N O T E

Developmental Delay and Evidence for Reduced Cannibalism in Corn Earworm (Lepidoptera: Noctuidae) Larvae Feeding on Transgenic Bt Sweet Corn[1]

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Corn earworm, *Helicoverpa zea* (Boddie), is a devastating pest of sweet corn, *Zea mays* L., in the midwestern United States. Annual losses attributed to *H. zea* in Minnesota have been estimated at $9.3 million, second only to damage caused by European corn borer, *Ostrinia nubilalis* (Hübner), in Minnesota (Noetzel et al. 1986, Estimated annual losses due to insects in Minnesota 1981-1983. University of Minnesota AES. AG-BU-2541). *Helicoverpa zea* adults fly north from southern U.S. locations, typically arriving in southern Minnesota from mid-to-late August, coinciding with sweet corn nearing maturity (Bartels and Hutchison 1995, J. Econ. Entomol. 88: 380-386). Both processing and fresh-market sweet corn hybrids are susceptible to *H. zea* and can experience significant damage during years of high pressure. Growers regularly apply insecticides on 2 to 3 day schedules for up to 3 wks in the southern U.S. to control *H. zea* (Wiseman 1999, Corn earworm. *In* Steffey, K., M. Rice, J. All, D. Andow, M. Gray and J. Van Duyn [eds.], Handbook of corn insects. Entomol. Soc. America, Lanham, MD. 164 pp.), or on 4 to 5 day schedules in the midwestern U.S. when infestations are high (Foster and Flood 1995, Vegetable insect management: with emphasis on the Midwest, Meister Pub. Co, Willoughby, OH, 206 pp.). The introduction of transgenic 'Bt' sweet corn, expressing protein toxins from the bacterium *Bacillus thuringiensis* Berliner, offers growers an alternative to traditional insecticide-intensive sweet corn production (Lynch et al. 1999, J. Econ. Entomol. 92: 246-252; Burkness et al. 2002, Crop Prot. 21: 157-169). Although several Bt sweet corn hybrids have shown 99 to 100% control of *O. nubilalis,* efficacy against *H. zea* has been good, but less dramatic (Burkness et al. 2002). In this paper, we present field data that suggest a developmental delay and reduced cannibalism of *H. zea* larvae on transgenic 'Bt' sweet corn.

Experimental plots containing both transgenic Bt and non-transgenic sweet corn

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hybrids were planted in Minnesota in 1998 as part of a larger study to monitor the frequency of surviving late-instar *O. nubilalis* and *H. zea* larvae on Bt sweet corn (Bolin et al. 1998, J. Agric. Entomol. 15: 231-238; Venette et al. 2000, J. Econ. Entomol. 93: 1055-1064). The Bt sweet corn hybrid ('GH-0937') used in this study expressed the Cry1Ab protein toxin derived from the bacterium *B. thuringiensis* var. *kurstaki* (i.e., Bt corn), using 'Event BT11' (Lynch et al. 1999). The Bt hybrid, 'GH-0937', and its accompanying non-transgenic isoline, 'Bonus', were evaluated at maturity for the presence and growth stage of *H. zea* larvae. Both the Bt and non-Bt isoline are known to have similar silking phenology and ear maturity characteristics (Lynch at al. 1999, Burkness et al. 2002), and could therefore be harvested on the same date.

Research was conducted at University of Minnesota Agricultural Research and Outreach Centers in Becker, Crookston, Lamberton, Morris, Rosemount and Waseca, MN (O'Rourke and Hutchison 2000, J. Agric. Urban Entomol. 17: 213-217). The commercially available sweet corn varieties 'GH-0937' (Bt) and 'Bonus' (non-Bt) were provided by Novartis Seeds Inc. (Syngenta Corp.), Nampa, ID. Sentinel plots consisting of 'GH-0937' and 'Bonus' were planted at Becker (25 Jun), Crookston (03 Jun), Lamberton (01 and 23 Jun), Morris (01 Jun and 01 Jul), Rosemount (10 and 29 Jun) and Waseca (02 Jul). Multiple late-season planting dates were used at select locations to maximize natural insect pressure. Sentinel plots consisted of adjacent 'GH-0937' (15m × 30m) and 'Bonus' (4.5 m × 30 m) plantings separated by a 3 m alley. The late-planted Rosemount plot (29 Jun) contained larger plantings of 'GH-0937' (64.5 m × 64.5 m) and 'Bonus' (9 m × 64.5 m) separated by a 3 m alley.

For all locations, the sweet corn hybrids were evaluated for insect damage by randomly selecting 40 ears per sample date in the non-Bt corn, and up to 10,000 ears in the Bt corn. Primary ears were hand-harvested at maturity, approximately 90 d after planting, placed in burlap sacks and taken to the field edge for immediate evaluation. Ear evaluation included husk removal and examination of the ear for the presence of lepidopteran larvae; feeding damage was also noted (e.g., O'Rourke and Hutchison 2003, Crop Prot. 22: 903-909). All larvae were classified as either early (first to second instars) or late (third to sixth instars) with confirmation of Bt expression among 'GH-0937' ears being made using Gene Check™ strip tests on select ear samples (Monsanto Co. 1995, GeneCheck™ *B.t.k.* lab screening instructions).

A total of 4,547 *H. zea* larvae was observed on both hybrids at all locations surveyed in 1998, including 319 on 'Bonus' (non-Bt) and 4,228 on 'GH-0937' (Bt) (Table 1). However, because many more Bt ears were sampled compared with the non-Bt hybrid, larval density was lower in 'GH-0937'; overall larval density was 0.80 larvae/ear for 'Bonus' and 0.20 larvae/ear for 'GH-0937'. In addition, only 3% of the larvae on 'GH-0937' were late instars compared with 67% late instars on non-Bt 'Bonus' (Table 1). Larval density varied across locations due to natural population fluctuation, weather conditions, host availability and sample date. The greatest number of larvae were observed on 'GH-0937' (Bt) at Rosemount on 21 September. Of the 2,323 larvae observed on that date, 2,262 (97%) were early instars and only 61 (3%) were late instars (Table 1). In addition to the lower *H. zea* density on 'GH-0937', the proportion of early instars was significantly different from the proportion of early instars on 'Bonus' ears (Chi square test; $\chi^2 = 48.40$, df = 9, $P < 0.001$) (Table 1). The proportion of late instars on 'GH-0937' was also significantly different from the proportion of late instars on 'Bonus' (Chi square test; $\chi^2 = 514.48$, df = 7, $P < 0.001$) (Table 1).

Table 1. Number (and proportion) of *Helicoverpa zea* larvae observed on Non-Bt, 'Bonus' and Bt, 'GH-0937' sweet corn hybrids in Minnesota, 1998

		Non-Bt, 'Bonus', ears				Bt, 'GH-0937', ears			
Location	Date harvested	# Ears	Early instars	Late instars	Total larvae	# Ears	Early instars	Late instars	Total larvae
Becker	14 Sep	40	1 (0.03)	37 (0.97)	38	1254	144 (1.0)	0 (0)	144
Crookston	10 Sep	40	11 (0.46)	13 (0.54)	24	1241	75 (1.0)	0 (0)	75
Lamberton	28 Aug	40	2 (1.00)	0 (0)	2	1250	110 (1.0)	0 (0)	110
Lamberton	16 Sep	40	9 (0.22)	32 (0.78)	41	1251	123 (0.98)	3 (0.02)	126
Morris	03 Sep	40	9 (0.82)	2 (0.18)	11	359	29 (1.0)	0 (0)	29
Morris	11 Sep	40	36 (0.72)	14 (0.28)	50	619	9 (0.90)	1 (0.10)	10
Morris	17 Sep	40	14 (0.29)	34 (0.71)	48	732	406 (0.90)	44 (0.10)	450
Rosemount	01 Sep	40	6 (1.00)	0 (0)	6	1220	78 (1.0)	0 (0)	78
Rosemount	21 Sep	40	3 (0.08)	36 (0.92)	39	10,131	2262 (0.97)	61 (0.03)	2323
Waseca	18 Sep	40	14 (0.23)	46 (0.77)	60	1331	873 (0.99)	10 (0.01)	883
Total	—	400	105	214	319	19,388	4109	119	4228
Proportion Total Larvae	—	—	33*	67**	—	—	97*	3**	—
Mean (±SEM)	—	—	0.263† (±0.080)	0.535†† (±0.138)	0.798§ (±0.158)	—	0.195† (±0.071)	0.008†† (±0.006)	0.203§ (±0.075)

* Proportion of early instar larvae on Bt ears significantly different from Non-Bt ears, Chi square test ($\chi^2 = 48.40$, df = 9, $P < 0.001$).

** Proportion of late instar larvae on Bt ears significantly different from Non-Bt ears, Chi square test ($\chi^2 = 514.48$, df = 7, $P < 0.001$).

† Means not significantly different, Students *t* test ($t = 0.63$, df = 18, $P = 0.54$).

‡ Means significantly different, Students *t* test ($t = 3.81$, df = 9, $P = 0.004$).

§ Means significantly different, Students *t* test ($t = 3.39$, df = 13, $P = 0.005$).

The presence of high numbers of early-instar *H. zea* larvae on 'GH-0937' can be partially explained by retarded growth, or a developmental delay induced by exposure to *B. thuringiensis* (Sims et al. 1996, J. Entomol. Sci. 31: 340-346; Storer et al. 2001, J. Econ. Entomol. 94: 1268-1279). The relatively high number of early-instar *H. zea* is likely due to a developmental delay and subsequently, reduced levels of cannibalism within a given cohort because there are fewer larvae developing beyond the third instar. Cannibalism in *H. zea* is believed to begin primarily in the third instar (Barber 1936, The cannibalistic habits of the corn ear worm. Tech. Serv. Bull. No. 499. U.S. Dept. of Agric., Washington, DC. 18 pp.). In pest management studies with non-Bt hybrids, it is common to observe a maximum of only 1 to 2 late-instar larvae/ear (e.g., Burkness et al. 2002) which is often attributed to cannibalism (Barber 1936).

Detection of *H. zea* infestations on 'GH-0937' corroborates other studies' findings that event BT11 expressing the Cry1Ab toxin provides <100% control of *H. zea* (Sims et al. 1996, Storer et al. 2001, Burkness et al. 2002). This is not surprising given the known differential susceptibility of Lepidoptera to *B. thuringiensis* toxins (MacIntosh et al. 1990, J. Invert. Path. 56: 258-266; O'Rourke and Hutchison 2000, J. Agric. Urban Entomol. 17: 213-217) and that the first Bt corn events were developed primarily for control of *O. nubilalis* (Ostlie et al. 1997, Bt corn and European corn borer. NCR Publication 602, University of Minnesota). Although high numbers of *H. zea* larvae were observed on 'GH-0937', the Bt hybrid provided approximately a 4-fold reduction in larval density compared to adjacent non-Bt 'Bonus' plantings (Table 1).

Despite the fact that *H. zea* larvae were observed on transgenic Bt ears in 1998, the potential for these larvae to have any long-term impact appears minimal in the midwestern U.S. because the insects are not known to survive winters above 40° N latitude (Metcalf and Metcalf 1993, Destructive and useful insects: their habits and control. McGraw-Hill, Inc., NY). Successful overwintering is further unlikely given the current resistance management protocol requiring transgenic sweet corn growers to destroy Bt-planted fields within one month of harvest by plowing or chopping, which should destroy most larvae remaining in plant material (Lynch et al. 1999).

Although current Bt hybrids such as 'GH-0937' provide less overall efficacy to *H. zea* compared with *O. nubilalis* (Burkness et al. 2002), the possibility that the majority of surviving *H. zea* larvae will be early instars indicates that the "in-field screen" method could be useful for late instars (e.g., Venette et al. 2000a, 2000 Proc. Beltwide Cotton Conf. 1058-1060; Venette et al. 2000b, J. Econ. Entomol. 93: 1055-1064). The use of an in-field monitoring approach could play a key role in developing a sound resistance monitoring program by determining baseline frequencies of resistance, and could be used in tandem with traditional lab bioassays for resistance screening (e.g., Siegfried et al. 2000, J. Econ. Entomol. 93: 1265-1268).

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