissue of MN3152, MN3153, and 'Apache' detrimentally affected 10 d weights compared with the controls and with diet + 'Jubilee' silk tissue (Fig. 1). Mean weight of larvae exposed to the diet alone control exceeded that from the diet + tissue combinations and the diet + cellulose control, suggesting that the addition of tissues may be diluting the nutritional value of the diet (Fig. 1). The diet + cellulose control more closely mimics the effects of corn tissue additions than diet alone. Larvae on all treatment combinations had pupated by 20 d (Fig. 2). Time to pupation for larvae on diet with MN3152 silk, MN3153 silk, 'Apache' silk, MN3152 kernel, and

Table 2. Mean *O. nubilalis* larval weight (± SEM), relative growth rate (RGR) (± SEM) at 7 and 14 d, and time to pupation (± SEM) when reared on either of two diets plus sweet corn kernel or silk tissue (MN365) incorporated at 1 g tissue/20 ml diet (Bioassay 1). Controls were Diet 1, which contained contaminant control agents, and Diet 2, which had limited contaminant control agents, alone.

	7 Day*			
	Larval weight (mg)		RGR (mg/d/mg)	
Treatment	Mean	SEM	Mean	SEM
Diet 1 + kernel	2.60 a	0.18	0.158 ab	0.009
Diet 1 + silk	2.76 a	0.17	0.173 a	0.006
Diet 1	2.13 b	0.16	0.137 b	0.009
Diet 2 + kernel	6.50 a	0.24	0.234 a	0.002
Diet 2 + silk	3.93 b	0.30	0.197 b	0.007
Diet 2	6.79 a	0.23	0.236 a	0.002

	14 Day			
	Larval weight (mg)		RGR (mg/d/mg)	
Treatment	Mean	SEM	Mean	SEM
Diet 1 + kernel	63.97 a	4.32	0.262 a	0.001
Diet 1 + silk	53.84 b	2.74	0.257 a	0.002
Diet 1	48.22 b	3.76	0.256 a	0.004
Diet 2 + kernel	80.24 b	3.09	0.241 a	0.002
Diet 2 + silk	40.56 c	4.73	0.223 b	0.005
Diet 2	96.45 a	3.73	0.247 a	0.002

	Time to Pupation (d)**	
Treatment	Mean	SEM
Diet 1 + kernel	19.5 b	0.32
Diet 1 + silk	–	–
Diet 1	21.0 a	0.31
Diet 2 + kernel	17.6 a	0.27
Diet 2 + silk	–	–
Diet 2	17.4 a	0.28

*Means within a column (for each diet) followed by different letters are significantly different, Least Squares Means ($P \leq 0.05$, SAS Institute, [1990]).

**– data not available due to limited tissue quantities.

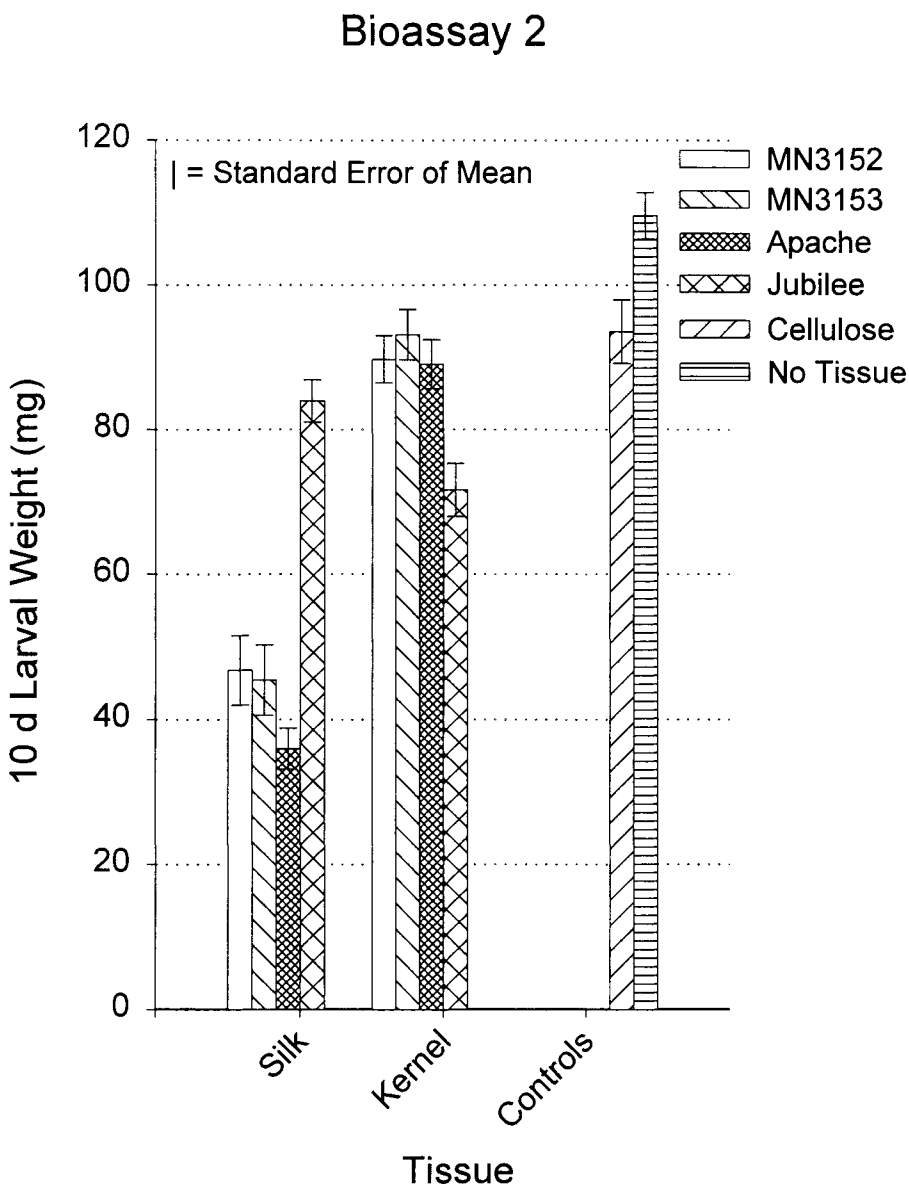

Fig. 1. Mean 10 d larval weight of *O. nubilalis* reared on meridic diet plus silk or kernel tissue of four sweet corn genotypes. Bioassay controls were diet alone and diet plus cellulose.

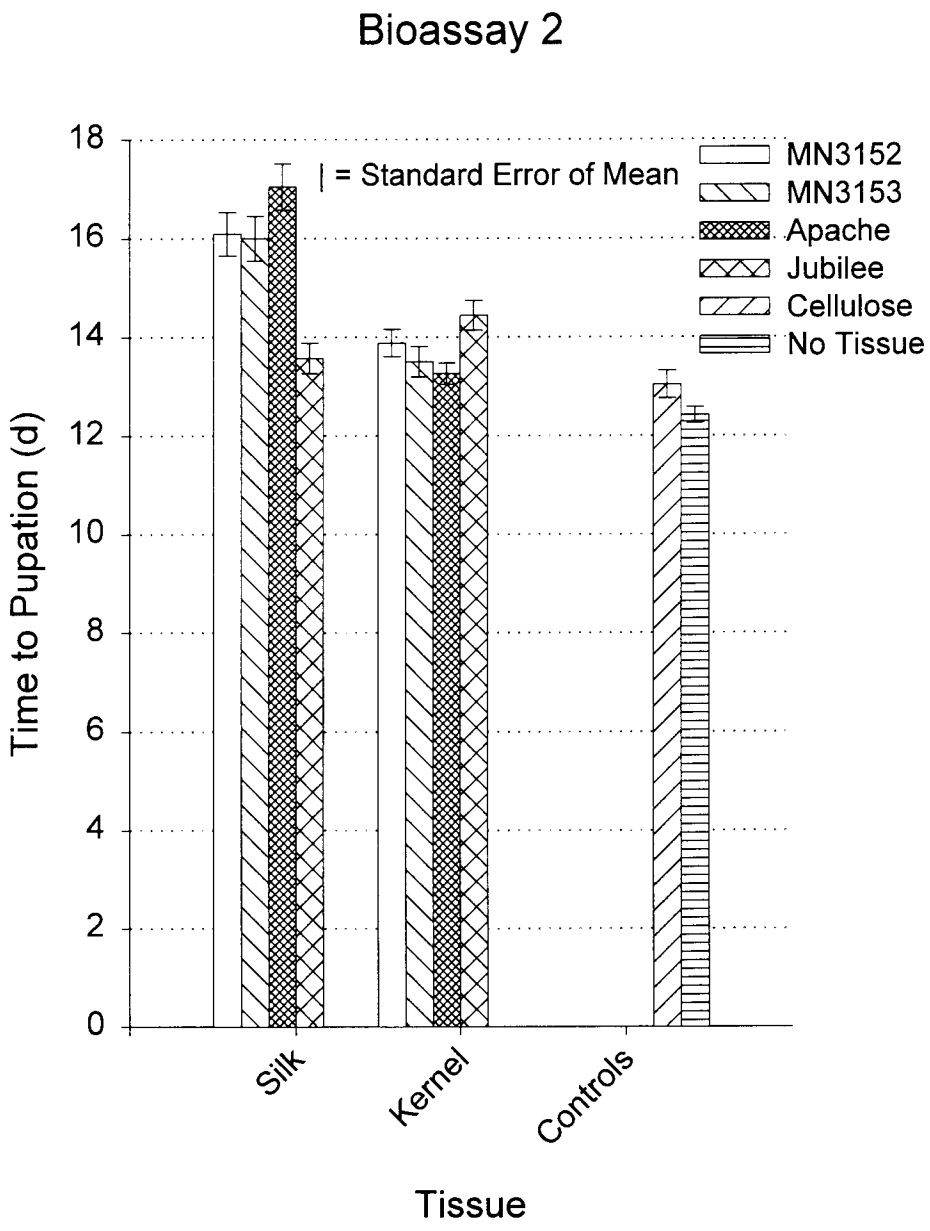

Fig. 2. Mean time to pupation of *O. nubilalis* larvae reared on meridic diet plus silk or kernel tissue of four sweet corn genotypes. Bioassay controls were diet alone and diet plus cellulose.

'Jubilee' kernel was longer than for the controls (Fig. 2). Time to pupation for larvae reared on diet + silk, excluding 'Jubilee' silk, was significantly longer than for all other combinations (Fig. 2).

Generally, diet containing silk tissue from the field resistant genotypes MN3153 and 'Apache' resulted in reduced larval weights and increased developmental time compared with the diet containing silk tissue from the field susceptible genotype 'Jubilee'. Surprisingly, diet containing silk tissue from the field susceptible MN3152 inbred reduced larval development, similar to that of silk tissue from the field resistant genotypes 'Apache' and MN3153 (Figs. 1-2). The field susceptible MN3152 inbred has a loose silk channel compared with 'Apache' and MN3153 (Warnock et al. 1997). This morphological feature may negate any chemical resistance factors in the silk by allowing *O. nubilalis* larvae to reach the kernel tissue without feeding extensively on the silk tissue. Two observations, (1) dramatic 10 d larval weight differences between diet containing silk and kernel tissue and (2) reduced larval weights observed with the diet + cellulose control versus diet alone, led to the hypothesis that tissue incorporation on a weight basis may dilute the nutritive value of the diet. The volume of 20 g of silk tissue is about 3 x the volume of 20 g of kernel tissue, suggesting the nutritional value of the diet easily might be compromised with tissue additions.

Bioassay 3. Larval survivability did not differ after 10 d ($P > 0.10$); however, survivability was affected by tissue ($F = 202.87$; df = 1, 1233; $P \leq 0.001$) at 20 d. Unlike Bioassay 2, few larvae (11%) reared on diet + silk had pupated at 20 d. In contrast, most larvae reared on diet + kernel (>99%) and all larvae reared on diet + cellulose (100%) had pupated at 20 d. Pupated larvae were not included in the number of larvae surviving at 20 d. Thus, the 20 d survivability differences were not due to increased deaths with silk incorporation but to a developmental delay.

Silk and kernel tissue incorporation resulted in different mean larval weights at 10 d ($F = 2109.50$; df = 1, 1216; $P \leq 0.001$; Fig. 3) and time to pupation ($F = 1006.83$; df = 1, 1122; $P \leq 0.001$; Fig. 4) regardless of the tissue concentration incorporated. The responses to tissue quantities in the diet were inconsistent (Figs. 3-4), affecting 10 d larval weights differently across three genotype + tissue combinations: MN3152 kernel, 'Jubilee' kernel, and 'Jubilee' silk (Fig. 3). These differences could not be attributed to sex as the female to male ratio between treatments was approximately 1:1 (females ranged from 41.7 to 55.0% for the various treatment combinations). Within a specific genotype, the proportion of females between the two tissue concentrations never differed by > 3.9%. The V/V measurement method increased the time to pupation only when the diet contained 'Jubilee' silk (Fig. 4). Females represented 48.3 and 46.7% of the population for the V/V and W/V treatments, respectively. For most genotype + tissue combinations, the concentration of incorporated tissue did not affect larval growth and development (Figs. 3-4).

The dramatic differences between silk and kernel tissues of individual genotypes observed in Bioassay 2 also were apparent in Bioassay 3 (Figs. 3-4). Silk tissue from MN3152 inbred, MN3153 inbred, and 'Apache' always resulted in reduced larval weight and increased time to pupation compared with kernel tissue from these lines, 'Jubilee' tissue combinations, and the controls. Silk tissue of these genotypes detrimentally affected larval development. Silk tissues may contain a chemical resistance factor or have a nutritional deficiency. However, a nonreplicated analysis for

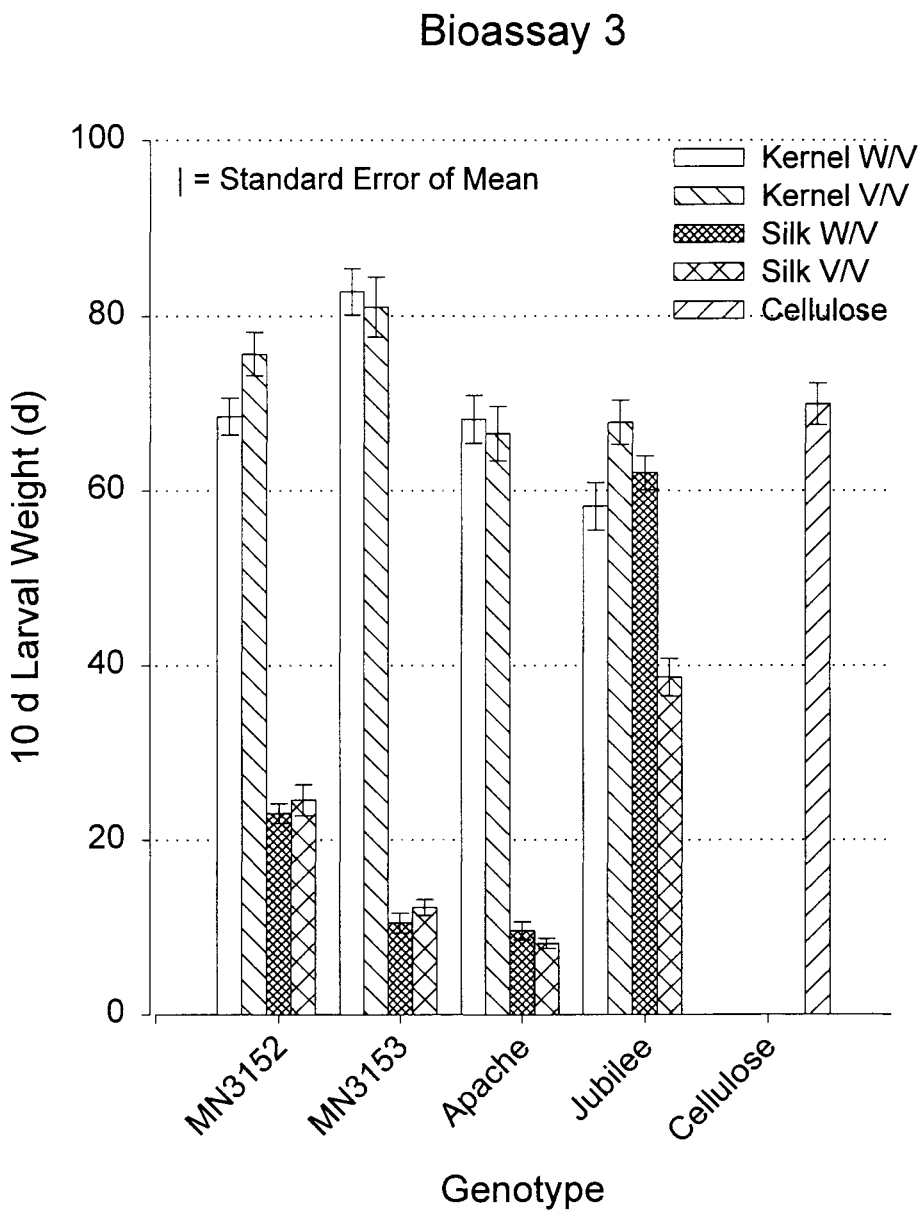

Fig. 3. Mean 10 d weight of *O. nubilalis* larvae reared on meridic diet plus silk or kernel tissue of four sweet corn genotypes. Tissues were incorporated into the diet on a tissue-to-diet weight/volume (W/V) and a tissue-to-diet volume/volume (V/V) basis. Bioassay control was diet plus cellulose.

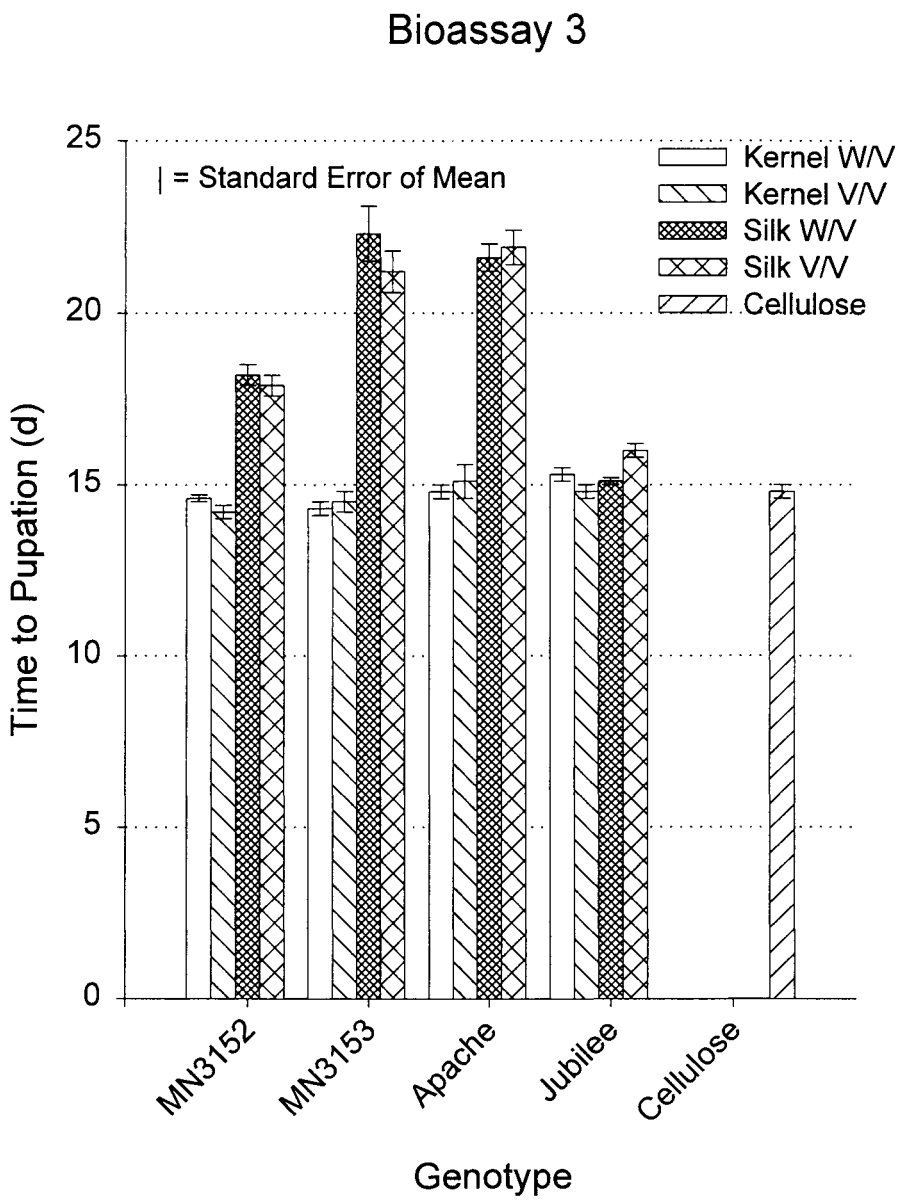

Fig. 4. Mean time to pupation of *O. nubilalis* larvae reared on meridic diet plus silk or kernel tissue of four sweet corn genotypes. Tissues were incorporated into the diet on a tissue-to-diet weight/volume (W/V) and a tissue-to-diet volume/volume (V/V) basis. Bioassay control was diet plus cellulose.

Table 3. Mean level (mg/kg) of 16 elements in sweet corn silk and kernel tissue as determined by inductively coupled plasma – atomic emission spectrometry using the dry ash method. Nitrogen levels were determined using the Kjeldahl digestion method. (Bioassay 3).

Element	Level in tissue (mg/kg)* Silk	Kernel	*F; P* Values**
Aluminum (Al^2)	25.72 a	3.58 b	23.71; 0.017
Boron (B)	13.79 a	3.01 b	83.63; 0.003
Cadmium (Cd)	0.13	0.12	1.00; 0.391
Calcium (Ca)	940.00 a	113.00 b	35.35; 0.001
Copper (Cu)	6.28	4.87	0.16; 0.716
Chromium (Cr)	0.48 a	0.32 b	91.19; 0.002
Iron (Fe^1)	461.30	368.00	0.07; 0.811
Lead (Pb)	1.71	1.73	0.09; 0.783
Magnesium (Mg)	1,468.32 a	1,167.83 b	25.45; 0.015
Manganese (Mn)	14.77	6.93	7.61; 0.070
Nickel (Ni)	0.83	0.49	1.02; 0.388
Nitrogen (N)†	2.72	2.44	3.48; 0.159
Phosphorous (P^1)	4,066.50	4,239.90	0.62; 0.490
Potassium (K)	19,058.80 a	11,645.70 b	88.8; 0.003
Silica (Si)	645.85 a	39.12 b	52.37; 0.005
Sodium (Na)	108.95 a	20.06 b	18.67; 0.023
Zinc (Zn)	58.85 a	31.48 b	121.83; 0.002

*Means within a row with different letters are significantly different, Least Squares Means ($P \leq 0.05$, SAS Institute [1990]).
**df = 1, 3 for all elements.
†Nitrogen levels are presented as % total Kjeldahl nitrogen per 150 mg sample.

nitrogen levels determined with the Kjeldahl digestion method (Bremner 1965) and for levels of 16 elements by inductively coupled plasma – atomic emission spectrometry using the dry ash method failed to reveal differences between genotypes ($P > 0.05$). Silk and kernel tissues differed in elemental amounts for nine of the 17 elements (See Table 3 for F, df, and P values). As the meridic diet was nutritionally complete and the nine element amounts always were greater in silk than kernel tissues, nutritional deficiencies caused by silk incorporation probably were not responsible for the detrimental effects on larvae in these assays. Though toxic effects of individual elements cannot be ruled out, chemical resistance factors may reside in silk and should be tested in future assays. Finally, the previously used W/V method was most time efficient and should be used for future bioassays.

Integration of the results of the three bioassays lead us to suggest that bioassays for screening sweet corn breeding lines and hybrids for resistance to *O. nubilalis* should use a diet with limited contaminant control chemicals and should focus on the use of silk tissue incorporated on a tissue-to-diet W/V basis. A diet + cellulose control should be included in each bioassay to ensure consistency among bioassays. Because larval weight differences among genotypes are apparent within 10 d of infestation (at 25° C), and host plant resistance screening techniques ideally must allow many genotypes to be tested, future bioassays in breeding programs could be limited to this time period. The primary limitation of the technique occurs during tissue lyophilization. Potentially, one may be able to overcome this limitation by oven-drying tissues; however, this may alter tissue chemical composition and possibly reduce the effectiveness of the assay.

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