

Evaluation of *Bt* Transgenic Sweet Corn Hybrids for Resistance to Corn Earworm and Fall Armyworm (Lepidoptera: Noctuidae) using a Meridic Diet Bioassay^{1,2}

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J. Entomol. Sci. 34(4): 415-425 (October 1999)

Abstract A laboratory bioassay was used to evaluate *Bt* transgenic sweet corn hybrids for resistance against the corn earworm, *Helicoverpa zea* (Boddie), and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Whorl leaves, silks, and kernels, either fresh or oven-dried and ground with a mill, were incorporated into a dilute pinto bean diet and bioassayed against neonate, 3-, or 6-day-old larvae. Regardless of age of the larvae, results with the diet bioassay using fresh silks, oven-dried silks or fresh kernels were highly correlated with those for the fresh silk bioassay. Differences in susceptibility between insect species to the CryIA(b) toxin produced in the transgenic plants were also readily discernable using the diet bioassay. Based on results of the bioassays, Novartis sweet corn hybrids containing a *cryIA(b)* gene for δ -endotoxin production were very highly resistant to leaf, silk and kernel feeding by the corn earworm and highly resistant to leaf and silk feeding by the fall armyworm.

Key Words *Helicoverpa zea*, *Spodoptera frugiperda*, transgenic plants, *Bt* corn.

Genetically-engineered organisms containing a codon-modified gene from either the same species or from a different species are rapidly changing many segments of our society. This is especially true for the pharmaceutical and agricultural fields. In agriculture, genetically-engineered cotton (Jenkins et al. 1993, Benedict et al. 1992, 1996) and potato (Cheng et al. 1992) containing modified genes from *Bacillus thuringiensis* Berliner (*Bt*) to express a cry protein were first developed in the early 1990's and are now commercially available to help manage important insect pests.

Initially, this new technology was applied only to dicotyledonous plant species (Fromm et al. 1990), but more recently the technology has also been applied to monocots such as corn (Fromm et al. 1990, Koziel et al. 1993, Armstrong et al. 1995). Presently, four unique transformation events in corn (Event 176, Novartis Seeds and Mycogen Seeds; Event BT11, Northrup King/Novartis Seeds; Event MON810, Monsanto; and Event DBT418, DEKALB Genetics Corp.) have been registered with EPA

¹Received 30 September 1998; accepted for publication 22 December 1998.

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for commercial use (Ostlie et al. 1997). *Bt* protein is expressed in pollen and green silks for Event 176 and in all tissue in BT11 and MON810 (Ostlie et al. 1997). DBT418 also expresses *Bt* protein in all plant parts, but the expression varies by tissue type (D. Isehour, DEKALB Genetics, pers. commun.). Hybrid field corn plants resulting from crosses with transgenic inbreds containing a codon-modified *cryIA(b)* or *cryIA* gene from *B. thuringiensis* resulted in an extremely high level of resistance to the European corn borer, *Ostrinia nubilalis* (Hübner) (Koziel et al. 1993, 1996, Armstrong et al. 1995), and southwestern corn borer, *Diatraea grandiosella* Dyar (Williams et al. 1997), and a good level of resistance to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Williams et al. 1997) and corn earworm, *Helicoverpa zea* (Boddie) (Sims et al. 1996). Sweet corn hybrids containing a *cryIA(b)* gene (Novartis Seeds, BT11 event) are also highly resistant to leaf feeding by the fall armyworm and ear feeding by the corn earworm and fall armyworm (Lynch et al. 1999).

Natural infestations with insects are not always adequate to provide feeding damage sufficient to differentiate resistance among plant genotypes in the field. Likewise, artificially inoculating plants with insects does not always provide sufficient plant feeding damage due to environmental conditions, infertility of insect eggs, predators, and/or other extenuating circumstances. Wiseman (1989) and Wiseman and Isehour (1990) developed a laboratory bioassay procedure to evaluate plant tissue for resistance to the corn earworm and fall armyworm by incorporating tissue from field-grown plants into a modified pinto diet. We report here the evaluation of *Bt* transgenic sweet corn by incorporating different plant tissue into a meridic diet, and a correlation of the results from the diet bioassay with those where insects were fed silks from the *Bt* sweet corn lines.

Materials and Methods

The diet bioassay developed by Wiseman (1989) was used to evaluate four transgenic sweet corn hybrids, *Bt*-0906-*Bt*-0909, developed by Novartis Seeds, Inc. and expressing a synthetic *cryIA(b)* gene (BT11 event), for resistance to the corn earworm and fall armyworm. Non-transgenic donor sweet corn hybrids (GH-1355, GH-2628, and Heritage) were used as checks along with conventional, non-transgenic susceptible and resistant check genotypes (Zapalote Chico and Stowell's Evergreen for corn earworm tests and FAWCC(C5) and Pioneer 3369A for fall armyworm tests) also were included in the evaluations. Corn for all tests was planted on 25 April 1996 on the Belflower Farm near Tifton, GA, in rows 3.04 m long and 0.91 m between rows in a randomized complete block with eight replications. Fertilization and herbicides were those recommended by the Georgia Cooperative Extension Service. The *Bt* sweet corn also contained the selectable marker PAT gene which confers tolerance to glufosinate herbicides. Thus, we were able to eliminate plants that did not contain the *cryIA(b)* gene by spraying all plants in the 3 to 5 leaf stage with a 1% solution of Ignite®³ (AgrEvo USA Co., Wilmington, DE) using a hand sprayer.

Laboratory bioassays were conducted using whorl, silk and kernel tissue incorporated into a modified pinto bean diet (Perkins 1979). Plants in the midwhorl stage (stage V5-V6) (Ritchie et al. 1997) of plant development were removed from plots in the field, placed in labeled plastic bags, and transported to the laboratory. Approximately 3 to 6 cm of whorl tissue was removed from each plant and either incorporated fresh into the modified diet or oven-dried at 41°C for 10 d and ground with a Cyclotec mill with a 1-mm screen before incorporation into the diet. Likewise, 1- to 3-day-old

silks (stage R1) were collected from plots in the field, placed in labeled plastic bags, and brought into the laboratory. Each ear was cut approximately 5 to 6 cm from the tip of the ear, and the silks were removed and either incorporated fresh or oven-dried and ground before bioassay as described above.

Bioassays were conducted by mixing plant material into the pinto bean diet that had been diluted by the addition of 250 ml distilled water to 350 ml of diet. The plant tissue was thoroughly mixed in the diet with a blender for approximately 1 to 2 min and the mixture was dispensed into 30 30-ml diet cups and allowed to solidify 1 to 2 h. Each cup was infested with either one neonate, 3-day-old, or 6-day-old corn earworm or fall armyworm larva. Larvae for the bioassay were from a laboratory colony maintained at the Insect Biology and Population Management Research Laboratory (Perkins 1979, Perkins et al. 1973). The 3- and 6-day-old larvae had been reared on meridic diet in cups prior to being transferred to cups containing the leaf- or silk-diet. After being infested, cups were capped with a paper lid, arranged in a randomized complete block with 30 replications, and placed in an incubator operated at 26.7°C, 75% RH and a 16:8 (L:D) photoperiod. Data were recorded on survival of larvae and weight of the survivors after 7 to 10 d. Bioassays with fresh silks were conducted by placing a mass of silks, approximately 3 to 4 cm long by 2 to 3 cm wide, in a diet cup and infesting with an insect. Percentage data were transformed to $\arcsin \sqrt{\%}$, all data were analyzed by analysis of variance, and significantly different means were separated by LSD (SAS 1989). Data for bioassays with fresh silks were correlated with data for the diet bioassay by Proc Correlation to generate Pearson Correlation Coefficients.

Results and Discussion

Survival of corn earworm larvae fed diets containing fresh whorl leaves from *Bt* transgenic plants was significantly lower at 9 d than survival for larvae fed diets containing fresh leaves from non-transgenic corn plants (Table 1). Larvae that were alive after feeding for 9 d on the *Bt* transgenic leaf diets weighed significantly less than their counterparts which fed on non-transgenic leaf diets. These relationships were true when fresh leaves or oven-dried leaves were used to conduct the bioassay regardless of the age of the corn earworm used in the bioassay. Corn earworm larvae initially weighed an average 1.3, 4.8, and 89.5 mg at 0, 3, and 6 days of age when first placed on the leaf diets. Neonate corn earworms showed only a slight increase in weight after 9 d on the *Bt* transgenic diets and weighed significantly less at 9 d of age than their counterparts which fed on the non-*Bt* diets (Table 1). Similarly, 3-day-old larvae that were fed diet containing leaves of *Bt*-0907 and *Bt*-0908 weighed only slightly more after 6 d on the *Bt* corn diets than they weighed when first placed on the diet. Conversely, the average weight of 3-day-old corn earworms that were fed diet containing leaves of *Bt*-0906 and *Bt*-0909, and all 6-day-old larvae was actually less than their average weight when first placed on the *Bt* leaf diet. Diet containing *Bt* corn leaves significantly reduced survival of neonate and 3-day-old larvae but not 6-day-old larvae as compared with survival of larvae that fed on the non-*Bt* diets.

No significant difference in survival of fall armyworm neonates, 3-day-old (Table 2), or 6-day-old larvae were noted when they were fed diet containing *Bt* transgenic and non-transgenic whorl leaf tissue. However, results similar to that for the corn earworm above were noted for weights of the surviving fall armyworms when they were fed diet containing whorl leaf tissue from the *Bt* transgenic plants. Larvae fed the

Table 1. Survival and weight of corn earworm larvae fed leaves of Novartis Bt-corn lines incorporated in a meridic diet*

| Corn line | Neonates | | | 3-day-old larvae | | 6-day-old larvae | |
|-----------------|------------------------------|-------------------------------|------------------------------------|-------------------------------|------------------------------|-------------------|-------------------------------|
| | 30 g fresh leaves/40 ml diet | 30 g fresh leaves/400 ml diet | 24 g oven-dried leaves/500 ml diet | 30 g fresh leaves/400 ml diet | Weight (mg) of larvae at 9 d | % survival at 9 d | 30 g fresh leaves/400 ml diet |
| | % survival at 9 d | Weight (mg) of larvae at 9 d | Weight (mg) of larvae at 9 d | % survival at 9 d | Weight (mg) of larvae at 9 d | % survival at 9 d | Weight (mg) of larvae at 9 d |
| Bt-0906 | 30.0 c | 3.1 e | 2.3 f | 53.3 c | 3.7 d | 86.7 a | 26.7 d |
| Bt-0907 | 40.0 bc | 2.2 e | 0.9 f | 76.7 b | 7.8 d | 90.0 a | 35.2 d |
| Bt-0908 | 50.0 b | 4.7 e | 1.9 f | 76.7 b | 8.0 d | 90.0 a | 22.1 d |
| Bt-0909 | 37.0 c | 1.6 e | 0.2 f | 6.7 c | 4.2 d | 90.0 a | 25.1 d |
| Heritage** | 100.0 a | 313.7 d | 189.0 d | 100.0 a | 510.0 c | 100.0 a | 466.1 c |
| GH2628** | 96.7 a | 333.1 d | 107.6 e | 100.0 a | 611.5 bc | 100.0 a | 588.8 ab |
| GH1355** | 96.7 a | 346.5 d | 538.9 c | 100.0 a | 641.8 bc | 100.0 a | 572.3 b |
| GT-FAWCC(C5)† | 100.0 a | 533.8 b | 231.2 d | 100.0 a | 722.9 ab | 100.0 a | 638.7 a |
| Pioneer 3969A‡ | 100.0 a | 441.6 c | 699.8 a | 100.0 a | 829.3 a | 90.0 a | 588.8 ab |
| Bean Diet Check | 96.7 a | 649.2 a | 648.5 b | 100.0 a | 740.8 ab | 100.0 a | 615.2 ab |

* Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) using Waller-Duncan k-ratio t test.

** Non-Bt sweet corn lines used as a control.

† A fall armyworm resistant line included as a resistant control.

‡ A susceptible corn line included as a susceptible control.

Table 2. Survival and weight of fall armyworm larvae fed leaves of Novartis *Bt*-corn lines incorporated in a meridic diet*

| Corn line | Neonates | | | 3-day-old larvae | | 6-day-old larvae | |
|-----------------|-------------------------------|-------------------------------|------------------------------------|-------------------------------|-------------------------------|--------------------|-------------------------------|
| | 30 g fresh leaves/400 ml diet | 30 g fresh leaves/400 ml diet | 24 g oven-dried leaves/500 ml diet | 30 g fresh leaves/400 ml diet | Weight (mg) of larvae at 10 d | % survival at 10 d | 30 g fresh leaves/400 ml diet |
| | % survival at 10 d | Weight (mg) of larvae at 10 d | Weight (mg) of larvae at 10 d | % survival at 10 d | Weight (mg) of larvae at 10 d | % survival at 10 d | Weight (mg) of larvae at 10 d |
| <i>Bt</i> -0906 | 100.0 a | 123.2 d | 141.3 e | 100.0 a | 90.3 e | 100.0 a | 109.3 cd |
| <i>Bt</i> -0907 | 100.0 a | 100.9 de | 157.7 e | 100.0 a | 93.3 e | 100.0 a | 119.7 cd |
| <i>Bt</i> -0908 | 100.0 a | 112.4 d | 137.2 e | 100.0 a | 98.2 e | 100.0 a | 140.9 c |
| <i>Bt</i> -0909 | 100.0 a | 80.8 e | 128.4 e | 100.0 a | 82.9 e | 100.0 a | 95.8 d |
| Heritage** | 100.0 a | 326.2 c | 275.9 cd | 100.0 a | 341.2 c | 100.0 a | 335.2 ab |
| GH2628** | 100.0 a | 308.2 c | 312.3 bc | 100.0 a | 282.9 d | 100.0 a | 323.8 ab |
| GH1355** | 100.0 a | 296.2 c | 357.4 ab | 96.7 a | 274.2 d | 100.0 a | 326.1 ab |
| GT-FAWCC(C5)† | 100.0 a | 320.2 c | 275.6 cd | 100.0 a | 393.0 b | 96.7 a | 338.3 a |
| Pioneer 3369A‡ | 100.0 a | 379.4 b | 381.7 a | 96.7 a | 326.2 c | 100.0 a | 299.2 b |
| Bean Diet Check | 100.0 a | 519.7 a | 358.5 ab | 100.0 a | 434.9 a | 100.0 a | 303.8 ab |

* Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) using Waller-Duncan k-ratio t test.

** Non-*Bt* sweet corn lines used as a control.

† A fall armyworm resistant line included as a resistant control.

‡ A susceptible corn line included as a susceptible control.

Bt transgenic diets always weighed significantly less than their counterparts that were fed non-transgenic leaf diets, regardless of the method of preparing the leaf tissue before incorporation into the diet. However, as noted by Lynch et al. (1999), the level of resistance noted for fall armyworms which fed on the *Bt* diets was not as great as the level of resistance noted against the corn earworm. Fall armyworms weighed an average of 1.1, 2.0, and 32.5 mg at 0, 3, and 6 days old, respectively, when they were placed on the diet. Regardless of the age when first placed on the diet, fall armyworm larvae actually gained weight when fed diet containing leaves of transgenic plants; whereas, corn earworm larvae either gained weight much more slowly or lost weight when fed diet containing leaves of the *Bt* transgenic plants.

None of the corn earworm neonate or 3-day-old larvae survived when fed fresh silks from the *Bt* transgenic plants, and 6-day-old larvae lost weight when fed fresh silks from the *Bt* sweet corn hybrids (Table 3). Results of the bioassays with corn earworms where fresh silks, fresh unpollinated silks, oven-dried silks, or kernels were incorporated into the diet were very similar to results where leaves were incorporated into the diet, regardless of the initial age of the larvae when placed on the diets. Corn earworm larvae always weighed significantly less when fed diet containing tissue from transgenic *Bt* sweet corn hybrids than when fed diets containing tissue from non-transgenic corn lines. The damage ratings for silks on which 6-day-old larvae had fed reveal that larvae readily consumed the silks of non-*Bt* corn, but essentially refused *Bt* corn silks after initial feeding.

With the exception of larval weights and damage ratings when larvae were fed silks of Zapalote Chico, results of bioassays with fall armyworms of various ages fed fresh silks, fresh silks incorporated into the diet, or oven-dried silks incorporated into the diet were similar to those for fall armyworms fed leaf tissue. Fall armyworm larvae always weighed significantly less, regardless of age when placed on the diet, when fed silks from transgenic plants than when fed silks from susceptible or donor sweet corn lines (Table 4). Zapalote Chico has a high level of resistance to the fall armyworm (Wiseman and Widstrom 1986) (Table 3). However, the level of resistance noted when fall armyworm were fed fresh silks from *Bt* sweet corn hybrids was actually greater than that when larvae were fed fresh silks of Zapalote Chico (Table 3) as noted by Lynch et al. (1999). Although larvae gained weight much faster when fed diets containing corn silks, the same basic relationships were apparent when fall armyworms were fed *Bt* corn silks incorporated into the diet as they were when armyworms were fed fresh silks.

Results of the bioassays with fresh silks show a high degree of correlation with results from bioassays with fresh silks incorporated into the diet, oven-dried silks incorporated into the diet, or corn kernels incorporated into the diet (Table 5) for both the corn earworm and fall armyworm. In all instances, the correlations were significant and explained 62 to 97 percent of the variation of the results.

Pilcher et al. (1997) also reported a bioassay procedure where freeze-dried *Bt* corn leaf tissue was incorporated into an insect diet. These authors extracted *Bt* protein from freeze-dried corn leaves and topically applied the extract on the surface of an insect diet. They showed little effect of *Bt* corn on the black cutworm, *Agrotis ipsilon* (Hufnagel), and delayed development of the armyworm, *Pseudaletia unipuncta* (Haworth) and corn earworm using these bioassays. Results reported here show that bioassays conducted by feeding either fresh or oven-dried tissue of *Bt* sweet corn incorporated into a meridic diet was highly correlated with bioassays where the corn earworm or fall armyworm were fed fresh silks.

Table 3. Survival and weight of corn earworm larvae fed corn silk-diets, kernel-diets, or fresh silks from Novartis *Bt*-corn lines and damage ratings to the silks due to corn earworm feeding*

| Corn line | Neonates | | | | | | 3-day-old larvae | | | 6-day-old larvae | | |
|-----------------|--------------------------------|-------------------------------------|---|-----------------------------------|-----------------------------------|--------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Fresh silks | | 80 g fresh unpollinated silks/400 ml diet | | 20 g oven dried silks/450 ml diet | | 80 g kernels/400 ml diet | | Fresh silks | 80 g fresh silks/400 ml diet | Fresh silks | 80 g fresh silks/400 ml diet |
| | Weight (mg) of larvae at 7-9 d | | Weight (mg) of larvae at 10 d | | Weight (mg) of larvae at 10 d | | Weight (mg) of larvae at 10 d | | Damage rating [†] | Weight (mg) of larvae at 10 d | Damage rating [†] | Weight (mg) of larvae at 10 d |
| | 80 g fresh silks/400 ml diet | 80 g unpollinated silks/400 ml diet | 80 g fresh unpollinated silks/400 ml diet | 20 g oven dried silks/450 ml diet | 20 g oven dried silks/450 ml diet | 80 g kernels/400 ml diet | Fresh silks | 80 g fresh silks/400 ml diet | Weight (mg) of larvae at 10 d | Weight (mg) of larvae at 10 d | Weight (mg) of larvae at 10 d | Weight (mg) of larvae at 10 d |
| <i>Bt</i> -0906 | 0.0 d | 1.1 d | — | 2.4 f | — | — | 0.0 e | 9.7 c | 1.00 d | 0.0 f | 59.2 fg | |
| <i>Bt</i> -0907 | 0.0 d | 3.4 d | 1.6 e | 3.2 f | 9.2 e | 9.2 e | 0.0 e | 11.5 c | 1.00 d | 8.4 f | 130.4 e | |
| <i>Bt</i> -0908 | 0.0 d | 2.1 d | 1.6 e | 2.0 f | 20.8 e | 20.8 e | 0.0 e | 13.7 c | 1.00 d | 22.0 f | 102.5 ef | |
| <i>Bt</i> -0909 | 0.0 d | 1.2 d | 1.9 e | 2.4 f | 7.0 e | 7.0 e | 0.0 e | 10.4 c | 1.00 d | 0.0 f | 43.5 g | |
| GH1355* | 164.6 a | 431.2 c | 655.7 b | 408.7 d | 472.1 d | 472.1 d | 342.3 b | 620.5 b | 5.00 a | 364.2 c | 208.5 d | |
| GH2628** | 149.2 a | 516.0 b | 396.8 d | 372.1 d | 619.7 c | 619.7 c | 472.6 a | 649.2 b | 4.54 b | 452.0 b | 838.9 a | |
| Heritage** | 81.9 b | 484.1 b | 599.2 c | 515.1 c | 711.7 b | 711.7 b | 298.0 bc | 633.3 b | 4.96 a | 610.9 a | 805.1 a | |
| Zapalote Chico† | 33.3 c | 11.3 d | — | 13.2 f | — | — | 229.7 d | 90.8 c | 4.73 ab | 285.9 e | 370.6 c | |
| Stowell's | | | | | | | | | | | | |
| Evergreen‡ | 161.3 a | 487.0 b | — | 228.8 e | — | — | 263.5 cd | 728.5 ab | 4.18 c | 328.0 d | 796.1 a | |
| Bean Diet | | | | | | | | | | | | |
| Check | — | 720.1 a | 743.9 a | 686.7 a | 798.8 a | 798.8 a | — | 787.6 a | — | — | 693.2 b | |

* Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) using Waller-Duncan k-ratio t test.

** Non-*Bt* sweet corn lines used as a control.

† A fall armyworm resistant line included as a resistant control.

‡ A susceptible corn line included as a susceptible control.

* Damage rated on a 1-5 scale where 1 = no damage and 5 = extensive damage.

Table 4. Survival and weight of fall armyworm larvae fed silks or silk-diets from Novartis Bt-corn lines and damage ratings to the silks due to fall armyworm feeding*

| Corn line | Neonates | | | | 3-day-old larvae | | | 6-day-old larvae | | | |
|----------------------|-------------|---------------------------------|---------------------------------|-----------------------------------|-------------------------------|-------------|-------------------------------|-------------------------------|-------------|-------------------------------|----------------|
| | Fresh silks | 80 g fresh silks/400 ml diet | | 20 g oven-dried silks/450 ml diet | | Fresh silks | 80 g fresh silks/400 ml diet | | Fresh silks | 80 g fresh silks/400 ml diet | |
| | | Weight (mg) of larvae at 7-10 d | Weight (mg) of larvae at 7-10 d | Weight (mg) of larvae at 7-10 d | Weight (mg) of larvae at 10 d | | Weight (mg) of larvae at 10 d | Weight (mg) of larvae at 10 d | | Weight (mg) of larvae at 10 d | Damage rating† |
| Bt-0906 | 3.3 e | 97.0 e | 176.3 e | 33.5 e | 96.5 e | 1.17 e | 50.3 f | 85.0 f | | | |
| Bt-0907 | 4.8 e | 91.1 e | 154.8 ef | 91.3 d | 105.9 e | 1.14 e | 102.8 e | 162.8 cd | | | |
| Bt-0908 | 6.6 e | 125.1 d | 154.3 ef | 36.0 e | 78.6 e | 1.37 de | 82.3 ef | 143.7 de | | | |
| Bt-0909 | 5.2 e | 57.0 f | 127.1 f | 33.7 e | 77.5 ef | 1.30 e | 54.0 f | 106.8 ef | | | |
| GH1355** | 75.5 a | 201.6 c | 400.2 bc | 413.2 a | 245.0 c | 4.13 a | 207.3 d | 195.7 c | | | |
| GH2628** | 54.8 b | 241.0 b | 395.5 c | 391.9 a | 222.1 c | 3.23 b | 407.9 a | 475.1 a | | | |
| Heritage** | 44.4 c | 202.1 c | 441.2 b | 306.5 b | 330.1 b | 2.86 bc | 352.0 b | 301.5 b | | | |
| Zapalote Chico† | 18.3 d | 8.8 d | 2.1 g | 172.1 c | 45.3 f | 1.79 d | 181.6 d | 127.0 de | | | |
| Stowell's Evergreen‡ | 52.3 b | 226.3 b | 255.2 d | 285.6 b | 187.9 d | 2.60 c | 305.6 c | 312.6 b | | | |

* Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) using Waller-Duncan k-ratio t test.

** Non-Bt sweet corn lines used as a control.

† A fall armyworm resistant line included as a resistant control.

‡ A susceptible corn line included as a susceptible control.

+ Damage rated on a 1-5 scale where 1 = no damage and 5 = extensive damage.

Table 5. Pearson Correlation Coefficients for corn earworm and fall armyworm fed fresh silks versus silks of Novartis *Bt* sweet corn incorporated into a meridic diet

| Variable | Neonate | | | 3-day-old larvae | | 6-day-old larvae | | |
|--------------------------|-------------------|---------------------|--------------------------|-----------------------|------------------|---------------------|---------------------|------------------|
| | Fresh silks | Fresh silks in diet | Oven-dried silks in diet | Fresh kernels in diet | Fresh silks | Fresh silks in diet | Fresh silks in diet | |
| Fresh silks | — | 0.9290* 0.0003** | 0.7998 0.0097 | 0.8345 0.0388 | — | 0.8762 0.0019 | — | 0.8603 0.0029 |
| Fresh silks in diet | 0.9290 0.0003 | — | 0.9307 0.0003 | 0.9848 0.0003 | 0.8762 0.0019 | — | 0.8603 0.0029 | — |
| Oven-dried silks in diet | 0.7998 0.0097 | 0.9307 0.0003 | — | 0.9820 0.0005 | — | — | — | — |
| Fresh kernels in diet | 0.8345 0.0388 | 0.9848 0.0003 | 0.9820 0.0005 | — | — | — | — | — |
| Fresh silks | — | 0.8012 0.0094 | 0.7863 0.0120 | — | — | 0.8055 0.0088 | — | 0.9338 0.0002 |
| Fresh silks in diet | 0.8012 0.0094 | — | 0.8991 0.0010 | — | 0.8055 0.0088 | — | 0.9338 0.0002 | — |
| Oven-dried silks in diet | 0.78634 0.0120 | 0.8991 0.0010 | — | — | — | — | — | — |

* Pearson Correlation Coefficient.
 ** Level of significance.

The procedures reported here lend themselves to laboratory assays where corn tissue is incorporated into a diet and then fed to insects. If sufficient time is available during the growing season, the bioassays can be conducted by incorporating fresh leaf, silk, or kernel tissue directly into the diluted diet. If time is insufficient, as is often the case during the growing season, the corn leaves or silks can be oven-dried, ground into a fine powder, stored in a freezer and incorporated into the diet at a later date. In either case, the results of the bioassay are highly correlated with those where the insects are fed directly on corn tissue.

The results reported here compliment the field and laboratory data reported by Lynch et al. (1999) who reported bioassay results where corn earworm and fall armyworm were fed leaves, silks, and kernels of different *Bt* sweet corn lines. Bioassays using either fresh tissue or lyophilized tissue incorporated into a meridic diet also showed a high level of resistance in the *Bt* sweet corn to the corn earworm and a moderately high level to the fall armyworm. Based on the bioassay data, Novartis sweet corn hybrids containing a *cryIA(b)* gene were very highly resistant to leaf, silk, and kernel feeding by the corn earworm and highly resistant to leaf and silk feeding by the fall armyworm.

Acknowledgments

Appreciation is expressed to J. Skinner, C. Mullis, L. Copeland, and E. Harris for their assistance in the conduct of this research.

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